THE CONTRACTILE BASIS OF AMOEBOID MOVEMENT

V. The Control of Gelation, Solation, and Contraction in Extracts from *Dictyostelium discoideum*

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ABSTRACT

Motile extracts have been prepared from *Dictyostelium discoideum* by homogenization and differential centrifugation at 4°C in a stabilization solution (60). These extracts gelled on warming to 25°C and contracted in response to micromolar Ca^{++} or a pH in excess of 7.0. Optimal gelation occurred in a solution containing 2.5 mM ethylene glycol-bis(β-aminoethyl ether)N,N,N',N'-tetraacetate (EGTA), 2.5 mM piperazine-N-N'-bis[2-ethane sulfonic acid] (PIPES), 1 mM $MgCl_2$, 1 mM ATP, and 20 mM KCl at pH 7.0 (relaxation solution), while micromolar levels of Ca^{++} inhibited gelation. Conditions that solated the gel elicited contraction of extracts containing myosin. This was true regardless of whether chemical (micromolar Ca^{++}, pH >7.0, cytochalasin B, elevated concentrations of KCl, $MgCl_2$, and sucrose) or physical (pressure, mechanical stress, and cold) means were used to induce solation. Myosin was definitely required for contraction. During Ca^{++}- or pH-elicited contraction: (*a*) actin, myosin, and a 95,000-dalton polypeptide were concentrated in the contracted extract; (*b*) the gelation activity was recovered in the material squeezed out the contracting extract; (*c*) electron microscopy demonstrated that the number of free, recognizable F-actin filaments increased; (*d*) the actomyosin MgATPase activity was stimulated by 4- to 10-fold. In the absence of myosin the *Dictyostelium* extract did not contract, while gelation proceeded normally. During solation of the gel in the absence of myosin: (*a*) electron microscopy demonstrated that the number of free, recognizable F-actin filaments increased; (*b*) solation-dependent contraction of the extract and the Ca^{++}-stimulated MgATPase activity were reconstituted by adding purified *Dictyostelium* myosin. Actin purified from the *Dictyostelium* extract did not gel (at 2 mg/ml), while low concentrations of actin (0.7–2 mg/ml) that contained several contaminating components underwent rapid Ca^{++}-regulated gelation.

These results indicated: (*a*) gelation in *Dictyostelium* extracts involves a specific Ca^{++}-sensitive interaction between actin and several other components; (*b*) myosin is an absolute requirement for contraction of the extract; (*c*) actin-myosin interactions capable of producing force for movement are prevented in the gel,

while solation of the gel by either physical or chemical means results in the release of F-actin capable of interaction with myosin and subsequent contraction. The effectiveness of physical agents in producing contraction suggests that the regulation of contraction by the gel is structural in nature.

KEY WORDS actin · myosin · regulation · gelation · contraction

Dynamic structural changes are a common characteristic of cytoplasm during nonmuscle cell movements. One of the best examples is the cytoplasmic streaming in the large amoebae of the *Chaos* group in which cytoplasm undergoes a cyclic conversion from the less structured endoplasm or "sol" to the rigid ectoplasm or "gel" during normal movement. Consequently, theories of amoeboid movement have relied heavily on structural changes to explain movement (1, 39). Experiments designed to probe cytoplasmic organization have also demonstrated the necessity of structure in cell movements. The use of cold and pressure (32, 37), mechanical agitation (3), centrifugation (22, 2), and mechanical stress (18, 61) led to the conclusion that cytoplasm had a spatially variable structure and that the removal of structure disrupted cell movement or drastically altered the mode of movement. It was suggested that in some cases structural changes actually caused amoeboid movement (39).

Calcium has been demonstrated to regulate amoeba cytoplasmic contractions and cytoplasmic structure in single cell extracts (60), bulk extracts (62, 63), and intact cells (61, 67, 66). Furthermore, structural transformations of actin in cell-free extracts have been implicated in the dynamics of cell movement (62, 63). In addition, gradients of cytoplasmic structure and contractility have been suggested to control the extent, rate, and direction of movement (39, 1, 60, 61, 68, 10, 62, 63). However, the complete molecular basis and control of cytoplasmic structure and contractility must be determined in order to relate both of these processes to cell movements. The aim of the present investigation is to determine the ionic control of gel-sol transformations in relation to contraction.

MATERIALS AND METHODS

Materials

Materials were obtained as follows: ethylene glycol-bis(β-aminoethyl ether) N,N,N',N'-tetraacetate (EGTA), piperazine-N-N'-bis[2-ethane sulfonic acid] (PIPES), bovine serum albumin, myoglobin type I, ATP (Sigma Chemical Co., St. Louis, Mo.); ultrapure glutaraldehyde (PolySciences Corp., Niles, Ill.); Trasylol (Mobay Chemicals, New York); phosphorylase A (Worthington Biochemical Corp., Freehold, N. J.); osmium tetroxide (Fisher Scientific Co., Pittsburgh, Pa.); cytochalasin B (Worthington Biochemical Corp.); antibody to human erythrocyte spectrin was supplied by Dr. David Shotten (Biological Laboratories, Harvard University).

Methods

CULTURES: Amoebae of *Dictyostelium discoideum* (strain A3, a gift of Dr. Richard Kessin, Biological Laboratories, Harvard University) were grown in axenic culture (34) and harvested while in log growth phase at a concentration of ca. 1×10^7 cells/ml. The cells were collected by centrifugation at 200 g for 5 min and washed in cold phosphate buffer at pH 6.0.

The movement of washed amoebae was observed by placing a drop of phosphate buffer containing cells at a concentration of 1×10^7/ml on a glass microscope slide. Slides were degreased with acetone and washed in 7 × detergent before use.

PREPARATION OF THE MOTILE EXTRACTS, S1, S2, AND S3. Packed, washed ameobae were resuspended in an equal volume of 5 mM EGTA, 5mM PIPES, 1 mM dithiothreitol (DTT) at pH 7.0 containing 0.04 ml Trasylol/ml of suspension and chilled for 10 min on ice (Fig. 1). Cells were lysed by grinding in a tight-fitting Dounce homogenizer (Kontes Co., Vineland, N. J.) with 30 passes. This was sufficient to rupture all of the cells as monitored in the light microscope. Extracts were maintained at 4°C during the homogenization step and at each subsequent stage except where noted.

The pH of the homogenate, containing an average of 41 mg/ml protein, was adjusted slowly to 6.75 with 0.1 M KOH and centrifuged at 3,000 g for 5 min at 4°C (Fig. 1). The floating lipid fraction was aspirated off and the turbid supernate, designated S1, was carefully removed by pipetting. S1 was further fractionated by centrifugation at 45,000 g for 15 min at 4°C. This supernate, designated S2, was slightly turbid and had a light yellow color due to residual pigment. The pH of S2 was adjusted to 6.75 with 0.1 M KOH and centrifuged at 100,000 g for 30–60 min at 4°C. The final supernate was designated S3.

PREPARATION OF THE NONMOTILE EXTRACT, SB: 0.1 M $MgCl_2$, 0.1 M ATP, and 3 M KCl were added to S3 to final concentrations of 2 mM $MgCl_2$, 2 mM ATP, and 0.2 M KCl with stirring at 4°C. The pH was adjusted to 7.0 and the mixture was warmed to 25°C

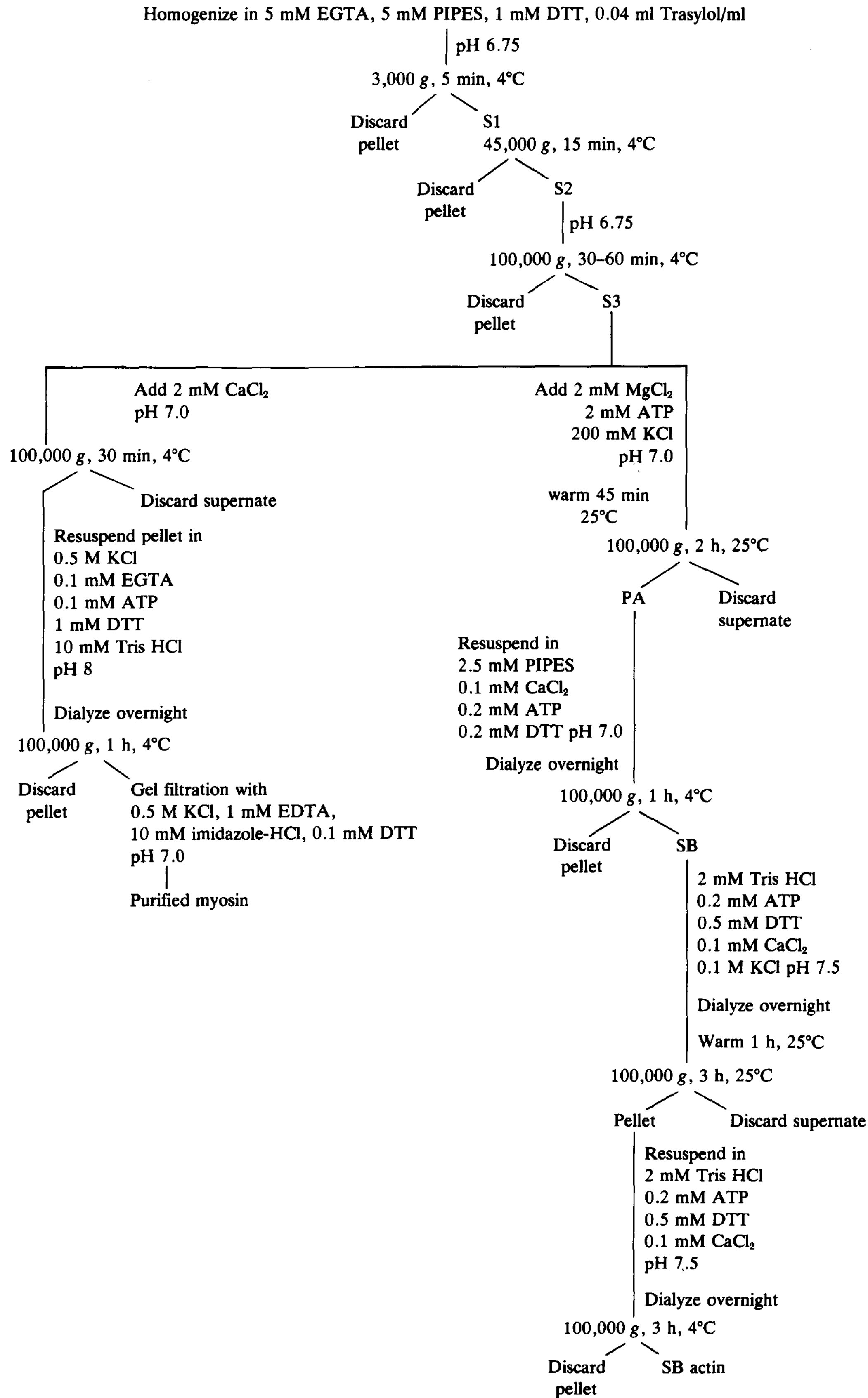

FIGURE 1 Methods of preparation of the extracts S3, SB, SB actin, and *Dictyostelium* myosin.

for 45 min. The viscosity of the extract increased during warming in 0.2 M KCl. The extract was then centrifuged at 100,000 *g* for 2 h at 25°C. The pellet (PA, Fig. 1) was resuspended in 2.5 mM PIPES, 0.1 mM $CaCl_2$, 0.2 mM ATP, 0.2 mM DTT, pH 7.0 (depolymerization buffer) by homogenization in 1/6 the original volume of S3. 0.04 ml of trasylol/ml of homogenate was added and the pH was adjusted to 7.0 with 0.1 M KOH. The solution was dialyzed overnight at 4°C against the depolymerization buffer. The turbid material containing myosin was removed by centrifugation at 100,000 *g* for 1 h at 4°C. The supernate was designated SB, the nonmotile extract (Fig. 1).

PREPARATION OF SB ACTIN: Actin was prepared by dialyzing SB overnight against 2 mM Tris HCl, 0.2 mM ATP, 0.5 mM DTT, 0.1 mM $CaCl_2$, 0.1 M KCl, pH 7.5. SB warmed to 25°C for 1 h in this solution, did not gel, but became viscous. Negatively stained preparations of the viscous solution demonstrated the presence of F-actin. The actin was pelleted by centrifugation at 100,000 *g* for 3 h at 25°C. The pellet was resuspended by homogenization in 2 mM Tris HCl, 0.2 mM ATP, 0.5 mM DTT, 0.1 mM $CaCl_2$, pH 7.5, and dialyzed overnight at 4°C. The solution was then clarified at 100,000 *g* for 3 h at 4°C. The supernate was designated SB actin (Fig. 1).

PREPARATION OF MYOSIN FROM S3: 0.1 M $CaCl_2$ was added to S3 at 4°C to a final concentration of 2 mM. This resulted in a large increase in turbidity of S3. The pH was adjusted to 7.0 with 0.1 M KOH, and the turbid material was pelleted at 100,000 *g* for 30 min at 4°C. The pellet was resuspended by homogenization in 0.5 M KCl, 0.1 mM EGTA, 0.1 mM ATP, 1 mM DTT, and 10 mM Tris HCl at pH 8.0 and dialyzed overnight. The insoluble material was pelleted by centrifugation at 100,000 *g* for 1 h at 4°C and the clear supernate was chromatographed on a 1.6- × 70-cm agarose column (Bio-Rad A15m, Bio-Rad Laboratories, Richmond, Calif.) equilibrated with 0.5 M KCl, 1 mM EDTA, 10 mM imidazole HCl, and 0.1 mM DTT at pH 7.0. The peak Ca^{++} ATPase fractions contained *Dictyostelium* myosin (Fig. 2).

PREPARATION OF OTHER PROTEINS: Muscle actin was purified to electrophoretic homogeneity from acetone powder of rabbit skeletal muscle according to the method of Spudich and Watt (55). Heavy meromyosin (HMM) was prepared from rabbit skeletal muscle according to Lowey and Cohen (35) and was chromatographed on agarose (Bio-Rad A15m) columns in 0.1 M KCl, 0.1 mM EDTA, and 10 mM imidazole HCl, pH 7.0, before use. *Dictyostelium* actin was prepared by the method of Spudich (56). In addition to the method outlined in Fig. 1, *Dictyostelium* myosin was also prepared according to the KCl-KI chromatography technique (49) as used by Clarke and Spudich (8). Human erythrocyte ghosts were prepared from outdated human blood according to the method of Dodge et al. (13).

ASSAYS: DNA was assayed according to the method of Burton (5). Protein was assayed by the Folin procedure with bovine serum albumin as standard (36).

The ATPase activity of the extracts and purified protein fractions was assayed according to a modified Martin and Doty procedure (47). Ca^{++} stimulation of the extract ATPase in the presence of Mg^{++} ATP (see figure and table captions for buffers) was followed by stopping the reaction at 2, 4, 6, 8, 10, 15, and 20 min. The peak activity expressed as micromoles P_i per minute before the activity plateaued was used to calculate the average stimulated activity of the extract at the various Ca/EGTA ratios.

The formation of the macroscopic gel was observed by inverting test tubes (8 × 75 mm) containing 0.1 ml of extract at various intervals after warming. The extent of gelation was scored at: +, viscous semisolid that poured slowly out of an inverted tube; ++, solid gel that incompletely supported its own weight and ran out of an

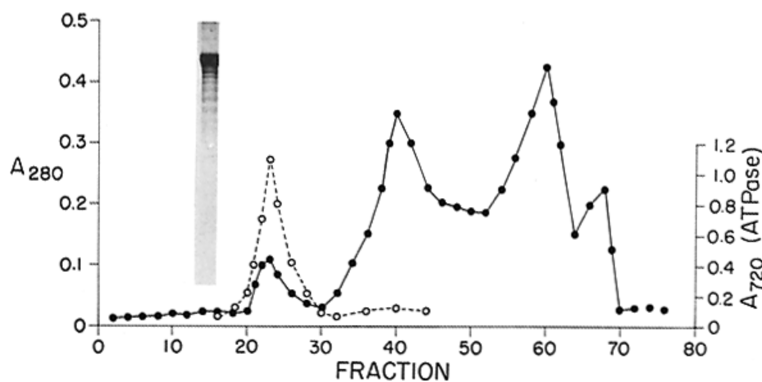

FIGURE 2 Purification of *Dictyostelium* myosin by gel filtration. The 1.6- × 70-cm column of agarose Bio-gel A-15m (200–400 mesh) was equilibrated and eluted at 4°C with 0.5 M KCl, 1mM EDTA, 10 mM imidazole-HCl and 0.1 mM DTT at pH 7.0. The sample was 2 ml of Ca^{++}-precipitated myosin from S3. A_{280} (●), Ca^{++} ATPase (○). Insert shows gel electrophoresis in SDS of the ATPase peak (fraction 22).

inverted tube; +++, solid gel that supported its own weight and did not run out of an inverted tube (63). Scoring the ability of the gelled extract to support its own weight in a test tube as a function of time afforded a semiquantitative estimate of the rate of gelation which was reproducible in replicate assays. The results of the test tube assays are shown in Tables II, III, V, VI, and VII.

POLYACRYLAMIDE GEL ELECTROPHORESIS: Protein samples were prepared for electrophoresis by mixing them 1: 1 with twice concentrated sample buffer (0.12 M Tris HCl, pH 6.8, 4% sodium dodecyl sulfate [SDS], 20% glycerol, 10% mercaptoethanol, and 0.01% bromophenol blue). The solution was then diluted to the desired protein concentration with sample buffer and heated for 5 min in a boiling water bath. The proteins were electrophoresed by the method of Laemmli (31), modified to a slab configuration. The slab gel was 0.8 mm thick with a 2.5-cm long 3% stacking gel and a 10-cm long 10% running gel.

Gels were stained at room temperature for 30 min with 1% Coomassie Blue in 10% acetic acid and 50% methanol, and destained at room temperature in 10% methanol and 7% acetic acid. Gels were scanned with a Joyce, Loebl microdensitometer (Joyce, Loebl and Co., Ltd., Gateshead-on-Tyne, England).

The molecular weights of polypeptides were calculated by the method of Weber and Osborn (75) using the following proteins as standards: human erythrocyte spectrin, 240,000 and 220,000; phosphorylase A, 95,000; bovine serum albumin, 68,000; muscle actin, 42,000; myoglobin, 18,700 daltons.

IMMUNOCHEMICAL ANALYSIS: Double diffusion was carried out on glass slides containing 1% agar prepared in 0.05 M NaCl, phosphate buffer at pH 7.0, by the Ouchterlony procedure (44). Samples to be analyzed were added in 2.5 mM EGTA and 2.5 mM PIPES, pH 7.0, or in the depolymerization buffer.

MEASUREMENT OF TURBIDITY: Turbidity changes in the extract were measured in 0.3-ml samples with a Beckman Acta III spectrophotometer (Beckman Instruments, Inc., Fullerton, Calif.). The sample temperature was controlled by a remote water bath circulator and monitored with a temperature probe supplied with the spectrophotometer.

Gelation of the extracts was accompanied by a rapid increase in turbidity that occurred in the absence of any contractile activity in the gel. The increased turbidity was always related temporally to gelation and was used to follow the gelation of extracts under a variety of conditions as described below.

To quantitate the initial rate of contraction of the extract (Tables II, III, V, and VI), the curvette carrier was fitted with an aperture in the bottom fifth of the curvette. Contraction of the extract always occurred toward the meniscus, resulting in an abrupt decrease in measured turbidity as the contracting cytoplasm pulled clear of the apertured region. The time to inflection of the turbidity curve (Figs. 5 A, 7 A, and 9) was equivalent to the time of initiation of contraction of the extract (Tables II, III, V, and VI).

ELECTRON MICROSCOPY: The gel and contracted gel were fixed at room temperature with 2% glutaraldehyde in either the relaxation or contraction solutions (Tables II and III) for 1 h. Fixed samples were overlayed with agar and postfixed in 1.0% OsO_4, 20 mM phosphate buffer, pH 7.0, at 4°C for 1 h, and embedded in Spurr's resin. Light gold and silver sections were stained with saturated uranyl acetate followed by lead citrate and observed with a Philips 301 electron microscope. Calibration was carried out with a no. 1002 cross-ruled optical grating replica (Fullam, Ernest, Inc., Latham, N. Y.).

Extracts were negatively stained by several methods: (*a*) the gelled or contracted extract was dispersed and diluted 1:15 (vol:vol) with the appropriate test solution and applied immediately to a Formvar-coated grid and stained with 1–2% uranyl acetate by the technique of either Moore et al. (42) or Huxley (27); (*b*) the extract was allowed to warm (and in some cases gel) on standard or poly-L-lysine-coated Formvar grids. The bulk of the material was then sheared away by flowing the test solution over the grid, leaving a thin layer of material behind which was negatively stained according to either of the above techniques.

OPTICAL METHODS: The extracts were observed with Zeiss Nomarski differential interference optics (Carl Zeiss, Inc., New York) and Nikon rectified polarized light optics (Nikon Inc., Garden City, N. Y.). Birefringence measurements were made with a new birefringence detection system: the Polar Eye (65, 62). Strain birefringence was induced in the extracts placed in observation chambers. The chambers were constructed from two strain-free cover glasses separated by a distance of 0.75 mm. A microcapillary held by a micromanipulator was inserted into the extract, and the extract was assayed for strain birefringence by moving the microcapillary 10 μm. The measuring beam (30 μm) was localized directly behind the microcapillary. This was the first quantitative assay for gelation (viscoelasticity).

AEQUORIN LUMINESCENCE: Aequorin luminescence was measured with a calibrated photometer (quanta per second) (23) in a light-tight chamber. 1-μl aliquots of purified aequorin from a 10 mg/ml stock were added to a 0.5-ml sample. The aequorin was placed in an empty vial, and the test solution or the extract (S3) was loaded into a syringe inserted through a light-tight seal directly over the vial. The solution was injected into the vial to initiate the experiment. Subsequent test solutions were injected into the vials with syringes. The luminescence of aequorin-Ca^{++}/EGTA test solutions was measured to calibrate the detection system. Ca^{++}/EGTA ratios of 0.1, 0.2, 0.4, 0.8, and 0.9 yielded luminescence values (quanta per second) of 1.5×10^9, 3.6×10^9, 5.3×10^9, 7.8×10^{10}, and 1.7×10^{11}. The relaxation solution and the contraction solution had luminescence

values of 1.0×10^9 and 1.3×10^{11}, respectively.

PRESSURE EXPERIMENTS: Whole cells or gelled extracts (S3) were placed in shell vials in a Yeda press. The pressure was raised to 2,000 lb/in^2 for 1 min at 25°C. Subsequent decompression occurred over a 1-min period.

RESULTS

General Description

Cells grown to a density of 1×10^7/ml in axenic culture were checked for motility before homogenization by plating the washed cells on cleaned glass slides. Normal motile behavior included a rapid flattening of the cells on the glass substrate accompanied by cytoplasmic cyclosis. Within a few minutes, the cells exhibited amoeboid movement using a combination of filopodia, pharopodia and lobopodia, resulting in aggregation of the cells (63).

Fig. 1 outlines the methods of preparation of the extracts after homogenization in 5 mM EGTA and 5 mM PIPES buffer. The extracts designated S1, S2, and S3 exhibited behavior that was similar to that of extracts prepared previously from *Dictyostelium* in the presence of a relaxing solution (63) and *Amoeba proteus* prepared in a stabilization solution (63).

Warming the S1, S2, and S3 to 25°C resulted in an increase in structure that could be viewed in the light microscope as an increase in the refractive index, relative to the surrounding buffer, and the appearance of cytoplasmic fibrils when the extract was disturbed with a microneedle. The increase in structure was observed in the test tube as a solidification or gelation of the extract (63).

The extracts remained completely nonmotile at room temperature, even in the presence of exogenous 1 mM $MgCl_2$ and ATP. Contractions and streaming (motility) could be elicited in the extracts by adding a contraction solution (1 mM $MgCl_2$, 1 mM ATP, 20 mM KCl, 10^{-6} M Ca^{++}, pH 7.0) to samples warmed in the light microscope observation chamber (60). Contraction began at the point of addition of the contraction solution and subsequently spread through the extract, contracting the entire preparation. Cytoplasm squeezed out from the contracting extract underwent vigorous cytoplasmic streaming.

The extract could also be contracted in large quantities in test tubes either by layering the contraction solution on top of the gelled extract at 25°C or by adding enough $CaCl_2$ to the extract on ice to bring the Ca^{++}/EGTA ratio above 0.2 and then warming to 25°C at pH 7.0. Contraction was observed in the test tube as a volume reduction of the extract as shown in Fig. 5 A (inset).

Raising the pH of S1, S2, or S3 above 7.0 resulted in the progressive loss of Ca^{++} regulation of contraction and in faster spontaneous[1] contraction and streaming. Reduction of the pH below 7.0 resulted in gradual loss of motility (contraction and streaming) in the extracts even in the presence of Ca^{++}, until at pH 6.6 no motility was observed. Low pH also reduced the rate of gelation in all three extracts (63). This effect of low pH on the rate of gelation and motility of the extract was reversible by raising the pH again to 7.0.

Periodically, some cultures plated on glass slides remained rounded and did not exhibit normal locomotory behavior. Extracts prepared from these cells did not exhibit gelation on warming and demonstrated feeble spontaneous contraction and streaming when observed in the light microscope. This problem was avoided by cloning motile cells every 6 wk, which resulted in a constant supply of cells exhibiting normal motile behavior and extracts capable of gelation and Ca^{++}-regulated contraction.

Although the behavior of the three extracts was similar in response to Ca^{++} and pH, S3 was chosen for detailed study because it contained the smallest number of components as indicated by SDS gel electrophoresis (Fig. 3*a*).

Composition of S3

S3 contained an average of 27 μg/ml of DNA. The polypeptides believed to play an important role in gelation and contraction are shown in Table I. The criteria used for identifying these components were as follows:

(*a*) The 250,000-, 95,000-, 75,000-, 50,000-, 38,000-, and 28,000-dalton components copurified with actin during the preparation of actin from the extract. The 55,000-dalton component had a mobility on SDS gels similar to that of components implicated in actin binding in other systems (30). The 250,000-dalton component did not appear to be a spectrin-like molecule since it did not precipitate in the presence of millimolar Ca^{++} during preparation of myosin (16) (Fig. 1). Furthermore, SB, containing substantial amounts of this component (Fig. 3*b*), did not cross-react with antibodies prepared against human erythrocyte spectrin. The mol wt of the 250,000-dalton

[1] Spontaneous contraction is defined as contraction of the extract in the absence of Ca^{++}

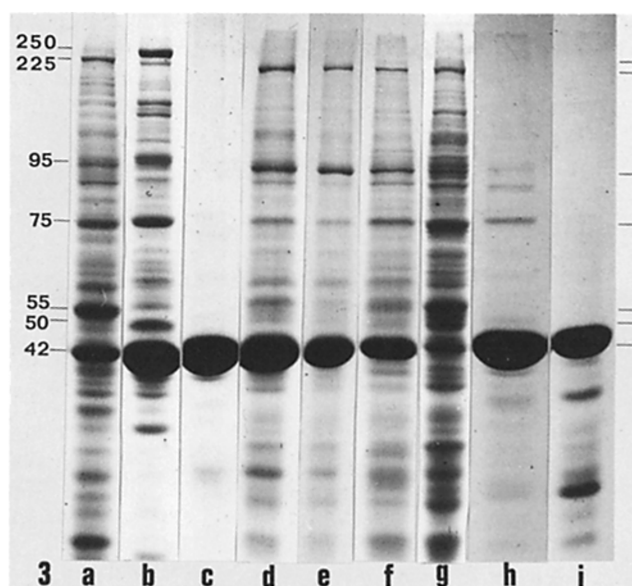

FIGURE 3 SDS-polyacrylamide slab gel electrophoresis of: (*a*) S3; (*b*) SB; (*c*) *Dictyostelium* actin purified according to the method of Spudich (56); (*d*) contracted gel resulting from a pH 7.4-elicited contraction; (*e*) contracted gel resulting from micromolar Ca^{++}-elicited contraction; (*f*) gelled S3 after centrifugation at 12,000*g* for 10 min at 25°C; (*g*) Ca^{++}-contracted gel supernate from (*e*); (*h*) SB actin; (*i*) *Dictyostelium* actin purified according to the method of Spudich (56), showing low molecular weight contaminants. The preparations of actin shown in (*c*) and (*i*) would not gel under any of the conditions tested (see text). S3(*a*), SB(*b*), and SB actin (*h*) rapidly gelled on warming in the relaxation solution. Numbers shown correspond to the polypeptide molecular weights in daltons × 10^{-3}.

TABLE I
*Composition of the Extracts**

	S3	SB	Ca^{++} contracted pellet	pH contracted pellet	SB actin
Average protein concentration (mg/ml)	16.2	4.4	NM‡	NM	0.73
250,000 (%)	0.85	4.65	ND§	ND	NM
Myosin	2.8	0.7	3.4	4.5	ND
95,000	3.9	6.7	7.6	6.0	1.26
75,000	5.0	6.1	2.4	3.7	5.2
55,000	7.3	2.1	1.7	4.0	ND
50,000	2.8	6.4	1.5	1.6	ND
Actin	8.6	24.0	49.0	32.0	73.5
38,000	1.2	2.0	NM	NM	1.2
28,000	0.9	3.8	ND	ND	ND

* Percent of each band was determined by quantitative densitometry of SDS-polyacrylamide gels. The numbers shown are the averages of five different experiments.
‡ NM, not measured.
§ ND, not detected.

band was determined relative to human erythrocyte spectrin on SDS gels.

(*b*) The 225,000-dalton polypeptide has been identified as myosin by isolating myosin from S3 as outlined in Figs. 1 and 2 and by demonstrating comigration with the isolated myosin on SDS gels. The molecular weight of the myosin heavy chain on SDS gels was determined relative to erythro-

cyte spectrin. The specific activity of *Dictyostelium* myosin isolated as in Fig. 1 averaged 0.07 μmol P_i/min per mg myosin in 10 mM $CaCl_2$, 10 mM imidazole HCl, pH 7.0, 0.5 M KCl, and 0.12 mg myosin/ml. Addition of purified *Dictyostelium* or rabbit skeletal muscle actin to *Dictyostelium* myosin in a weight ratio of 6:1 actin to myosin resulted in an average threefold stimulation of the myosin ATPase activity (0.02-0.06 μmol P_i/min per mg myosin) with *Dictyostelium* actin and fivefold stimulation with rabbit skeletal muscle actin (0.02 to 0.1 μmol P_i/min per mg myosin). Actin activation of the myosin ATPase was carried out in the relaxation solution (Table II) at pH 7.0 at 1 mg/ml of actin and 0.17 mg/ml of myosin. The properties of *Dictyostelium* myosin isolated from the motile extract S3 are comparable to those of *Dictyostelium* myosin prepared according to Clarke and Spudich (8).

(*c*) The 42,000-dalton polypeptide is actin as demonstrated by comigration with rabbit skeletal muscle actin on SDS gels and by purification from the extract as outlined in Fig. 1.

Together, these bands constituted over 33% of the protein in S3 while actin and myosin alone constituted over 11%.

Control of Gelation and Contraction in the Motile Extract S3

To assess the effect of the various salts on gelation in S3, disodium ATP, KCl, and $MgCl_2$ were added to freshly isolated S3 and to S3 after removal of the endogenous salts by dialysis.

KCl, ATP, and $MgCl_2$

Exogenous $MgCl_2$, ATP, or KCl was not required for gelation of S3, even after removal of

TABLE II

Effect of ATP, $MgCl_2$, and KCl on Gelatin and Contraction of S3

				Extent of gelation§§	Initiation of contraction
				avg time, min	*min*
S3*				++(24)	–
S3 +	1 mM Na_2ATP			++(15)	–
S3 +		1 mM $MgCl_2$		++(11)	–
S3 +		5 mM $MgCl_2$		+(9)	solation 30§
S3 +	1 mM Na_2ATP	+ 1 mM $MgCl_2$		++(8)‡	–
S3 +	"	+ 5 mM $MgCl_2$		++(14)	30
S3 +			20 mM KCl	++(8)	–
S3 +	1 mM Na_2ATP	+	"	++(5)‖	–
S3 +		1 mM $MgCl_2$ +	"	++(7)	–
S3 +		5 mM $MgCl_2$ +	"	+(5)	–¶
S3 +	1 mM Na_2ATP	+ " +	"	++(15)	20
S3 +	1 mM Na_2ATP	+ 1 mM $MgCl_2$ +	20 mM KCl**	+++(6)	–
S3 +	"	+ " +	40 mM KCl‡‡	+++(7)	7
S3 +	"	+ " +	60 mM KCl	++(2)	4
S3 +	"	+ " +	100 mM KCl	++(13)	27
S3 +	"	+ " +	150 mM KCl	+(5)	–
S3 +	"	+ " +	200 mM KCl	+(10)	–
S3 +	"	+ " +	300 mM KCl	–	–

* 10-ml samples of S3 containing an average protein concentration of 16.2 mg/ml were dialyzed to equilibrium (as determined by conductivity measurements) against two changes of 250 ml of 2.5 mM EGTA and 2.5 mM PIPES at pH 7.0. Care was taken to insure that the pH = 7.0 throughout the gelation assays, which were carried out at 25°C. Similar results were obtained with freshly prepared S3, except where noted.

‡ Freshly prepared S3 gelled to +++ in 15 min.

§ Freshly prepared S3 began to contract in 10 min.

‖ Freshly prepared S3 gelled to +++ in 5 min.

¶ Freshly prepared S3 began to contract in 15 min.

** This solution is designated the relaxation solution.

‡‡ This is the final concentration added to S3 from a 3 M KCl stock.

§§ The maximum extent of gelation was scored as described in Materials and Methods. The average time (in parentheses) refers to the length of time required for maximum gelation to the extent indicated after warming to 25°C. Incubation for longer times at 25°C did not increase the extent of gelation.

free ions by dialysis. Gelation occurred on warming to 25°C in the presence of 2.5 mM EGTA and 2.5 mM PIPES at pH 7.0 (Table II). However, optimal gelation was produced by a solution containing 1.0 mM $MgCl_2$, 1.0 mM ATP, and 20 mM KCl. This solution was designated the relaxation solution (Table II) since it resembled solutions used to relax whole amoeba cytoplasm obtained from single cells (60, 61). Concentrations of KCl above 20 mM resulted in a decrease in the extent of gelation and spontaneous contraction of the extract S3. However, concentrations of KCl greater than or equal to 150 mM inhibited contraction of S3 (Table II). This was consistent with the observation that the ATPase activity of purified *Dictyostelium* actomyosin was inhibited by KCl concentrations exceeding 150 mM (8).

Freshly prepared S3 containing exogenous $MgCl_2$ to 5 mM gelled poorly and contracted spontaneously[1] in the absence of Ca^{++}. The only exception to this was observed in S3 that was desalted before addition of the $MgCl_2$ (Table II). In this case, in the absence of ATP, spontaneous contraction did not occur. Instead, the gel solated slowly at room temperature after forming (Table II).

The Effect of Ca^{++} on S3

If the pH was maintained at 7.0 in the presence of the relaxation solution (Table II), the extract did not contract spontaneously even after incubation of the gelled extract for several hours at room temperature. In many cases, gelled samples left overnight at room temperature remained gelled and uncontracted. However, addition of Ca^{++} to these samples by layering the contraction solution (Table III) on top of the gel resulted in rapid contraction of the extract.

To determine the Ca^{++} requirement for contraction of S3 in the presence of the relaxation solution, enough 0.1 M $CaCl_2$ was added to S3 on ice to raise the Ca/EGTA ratio (Table III). The pH of the extract dropped on addition of Ca^{++} due to the presence of EGTA and possibly endogenous calcium-binding proteins (62). However, even in the absence of EGTA, the pH dropped when unbuffered Ca^{++} was added to the extract (0.1–0.2 pH U/0.01 mM Ca^{++}). Therefore, in either case it was necessary to return the pH to the desired value before warming. As shown in Table III, an increase in the Ca^{++} concentration resulted in the onset of contraction upon warming, the time

TABLE III
*Effect of Ca^{++} on Gelation and Contraction in S3 and SB**

	S3		SB	
Ca/EGTA¶	Extent of gelation‖	Initiation of contraction	Extent of gelation	Initiation of solation‡
	avg time, min	*min*	*avg time, min*	*min*
0.0	+++(5)	–	+++(5)	–
0.1	+++(5)	–	+++(5)	–
0.2	+++(5)	18	+++(5)	18
0.4	++(5)	13	+++(5)	11
0.6	+(5)	7	+(5)	10
0.8	–	superprecipitation	–	–
0.9§	–	superprecipitation	–	–
1.0	–	superprecipitation	–	–

* S3 and SB were warmed in the relaxation solution (Table II) to 25°C at pH 7.0 containing the Ca/EGTA ratios indicated.

‡ Solation of gelled SB was also achieved by layering the contraction solution on top of the gel as outlined in Table IV. The time to initiation of solation was determined by inverting the tubes containing the extract at various time intervals.

§ This solution containing 1 mM Na_2ATP, 1 mM $MgCl_2$, 20 mM KCl, and Ca/EGTA = 0.9 at pH = 7.0 is designated the contraction solution.

‖ See caption for Table II.

¶ The precise [Ca^{++}] representing each Ca/EGTA will depend on the Ca^{++}-EGTA binding constant chosen, pH, and the contribution of extract components to Ca^{++} binding. Since the latter is unknown, only estimates can be made for the [Ca^{++}]. At pH 7.0 (assuming $K_{EGTA} = 1.1 \times 10^6\ M^{-1}$), the [$Ca^{++}$] would vary from 1×10^{-7} M to 1×10^{-5} M in going from Ca/EGTA = 0.1 to 1.0, respectively.

of which was inversely proportional to the Ca/EGTA ratio. Contractions were usually complete at room temperature 5-15 min after initiation (Table III). Addition of Ca^{++} to S3 at 4°C did not cause contraction if the extract was not warmed. SDS gel electrophoresis of the contracted extract obtained by decanting the supernate squeezed out during Ca^{++}-elicited contraction (Fig. 5 A, inset) demonstrated that the contracted extract was composed primarily of actin, myosin, and the 95,000-dalton polypeptide (Fig. 3*e* and Table I). The contracted extract which contained actin (Fig. 3*e*) did not gel upon warming to 25°C when resuspended in the relaxation solution at pH 7.0. However, the supernate squeezed out during contraction (Fig. 3*g*), when dialyzed against the relaxation solution at pH 7.0, formed a solid gel at 25°C upon addition of *Dictyostelium* actin.

Ca/EGTA ratios above 0.6 resulted in a rapid increase in turbidity (Fig. 5 A) and superprecipitation of the extract. Collection of the turbid or superprecipitated material by centrifugation demonstrated that it was composed primarily of actin and myosin as demonstrated previously (63). Removal of ATP from the extract by dialysis abolished the Ca^{++}-elicited contraction and superprecipitation of the extract.

In addition to the onset of contraction in S3 in response to Ca^{++}, the ATPase activity of S3 was activated by Ca/EGTA ratios between 0.2 and 1.3 (Fig. 5 B). The largest activation occurred at Ca/EGTA = 1.1 where the activity was 3.4 nmol/min as compared with 0.7 nmol/min in the absence of Ca^{++}. This ATPase activity was strongly inhibited at Ca/EGTA > 1.3 (Fig. 5 B) (19). Removal of myosin from the extract either by Ca^{++} precipitation (Fig. 1) or by preparation of SB (Fig. 1) abolished the Ca^{++}-stimulated ATPase activity shown in Fig. 5 B (Table VIII). The ATPase activity of purified *Dictyostelium* actomyosin was not stimulated by Ca^{++} over the range of concentrations tested in Table VIII.

The effect of Ca^{++} on S3 that had already gelled in the presence of the relaxation solution was tested by layering the relaxation solution containing various Ca/EGTA ratios on top of the gelled extract S3 (63). Optimal contractions were obtained by lowering the free EGTA concentration in S3 (2.5 mM) by first bringing the Ca/EGTA ratio of S3 to 0.1 on ice before warming to form the gel. This Ca/EGTA ratio did not inhibit gelation or elicit contraction (Table III). As the Ca^{++} concentration layered on top of the gel was increased, the rate of contraction increased (Table IV). This was reminiscent of the response of cytoplasm isolated from single cells of *Chaos carolinensis* to Ca^{++} (60).

The Structure of Gelled and Ca^{++}-Contracted S3

Negatively stained preparations of S3 that had gelled to +++ in the presence of the relaxation solution at pH 7.0 exhibited a large number of aggregates with very few free F-actin filaments (Fig. 6*a*). Such aggregates have been observed previously in negatively stained extracts of *A. proteus* (62) where they were designated "amorphous aggregates" and in our earlier report of extracts from *D. discoideum* (63).

The Ca^{++}-contracted gel revealed the presence of large numbers of free F-actin filaments with an apparent decrease in the amount of aggregates on the grid relative to the gelled S3 preparations (Fig. 6*b*). Many actin filaments appeared to be associated with the fibrous aggregates that were present (Fig. 6*b*). The thin filaments in these preparations were identified as F-actin by HMM binding. No myosin thick filaments were observed in these preparations, in contrast to the large number of ca. 0.5-μm long filaments observed under similar conditions in extracts from *A. proteus* (62).

To determine the three-dimensional organization of the undisturbed gel and the contracted gel, they were fixed at room temperature and embedded for thin sectioning. No macroscopic contractions were observed during glutaraldehyde fixa-

Table IV

*Ca^{++}-Induced Contraction of Gelled S3**

Ca/EGTA	Time to 50% contraction
	min
0.0	–
0.2	6
0.4	4
0.6	3
0.9	2

* S3 at an average protein concentration of 16.2 mg/ml in the relaxation solution containing Ca/EGTA = 0.1 was assayed for contraction in a cuvette. 0.2 ml of S3 was placed in the cuvette and allowed to gel completely at pH 7.0 and 25°C (3 min). 0.02 ml of the test solution was then layered on top of S3. 50% contraction was estimated by comparing the volume of the extract in the cuvette to the initial volume of the extract before contraction.

tion of S3 that had gelled to +++ in the presence of the relaxation solution. The gel maintained its initial volume and did not become milky white in appearance as did contracting gels. Thin sections of gelled S3 demonstrated the presence of very few free, thin filaments with a majority of amorphous material (Fig. 6*c*) (62, 63).

The onset of gelation could be followed by using the polarizing light microscope to measure changes in retardation due to strain birefringence by applying a localized tension to the gelling or gelled extract with a micropipette. The results of such an experiment are shown in Fig. 4. Before the onset of gelation, no strain birefringence could be induced by stressing the extract. After gelation, the unstressed extract was optically isotropic (62). However, stressing the gelled extract resulted in a large increase in retardation which was stored partially by the gelled extract after removal of the stress. This behavior is characteristic of a viscoelastic material and is similar to that observed for cytoplasm isolated from single cells of *Chaos carolinensis* in the absence of exogenous ATP and a submicromolar free calcium ion concentration (60), and for anterior endoplasm (61, 18) and ectoplasm in intact cells (61).

Unlike the gelled extract, the majority of the material in thin sections of Ca^{++}-contracted extract was filamentous, with some regions containing many free 6–8 nm filaments while others consisted of twisted or lateral arrays of filaments (Fig. 6*d*). Furthermore, the birefringence of the extract increased during contraction. It was interesting that there were no obvious myosin thick filaments in the thin sections of the Ca^{++}-contracted extract, in contrast to extracts from *A. proteus* (62).

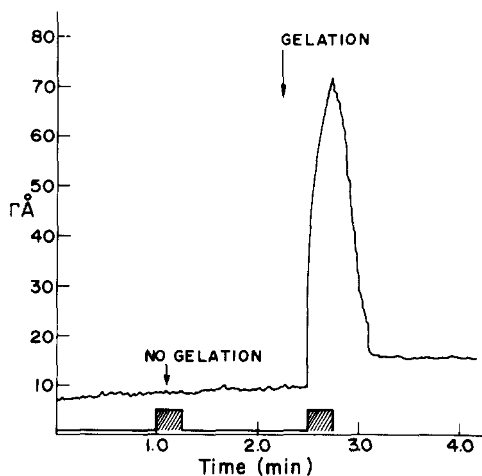

FIGURE 4 Strain birefringence assay for gelation. The phase retardation (Γ) was monitored vs. time and the application of 10-μm stretches by a micropippette inserted into the extract (bars). No strain birefringence could be induced until the extract gelled.

Effect of EGTA and Sucrose on S3

To test the effect of low divalent cation concentrations on the stability of the gel, the EGTA concentration in S3 was increased to a total of 5 or 10 mM. On warming the S3, the extent and rate of gelation were found to be decreased in the presence of relaxation solution containing additional EGTA (Table V). In addition, spontaneous contraction occurred and the time to onset of contraction decreased with increasing EGTA concentrations (Table V). Similar experiments with sucrose demonstrated that sucrose inhibited gelation while eliciting spontaneous contraction in the absence of Ca^{++} at pH 7.0 (Table V).

Effect of pH on S3

To test the effect of pH on the high-speed extract, S3, the pH was raised or lowered around 7.0 by adding KOH or HCl to S3 on ice in the presence of the relaxation solution. The intrinsic buffering capacity of the extract helped maintain the pH constant while warming. A slow downward pH drift occurred with time (0.1–0.2 pH U/15 min), so the experiments were carried out rapidly after reaching the desired pH.

Below pH 7.0, the rate of gelation on warming was decreased (Table VI) while spontaneous con-

TABLE V

*Effect of Sucrose and EGTA on Gelation and Contraction of S3**

	Extent of Gelation‡	Initiation of Contraction
	avg time, min	*min*
Sucrose (M)		
0.20	++(5)	28
0.34	++(5)	25
0.40	+(5)	35
EGTA (mM)		
5.0	++(10)	23
10.0	+(5)	15

* S3 was warmed in the relaxation solution (Table II) to 25°C at pH 7.0 containing the sucrose and EGTA concentrations indicated. The preparations of S3 used in these experiments were capable of gelation to +++ in 7 min in the absence of sucrose or extra EGTA.
‡ See last footnote to Table II.

traction did not occur (63). A decreased response to Ca^{++} also occurred at low pH, until at pH 6.5–6.7 no contraction could be elicited with Ca^{++} (63).

Above pH 7.0, gelation was inhibited and rapid contraction occurred on warming (Table VI) in the absence of Ca^{++} (63). The rise, and, at higher pH, the subsequent rapid fall of extract turbidity due to contraction was consistent with these observations (Fig. 7 A). SDS gel electrophoresis of the contracted extract resulting from contraction elicited at high pH demonstrated that actin, myosin, and the 95,000-dalton band were collected during pH contraction (Table I and Fig. 3*d*). As with the Ca^{++}-contracted extract, the pH-contracted extract would not gel at 25°C when resuspended in the relaxation solution at pH 7.0. However, the supernate squeezed out during contraction underwent rapid gelation in the relaxation solution at pH 7.0 in the presence of actin.

The ATPase activity of S3 was also sensitive to pH. The ATPase activity was depressed at pH 7.0 and below, but activated at pH above 7.0. The average activity at pH 7.0 was 0.2 nmol P_i/min, while at pH 7.6 it climbed to an average of 3.4 nmol/min (Fig. 7 B).

To determine whether contractions elicited by high pH were due to the release of Ca^{++} from some unknown source in the extract, aequorin luminescence was followed in the extract during pH-induced contraction. S3, containing aequorin, gelled upon warming at pH 7.0 and maintained a low "resting" luminescence. When the pH of S3 was raised to 7.6, contraction of S3 occurred 2 min after warming in the relaxation solution, but there was no increase in luminescence (Fig. 8). The addition of the contraction solution to a control sample of extract at pH 7.0 (1:20 vol:vol) resulted in a rapid rise in the luminescence coincident with contraction of the extract, demonstrating that submicromolar concentrations of Ca^{++} could easily be detected in the extract (Fig. 8).

Control of Gelation and Solation in the Nonmotile Extract SB

To determine the relationship between gelation and contraction, it was necessary to study the effect of Ca^{++} and pH on gelation in the absence of superimposed contraction. Therefore, an extract that was not capable of contraction (SB) was prepared from S3 as shown in Fig. 1 and described in Materials and Methods.

The composition of the nonmotile extract SB is demonstrated in Fig. 3*b* and Table I. Actin was the main component of SB, constituting an average of 24% of the protein, having been enriched by 2.8-fold over that present in S3. The 250,000-dalton band was the most enriched, constituting over 4% of the protein of SB or a 5.5-fold increase over that present in S3. The amount of myosin present was decreased in SB which contained only about 1/10 of the concentration present in S3 (Table I). SB was used at a protein concentration of 4–5 mg/ml for all experiments.

KCl, ATP, and $MgCl_2$

SB warmed to 25°C in the absence of KCl at pH 7.0 did not gel (Table VII), while the addition of 20 mM KCl caused gelation. However, as in S3 (Table II), the addition of the relaxation solution produced optimal gelation (Table VII). Higher concentrations of KCl resulted in progressively poorer gel formation in SB as observed in S3.

TABLE VI
*Effect of pH on Gelation and Contraction of the Extracts**

	Extent of gelation§			Initiation of contraction		
	pH 6.6	pH 7.0	pH 7.4	pH 6.6	pH 7.0	pH 7.4
	avg time, min				*min*	
S3	+++(60)	+++(6)	++(3)	–	–	7
Desalted S3	+(22)	+++(9)	++(3)	–	–	4
SB	++(5)	+++(5)	+(8)	–	–	solation initiated 15
SB + myosin‡	++(5)	+++(3)	–	–	–	10

* All extracts were warmed in the relaxation solution (Table II) to 25°C at the pH indicated.
‡ *Dictyostelium* myosin was added to a final concentration of 0.18 mg/ml.
§ See last footnote to Table II.

TABLE VII

Effect of ATP, $MgCl_2$, and KCl on Gelation and Solation of SB

	Extent of gelation¶	Initiation of solation‖
	avg time, min	*min*
SB*	–	–
SB + 1 mM Na_2ATP	–	–
SB + 1 mM $MgCl_2$	–	–
SB + 5 mM $MgCl_2$	–	–
SB + 1 mM Na_2ATP + 1 mM $MgCl_2$	–	–
SB + 20 mM KCl	+(16)	–
SB + 1 mM Na_2ATP + “	+(5)	–
SB + 1 mM $MgCl_2$ + “	+(15)	–
SB + 5 mM $MgCl_2$ + “	–	–
SB + 1 mM Na_2ATP + “ + “	+++(5)	–
SB + 1 mM Na_2ATP + 1 mM $MgCl_2$ + 20 mM KCl§	+++(5)	–
SB + “ + “ + 40 mM KCl‡	++(5)	7
SB + “ + “ + 60 mM KCl	+(5)	7
SB + “ + “ + 20 mM KCl + 5 mM EGTA	+++(11)	34
SB + 1 mM Na_2ATP + 1 mM $MgCl_2$ + 20 mM KCl + 10 mM EGTA	++(11)	23

* SB was transferred from the depolymerization buffer (Materials and Methods) into 2.5 mM EGTA and 2.5 mM PIPES at pH 7.0 before assay. SB was assayed for gelation at 25°C and an average protein concentration of 5 mg/ml.
‡ This is the final concentration added to SB.
§ Relaxation solution.
‖ The time to initiation of solation was determined by inverting the test tubes containing the extract at various time intervals.
¶ See last footnote to Table II.

Addition of ATP to SB increased the rate of gelation over that obtained with 1 mM $MgCl_2$ and 20 mM KCl alone and stimulated gelation in the presence of 5 mM $MgCl_2$ and 20 mM KCl (Table VII).

Chilling the SB on ice after warming it to form the gel resulted in solation that was usually completed by 15 min. Solation occurred in response to cold, regardless of the salts used to produce the gel. Cold did not precipitate any protein from SB during solation. The consistency of SB appeared to be greater after solation than that of SB maintained at 4°C as measured qualitatively in drawn-out pasteur pipettes. Upon rewarming the solated SB, feeble gelation occurred in SB that could be stimulated by additional exogenous ATP to 0.5 mM.

Effect of Ca^{++} on SB

Micromolar Ca^{++} was observed to control the onset of contraction both during (Table III) and after (Table IV) gelation in S3 and to stimulate the ATPase activity of S3 (Fig. 5*B*). To determine the effect of Ca^{++} on the formation and stability of the gel in SB, $CaCl_2$ was added to SB in the presence of the relaxation solution so that the Ca/EGTA was varied between 0 and 1.0 at pH 7.0. The results are shown in Table III. At Ca/EGTA <0.6, gelation of SB occurred on warming. However, the gel solated with time at higher Ca^{++} concentrations. The time-course of solation of SB was strikingly similar to the time of onset of Ca^{++}-elicited contraction in S3 at the same Ca/EGTA ratios (Table III).

At Ca/EGTA ratios above 0.6, no gelation occurred in SB (Table III). On warming the SB in the relaxation solution containing Ca/EGTA >0.6, the increase in turbidity (Fig. 10*b*) was less than that in the relaxation solution alone (Fig. 10*a*), in marked contrast to that observed in S3 (see Fig. 5 A).

Unlike S3, SB did not exhibit an increase in ATPase activity with increases in the Ca/EGTA ratio (Table VIII). The residual myosin concentration in SB averaged around 0.04 mg/ml compared to over 0.4 mg/ml in S3 (Table I).

The addition of purified *Dictyostelium* myosin to SB to a final concentration of 0.18 mg/ml reconstituted the ability of SB to contract. In the absence of Ca^{++}, spontaneous contraction did not

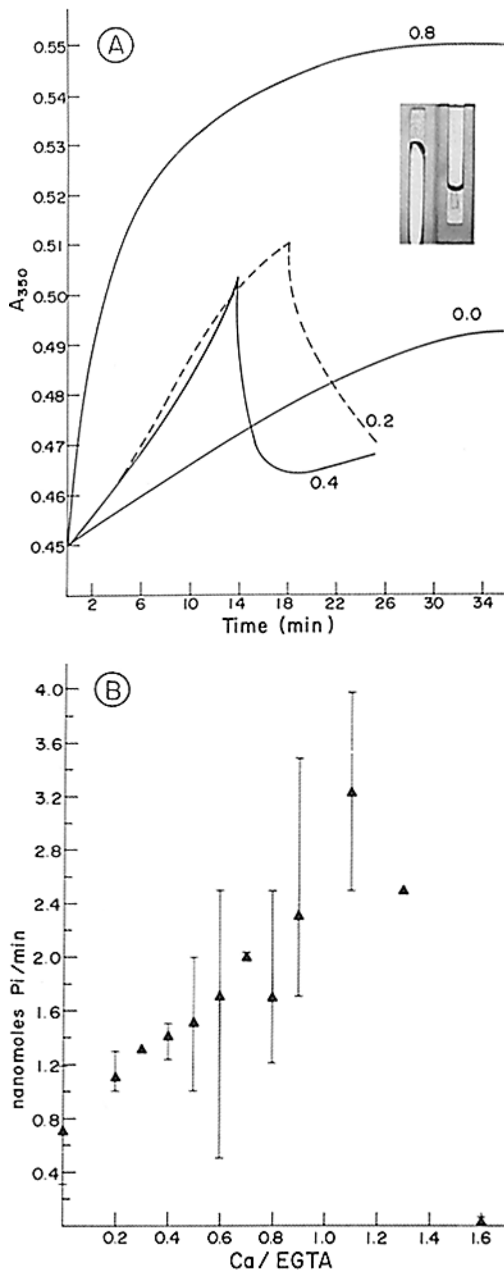

FIGURE 5 (A) Turbidity changes in extract S3 in response to Ca/EGTA ratios of 0.0, 0.2, 0.4, and 0.8. The extract was warmed from 4° to 25°C in the presence of the Ca/EGTA ratio indicated. The cuvette inserts show (left) the extract with Ca/EGTA = 0.0 after 18 min and (right) with Ca/EGTA = 0.4 after 18 min. Drop in turbidity is due to contraction. (B) Stimulation of the ATPase of extract S3 in response to increases in the Ca/EGTA ratio. S3 was warmed to 25°C in the presence of the relaxation solution (Table II) containing the Ca/EGTA ratio indicated at pH 7.0. The reaction was stopped by precipitating the protein, and the peak activities were averaged.

TABLE VIII
Ca^{++}-Stimulated ATPase Activity

Ca/EGTA*	Actin‡ + myosin	SB§	SB‖ + myosin
		nmol P_i/min ± SD	
0	1.0	1.2 ± 0.4	0.8 ± 0.15
0.2	1.0	1.3 ± 0.3	1.1 ± 0.08
0.4	1.0	1.1 ± 0.1	2.6 ± 0.6
0.6	0.8	1.5 ± 0.1	2.7 ± 0.62
0.8	1.0	1.3 ± 0.33	1.7 ± 0.5
1.0	–	1.3 ± 0.35	1.9 ± 0.3

* Assays were performed in the relaxation solution (Table II) at 25°C and pH 7.0 containing the Ca/EGTA ratios indicated.

‡ Purified *Dictyostelium* actin (56) and myosin (8) were assayed separately at a concentration of 1 mg/ml and 0.175 mg/ml, respectively. The activity of actin alone during polymerization in the presence of Ca^{++} averaged 0.35 nmol P_i/min. The activity of myosin in the absence of actin remained constant at 0.4 nmol P_i/min. (0.023 mmol P_i/min/mg myosin) in the presence and absence of Ca^{++}. In the range of Ca/EGTA ratios shown above, the specific activity of purified *Dictyostelium* actin plus *Dictyostelium* myosin (6:1 wt:wt) remained constant at 0.06 mmol P_i/min/mg myosin in the presence and absence of Ca^{++} as indicated.

§ SB was assayed at a protein concentration of 3.24 mg/ml and contained a residual endogenous myosin concentration of 0.03 mg/ml and an actin concentration of 0.78 mg/ml.

‖ Myosin was added to SB to a final concentration of 0.17 mg/ml. The sp act was 0.046 mmol P_i/min/mg myosin in the absence of Ca^{++} and climbed to an average of 0.16 mmol P_i/min/mg myosin at Ca/EGTA = 0.6.

occur for up to 30 min at 25°C in the presence of the relaxation solution at pH 7.0. Addition of Ca/EGTA ≥0.4 stimulated contraction on warming SB containing myosin to 25°C at pH 7.0. However, if higher concentrations of purified myosin were added to SB (0.3 mg/ml), spontaneous contraction occurred after warming SB containing myosin to 25°C in the relaxation solution.

The addition of purified *Dictyostelium* myosin to a final concentration of 0.18 mg/ml also reconstituted the Ca^{++}-stimulated ATPase activity of SB. The ATPase of SB containing myosin in the relaxation solution remained constant in the absence of Ca^{++}. However, on addition of intermediate Ca/EGTA ratios, the ATPase activity dem-

onstrated a three- to fourfold increase from a sp act of 0.04 to 0.16 μmol P_i/min per mg myosin at Ca/EGTA = 0.6 (Table VIII). This rise in ATPase activity was partially inhibited at higher Ca/EGTA ratios. These results are similar to the Ca^{++}-stimulated ATPase behavior of S3 (Fig. 5 B). Ca^{++} did not stimulate the ATPase activity of purified *Dictyostelium* actomyosin (Table VIII).

Effect of EGTA on SB

Increasing the concentration of EGTA in S3 resulted in poor gelation and spontaneous contraction. To test the effect of EGTA on SB, the EGTA concentration in the relaxation solution was raised. The higher EGTA concentrations decreased the rate of gelation on warming the SB (Table VII). Subsequently, solation occurred at 25°C, the rate of which was increased with increasing EGTA concentrations (Table VII). The time-course of solation of SB corresponded closely to the onset of spontaneous contraction observed in S3 containing the same EGTA concentrations (Table V).

Effect of pH on SB

The effect of pH on SB in the relaxation solution is summarized in Table VI. Low pH decreased the extent of gelation. However, high pH (7.4) resulted in partial inhibition of gelation with the eventual solation of any gel that did form at 25°C. Unlike S3 (Fig. 7 A), SB warmed in the relaxation solution at pH 7.4 exhibited a smaller continuous increase in turbidity than SB warmed at pH 7.0 (Fig. 10*c*). No contractions followed the pH-induced solation of gelled SB.

SB containing exogenous purified *Dictyostelium* myosin to a final concentration of 0.18 mg/ml exhibited behavior similar to that of S3 when warmed in the presence of the relaxation solution at different pH values. At low pH (6.6) gelation was decreased while at pH 7.0 rapid gelation (+++) occurred (Table VI). At both pH 6.6 and 7.0, spontaneous contraction did not occur. At pH 7.4, gelation was completely inhibited while contraction of SB containing exogenous myosin occurred.

Electron Microscopy of Structural Transformations Occurring in the Nonmotile Extract SB

Negatively stained preparations of SB that had gelled to +++ in the presence of the relaxation solution at pH 7.0 exhibited a few short, thin filaments and large amounts of aggregates (Fig. 12*a*) (20) that closely resembled the material observed in negatively stained preparations of gelled S3 (Fig. 6 A) and the motile extracts reported earlier (62, 63). In contrast, SB negatively stained at 4°C without prior warming, contained no detectable filaments.

To relate the dramatic effects of Ca^{++}, high concentrations of $MgCl_2$ and high pH on the formation and stability of the nonmotile gel, preparations of SB were also negatively stained in the presence of the relaxation solution at pH 7.0, containing micromolar Ca^{++}, 5 mM $MgCl_2$, or at pH 7.5 (Fig. 12*b*, *c*, and *d*, respectively). Under all these conditions and at the same protein concentration, the number of free thin filaments increased sharply (Fig. 12*b*, *c*, and *d*) over that observed in gelled SB in the relaxation solution at pH 7.0 (Fig. 12*a*). This apparent increase in the number of thin filaments resembled the increase in the number of filaments observed to occur in S3 contracted by a Ca/EGTA ratio of 0.4 (Fig. 6*c* and *d*).

Actin filaments observed in preparations "transformed" by high $MgCl_2$ concentrations, pH or micromolar Ca^{++}, were present as free filaments or bundles (Fig. 12*b*, *c*, and *d*) (62, 63). The thin filaments observed in the transformed preparations were identified as F-actin by the HMM-binding technique using rabbit skeletal muscle HMM in the absence of ATP. Attempts to identify the short thin filaments contained in negatively stained gelled S3 or SB by rinsing these grids with rabbit muscle HMM in the absence of ATP resulted in the production of large numbers of free HMM-labeled F-actin filaments (62) as shown for SB in Fig. 13. Rinsing with the HMM buffer alone produced no transformation and demonstrated that HMM was necessary to produce the increase in free filaments. Furthermore, addition of HMM to S3 elicited spontaneous contraction of S3 on warming in the relaxation solution at pH 7.0.

Relationship between Gelation and Contraction

The results presented above, as well as earlier studies on *Dictyostelium* extracts (63), demonstrate that physiological Ca^{++} and pH levels that optimize gelation in S3 and SB do not elicit contraction, while solation produced by elevated physiological Ca^{++} and pH levels is always accompanied by contraction of extracts containing

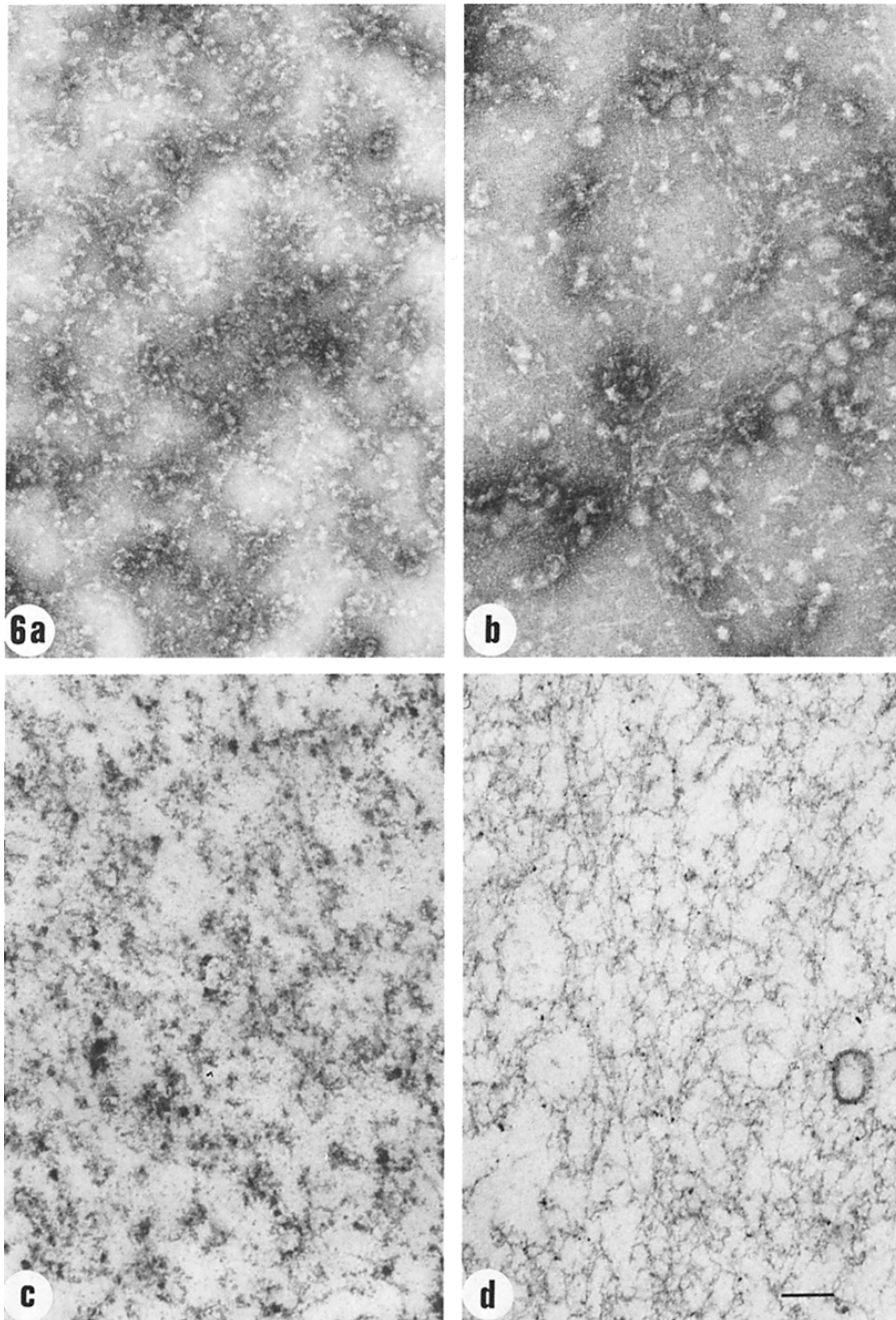

FIGURE 6 (*a*) Aggregates and short, thin filaments are observed after negatively staining the S3 gelled in relaxation solution at pH 7.0 in uranyl acetate; (*b*) S3 in relaxation solution at pH 7.0 after contraction with Ca/EGTA = 0.4 demonstrates an increase in the number of thin filaments measuring 6–8 nm in diameter. These filaments contain actin as demonstrated by the HMM binding technique; (*c*) a thin section of gelled S3 as in (*a*); (*d*) a thin section of contracted gel as in (*b*). Bar, 0.1 μm. × 81,500.

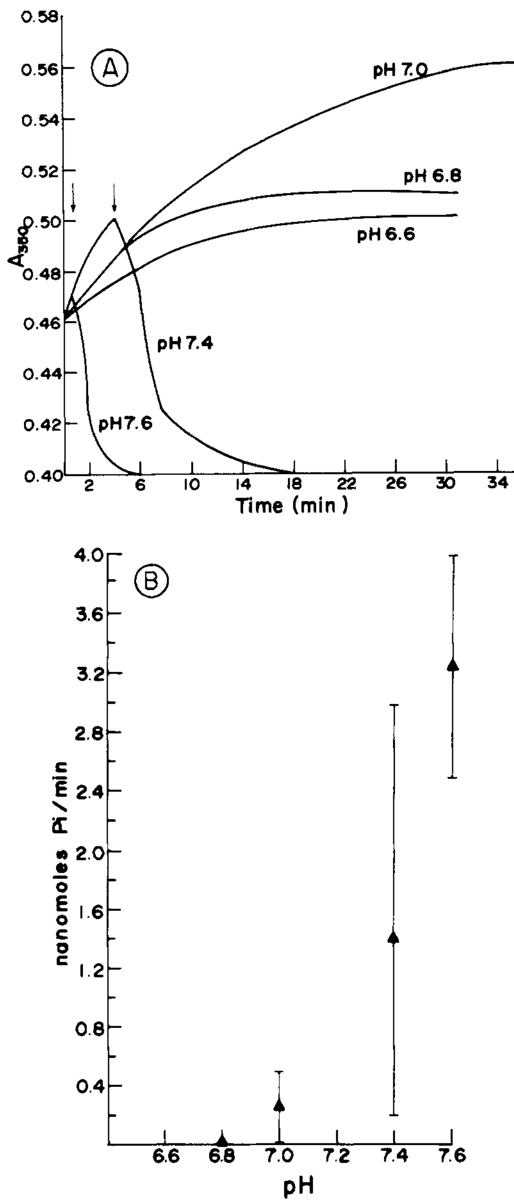

FIGURE 7 (A) Turbidity changes in extract S3 in response to pH. The extract was warmed to 25°C in relaxation solution at the pH indicated. Sharp drops in turbidity are due to contraction (arrows) (see text). (B) Stimulation of the ATPase of extract S3 in response to pH. S3 was warmed to 25°C in the presence of the relaxation solution at pH indicated.

myosin. This would suggest that the gel prevents contraction, and, therefore, that solation of the gel would be accompanied by contraction if the extract contained myosin (63). This hypothesis has been supported by the experiments described below.

Cold

Cooling the SB that had gelled in the relaxation solution at pH 7.0 resulted in solation of the gel as described above. However, if: (*a*) gelled S3 was used or (*b*) SB was mixed with purified *Dictyostelium* myosin to a final myosin concentration of 0.18 mg/ml and gelled by warming, then subsequent cold treatment induced solation of the gel which was always accompanied by contraction of the extract (Fig. 9). As shown in Fig. 9, solation of the gel that formed at 6 min, began at ~11°C (24 min) and was followed by contraction of the extract (indicated by the abrupt drop in turbidity) beginning at 6°C (30 min). Contraction of the extract at 6°C was much slower than contractions elicited at room temperature by pH or Ca^{++} (compare Fig. 9 and Figs. 5 A and 7 A). If the sample was warmed to 25°C after contraction was initiated at 6°C, the subsequent rate of contraction was similar to that observed at room temperature for Ca^{++}- or pH-elicited contractions (Figs. 5 A and 7 A). The ATPase of purified *Dictyostelium* actomyosin was not stimulated by cold treatment, and the pH remained constant at 7.0 during cooling.

A contraction supernate was prepared by pelleting extract contracted by cold treatment at 27,000

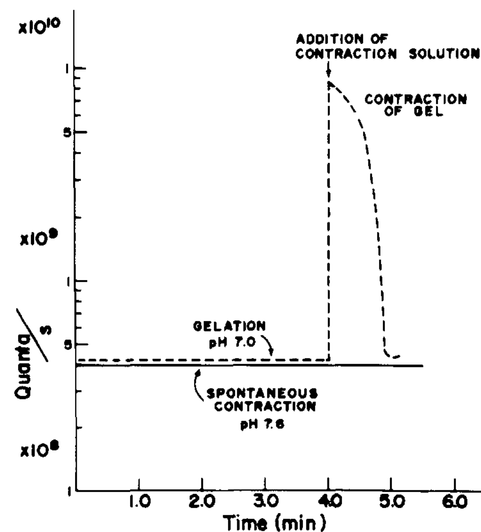

FIGURE 8 Extract S3 remained gelled with a low "resting" luminescence when warmed in the presence of aequorin. The addition of 1:10 vol:vol contraction solution to the gelled extract induced contractions and luminescence at the same time. However, there was no increase in luminescence when contractions were elicited with the relaxation solution at pH 7.6.

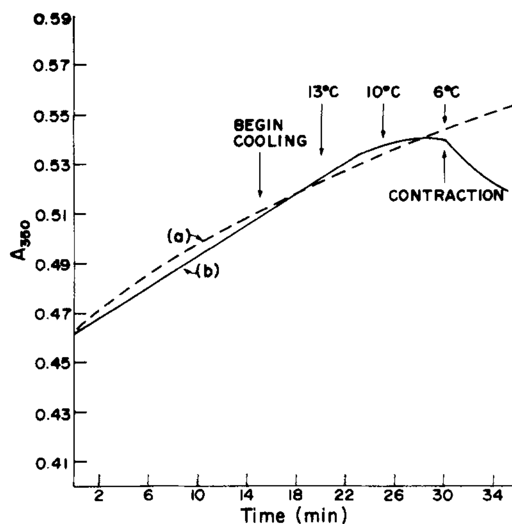

FIGURE 9 Turbidity changes in extract S3 (*a*) warmed and maintained at 25°C and (*b*) after cooling to 4°C. The cuvette was equilibrated at 25°C before addition of the extract at 4°C. The fall in turbidity in (*b*) results from contraction of the extract (see text).

g for 15 min. In six out of nine supernates, up to six cycles of gelation – solation could be produced over an 8-h period by alternate warming to 25°C and cooling to 4°C. In these samples, contraction did not occur during solation, while feeble contractions occurred in the remaining three samples. Up to three additional cycles of gelation – solation were produced by adding additional ATP to 1 mM.

Mechanical Stress

Gelled *Dictyostelium* extracts were mechanically unstable and the quantitation of gelation by methods dependent on stress or shear were not reproducible. Briefly shaking a test tube containing SB that had gelled in the relaxation solution at pH 7.0 and 25°C resulted in fragmentation of the gel and subsequent solation. However, gelled S3 or SB containing *Dictyostelium* myosin (0.18 mg/ml) contracted over a 10-min period after shaking, while control preparations that were not disturbed remained gelled and uncontracted at room temperature for at least 60 min after mechanically induced contraction of experimental preparations.

Pressure

A 1- to 5-min pressurization at 2,000 lb/in^2 and 25°C initiated solation of gelled S3 that was accompanied by contraction within 10 min after decompression. Control preparations that were not pressurized remained gelled and uncontracted over the same period at room temperature. Furthermore, whole cells were shown to round up upon the application of the same pressure and recovered after decompression (32).

Centrifugation of the gelled extract either at room temperature or at 4°C at 12,000 *g* for 10 min resulted in an opaque pellet. SDS gel electrophoresis revealed that this pellet resembled Ca^{++}- or pH-contracted pellets (Fig. 3*f*). The pressure in the tube at this speed was calculated to equal 2,520 lb/in^2, which is sufficient to produce contraction in S3 as described above. At lower speeds of centrifugation around 750 *g*, the gel did not compact completely. Hence, centrifugation was not used to collect samples of gelled S3 because of the possibility that the pressure produced by centrifugation might induce solation and, therefore, cause contraction of S3.

Cytochalasin B

Cytochalasin B has been shown to induce solation of gelled extracts prepared from a variety of cells (21, 51, 76). Cytochalasin B was layered on top of gelled S3 (1:10 vol:vol) in the relaxation solution at pH 7.0 containing 0.1% dimethyl sulfoxide (DMSO). The layering of cytochalasin B at 10^{-8} or 10^{-7} M had no effect on the consistency of the extract assayed by inverting test tubes at different time intervals as described in Materials and Methods. However, when 10^{-6} M cytochalasin B was layered on top of gelled extract S3, the gel began to solate, starting at the cytochalasin B-extract interface. During solation, the cytochalasin B-treated extract contracted very slowly over a 3-h period. Gelled S3 that was layered with relaxation solution containing DMSO remained gelled and uncontracted for at least 3 h at 27°C.

Purified Actin and Gelation

As described above, actin appears to play a major role in gelation of both the motile (S3) and nonmotile (SB) extracts. SB, which was capable of rapid gelation to +++, contained actin concentrations that varied between 0.76 and 1.3 mg/ml. To determine whether actin alone was capable of gelation, SB actin was prepared from SB as outlined in Fig. 1. For comparison, *Dictyostelium* actin and rabbit skeletal muscle actin were purified as described in Materials and Methods.

The composition of SB actin is shown in Fig. 3*h*

and Table I. The majority of the protein in SB actin was actin. In addition, several low molecular weight bands below actin and the 75,000-, 90,000-, and 95,000-dalton components were also present. A variable amount of the 250,000-dalton band was also observed on SDS gels. Together, these bands constituted <7% of the protein present in SB actin.

Actin purified according to the method of Spudich (56) (Fig. 3*c* and *i*) contained a variable amount of contamination with several low molecular weight components as shown in Fig. 3*i*. These preparations of purified *Dictyostelium* actin (either Fig. 3*c* or *i*) did not gel at concentrations up to 2 mg/ml, even in the presence of the relaxation solution at pH 7.0. However, a small increase in turbidity did occur on warming either preparation of *Dictyostelium* actin in the relaxation solution at a concentration of 1 mg/ml (Fig. 11*c*). This increase was comparable to the increase in turbidity resulting from warming purified rabbit skeletal muscle actin in the relaxation solution at a concentration of 1 mg/ml (Fig. 11*b*) and was due to polymerization of the actin.

However, warming 1 mg/ml of SB actin in the relaxation solution resulted in gelation (+++) in <3 min. A large increase in turbidity accompanied gelation of the SB actin (Fig. 11*a*). Solation occurred on chilling gelled SB actin samples on ice or upon addition of micromolar Ca^{++}. However, as with SB, cold-induced solation of SB actin did not elicit contraction in the absence of myosin. Furthermore, only feeble gelation resulted from rewarming SB actin after cold-induced solation.

DISCUSSION

Ionic Requirements for Gelation

The results presented in Tables II, III, V, and VI broadly define the ionic requirements for gelation in *Dictyostelium* extracts. The inhibitory effects of $MgCl_2$ and KCl on gelation in *Dictyostelium* extracts are consistent with the inhibition of gelation in sea urchin egg extracts by $MgCl_2$ (30) and in *Acanthamoeba* extracts by KCl (50). However, the inhibitory effect of sucrose and EGTA on gelation indicates that caution should be exercised when including sucrose or EGTA in solutions used to test gelation or in the preparation of motile extracts. Furthermore, spontaneous contraction in extracts prepared from various organisms in the presence of sucrose (50, 58) might result from a direct inhibitory effect of sucrose on gelation, thereby causing contraction.

Physiological variations in pH and/or Ca^{++} are likely candidates for the in vivo control of gelation and contraction as demonstrated previously (62, 63). Small variations in the pH around neutrality and in Ca^{++} at the micromolar level were sufficient to produce gel-to-sol transformations or the total inhibition of gelation in the *Dictyostelium* extracts. Hence, local variation of the Ca^{++} concentration or of the pH in vivo might control the spatial distribution of cytoplasmic gelation.

The cytoplasmic pH can be modulated by changes in the free Ca^{++} concentration in vivo (52). It has been demonstrated that an intracellular increase of Ca^{++} in a local region of the cell causes a corresponding local drop in pH. Contraction in this region would be expected to proceed until the pH dropped to a point where contraction would be depressed (Fig. 7 A) (62, 63). This could limit the spread and extent of contraction to local regions of the cell. Furthermore, variation of the intracellular pH independent from Ca^{++} might regulate the sensitivity of the cytoplasm to Ca^{++}-elicited contraction (63).

Molecular Basis of Gelation

Currently, there are several possible interactions that might be responsible for cytoplasmic structural changes associated with nonmuscle cell movements occurring in the absence of microtubules (64, 51). Actin-myosin interactions (60, 11), polymerization-depolymerization (68, 10, 46) of the contractile proteins, and cytoplasmic gel formation (61-63, 58, 30, 50) have been implicated in the structural changes in cell extracts and in vivo. Therefore, it is important to distinguish among these possibilities when working with structural changes occurring in cells and cell extracts.

The gelation of the motile *Dictyostelium* extracts S1, S2, and S3 on warming is not due to actin-myosin cross-linking (63) since optimal gelation occurred in a relaxation solution, conditions sufficient to dissociate actin-myosin cross-linking in amoeba cytoplasm (60, 42). Furthermore, optimal gel formation occurred in SB with a greatly reduced myosin concentration and in SB actin which contained no myosin.

Actin is the major component of the gel formed in *Dictyostelium* extracts. Polymerization of actin may be an important antecedent event in gel formation since warming the extracts in the presence

of solutions containing added KCl was necessary before optimal gel formation could occur. However, it is clear that the polymerization of *Dictyostelium* actin in the absence of contaminating proteins is not sufficient to produce gel formation since purified *Dictyostelium* actin at concentrations of ca. 2 mg/ml (exceeding those in the gelled extracts) was incapable of gelation. This is consistent with the properties of purified macrophage actin (58), muscle actin (6), and *Acanthamoeba* actin (38) which do not gel at low concentrations (ca. 2 mg/ml) in the absence of actin-binding proteins. However, SB actin containing "contaminating" proteins with mol wt of 250,000, 95,000, 90,000, and 75,000, 38,000 and 28,000 daltons will undergo a substantial Ca^{++}-regulated gelation.

Turbidity has been used to follow gelation in a variety of cell extracts (62, 63, 58, 50). The complete molecular bases of turbidity changes in the *Dictyostelium* extracts described here are unknown. However, depending on the ionic conditions, several interactions appear to contribute to changes in turbidity. The polymerization of actin contributes to the rise in turbidity on warming the extract, but this is small in comparison to the turbidity changes occurring in partially purified actin samples capable of gelation (Fig. 11). Warming the nonmotile extract (SB) under conditions that inhibit gel formation, i.e., micromolar free calcium, resulted in a smaller rise in turbidity than that occurring in the relaxation solution, a condition resulting in gel formation (Fig. 10). These results indicate that the molecular interactions involved in gel formation contributed to the rise in turbidity. This might result from conformational changes in the actin-binding proteins occurring under conditions that permit gelation (58) or from the formation of actin-actin-binding protein complexes. However, in extracts containing myosin, conditions that inhibit gel formation, such as micromolar Ca^{++} or high pH, cause very large increases in the turbidity resulting from Ca^{++}- or pH-stimulated actin-myosin interactions resulting in contraction (Figs. 5 A and 7 A).

The properties of the gels formed in the various cell extracts (30, 50, 62, 63) have been investigated by means of biochemical, ultrastructural, mechanical, and strain birefringence assays. However, from the current evidence it is not clear that the gels formed in the various cell extracts are equivalent structures. Indeed, several different types of gels might exist even within the same extract, differing from one another by the form of actin that is cross-linked and by the cross-linking proteins (actin binding proteins) involved.

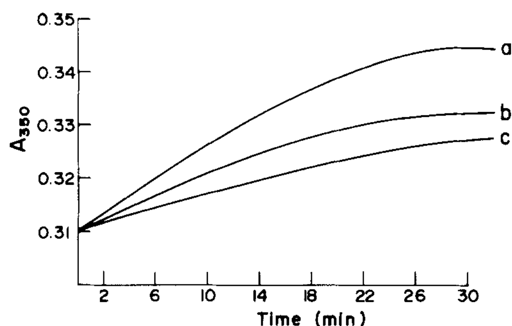

FIGURE 10 Turbidity changes in extract SB: (*a*) warmed in the relaxation solution pH 7.0; (*b*) warmed in the contraction solution (Table III) pH 7.0; (*c*) warmed in the relaxation solution at pH 7.4. Cuvette was equilibrated to 25°C.

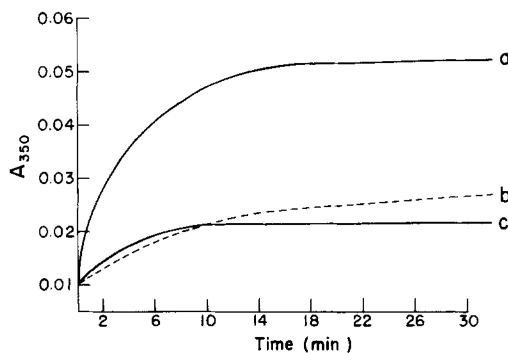

FIGURE 11 Turbidity changes on warming the samples to 25°C in relaxation solution at pH 7.0. (*a*) SB actin at 1 mg/ml (Fig. 3*h*); (*b*) rabbit skeletal muscle actin at 1 mg/ml; (*c*) *Dictyostelium* actin (Fig. 3*i*) (56) at a concentration of 1 mg/ml.

(*a*) Gelation may not require the formation of F-actin but may result from the formation of a flexible polymer of actin produced by warming G-actin in the presence of actin-binding proteins. Such a polymer of actin has been described by Hatano (25), and the protein responsible for this behavior (42K on SDS gels) has been isolated (26). The formation of the polymer requires the presence of millimolar Mg^{++} and ATP and is transformed to F-actin in the presence of concentrations of KCl above 50 mM (25). Similar transformations have been suggested to occur in *A. proteus* (62).

(*b*) Gelation may not require the formation of a polymeric form of actin but may result from bind-

ing G-actin to proteins that form an isotropic gel. Evidence for such a structure has been advanced by Tilney for the *Thyone* acrosome (71, 72). Isolation of the periacrosomal material demonstrated that it consisted of a gel of actin in association with two high molecular weight polypeptides (250,000 and 230,000 on SDS gels) in the range of sizes for actin-binding proteins from macrophages (58) and spectrin from erythrocytes (17). The periacrosomal material was partially dissolved by raising the pH to 8.0 or increasing the KCl concentration above 75 mM (72).

(*c*) Gelation may result from the simultaneous polymerization and random cross-linking of F-actin into a fibrous aggregate by actin-binding proteins.

(*d*) Gelation may result from the polymerization of F-actin followed by precise cross-linking of F-actin into parallel bundles by actin-binding proteins. Actin bundle formation during gelation has been observed in sea urchin egg extracts by Kane (30), and the actin-binding proteins involved have been tentatively identified (220,000 and 58,000 in SDS). Similar actin bundles have been identified in the acrosomal process of *Limulus* sperm and are composed of actin, 55,000- and 95,000-dalton polypeptides (70). Actin bundle formation has also been reported to occur in extracts of *A. proteus* on warming (69, 46), in cytoplasm isolated from *Chaos carolinensis* in millimolar EGTA (60), in cytoplasm isolated from pollen tubes (9), in extracts of *A. proteus* in the presence of a magnesium-enriched relaxation solution (62), and in partially purified *Dictyostelium* actin in response to high concentrations of Mg^{++} and Ca^{++} (57).

The properties of the gelled *Dictyostelium* extracts reported here are consistent with several of these possibilities. 250,000-, 95,000-, 55,000-, and 50,000-dalton polypeptides (SDS) are all present in these extracts. The undisturbed *Dictyostelium* gel is optically isotropic and the ultrastructural appearance of the *Dictyostelium* extracts negatively stained or prepared for thin sectioning in the relaxation solution (Figs. 6 and 12) is that of an "aggregate" (62, 63) which might arise from an Mg^{++} polymer-type interaction (25, 26, 62), the random cross-linking of short pieces of F-actin, and/or a structure similar to that observed in the periacrosomal material (71, 72, 62).

Elevated KCl concentrations that solate the *Dictyostelium* gel and cause transformation of the aggregate to free F-actin result in the transformation of Mg^{++} polymer to F-actin (25, 26). Furthermore, elevated pH, micromolar concentrations of Ca^{++}, and high concentrations of $MgCl_2$ and KCl that cause solation of the *Dictyostelium* extracts are sufficient to dissolve the periacrosomal material (71, 72).

It has been suggested that the absence of free F-actin in thin sections of gelled extracts is due to OsO_4 degradation of actin during fixation (51). However, this does not appear to be the case in gelled *Dictyostelium* extracts, since specimens prepared for negative staining in the absence of OsO_4 demonstrated the absence of well-defined filamentous actin and resembled thin-sectioned gels prepared by standard fixation (Fig. 6) (62, 63). Hence, the absence of recognizable free F-actin in gelled *Dictyostelium* extracts might reflect a property of the supramolecualr form of actin in the gel rather than fixation artifact alone (62, 63). This suggests that actin comprising gelled cytoplasmic structures may not be recognizable as free F-actin in thin sections of intact cells fixed under conditions that permit gelation. It must be emphasized that standard ultrastructural analyses of both extracts and intact cells are presently a subjective process. The experimenter must decide the ionic conditions of the fixative. Therefore, the results are biased, no matter what fixation methods are employed. The major point of our electron microscope results is that the structure of the extracts can be regulated by varying the physiological ionic environment while otherwise maintaining the fixation conditions constant. An absolute definition of the ultrastructure of contractile proteins in intact cells will require the knowledge of local ionic environments in cells.

Although current evidence indicates that any of the first three interactions described above might be involved in the formation of structures in the *Dictyostelium* extracts described here, it is unlikely that actin bundles compose a large part of the *Dictyostelium* gel since the undisturbed gel is optically isotropic, indicating the absence of aligned filamentous structure. In addition, bundles of actin are observed in these extracts only under conditions that solate the gel, such as mechanical stress, elevated pH and Mg^{++}, and micromolar Ca^{++} (conditions that induce contraction).

Further work is needed to clarify the role of the proteins corresponding to the 250,000-, 95,000-, 90,000-, 55,000-, 50,000-, 38,000-, and 28,000-dalton polypeptides in the *Dictyostelium* extracts to determine which ones are involved in calcium-sensitive actin binding and what types of structures

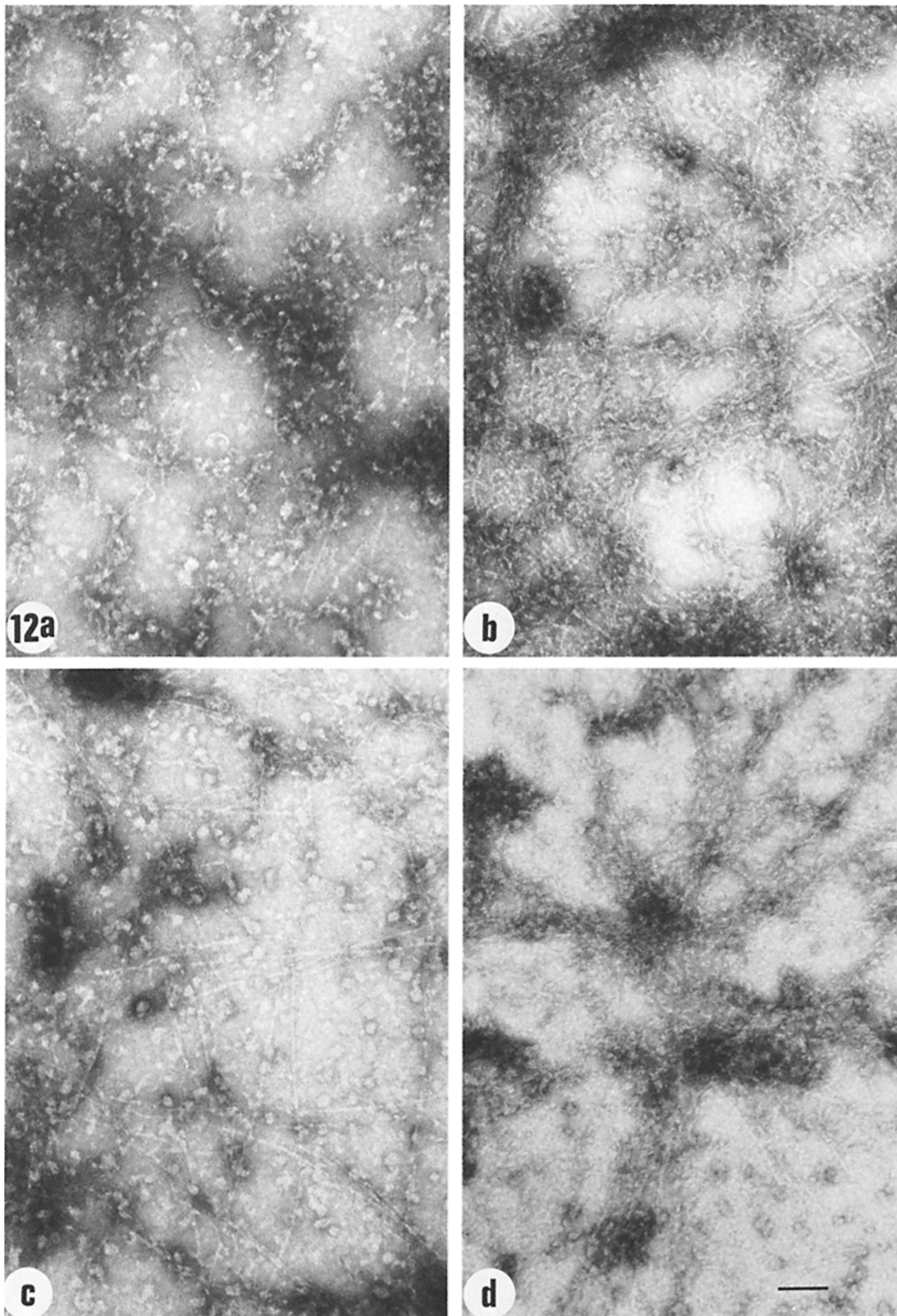

FIGURE 12 Electron micrographs of negatively stained SB: (*a*) after gelation in relaxation solution at pH 7.0; (*b*) after warming in the contraction solution at pH 7.0; (*c*) after warming in the relaxation solution at pH 7.0 containing 5 mM $MgCl_2$; (*d*) after warming in the relaxation solution at pH 7.5. Under conditions that solate gelled SB (*b*, *c*, and *d*), numerous 6–8 nm actin containing filaments are visualized. Bar, 0.1 μm. × 81,500.

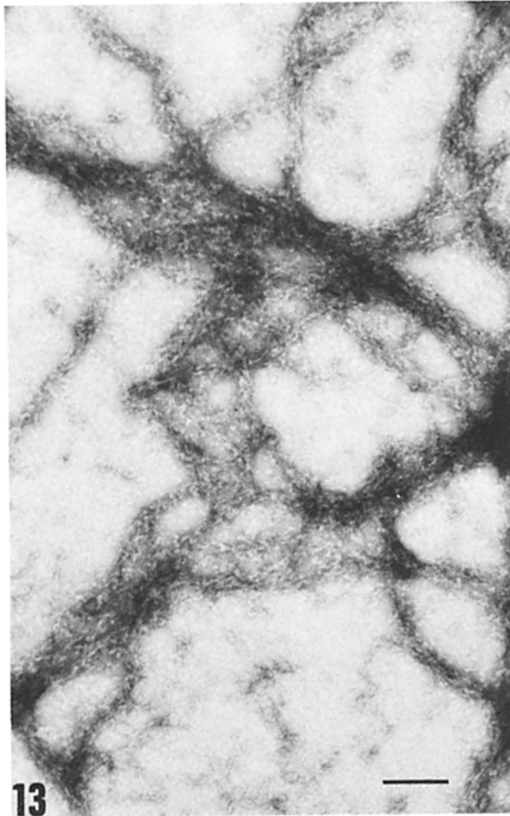

FIGURE 13 SB gelled in the relaxation solution at pH 7.0 was prepared for electron microscopy as in Fig. 12*a*. However, before negatively staining the preparation, the grid was rinsed with 0.4 mg/ml HMM in the absence of ATP, resulting in the visualization of numerous filaments. Compare with Fig. 12*a*. Bar, 0.1 μm. × 81,500.

result from their individual interactions with actin. Furthermore, the long list of actin-binding proteins in all of the above experimental systems must be analyzed to identify specific actin-binding proteins and the ionic regulation of the interactions.

The Requirement of Myosin for Contraction

A basic assumption of most investigators who work on cell motility is that myosin is the motor for cell movements involving actin. Therefore, it is crucial to demonstrate the requirement of myosin for contraction of actin-containing extracts.

Contraction of *Dictyostelium* extracts occurred only in the presence of myosin. In the nonmotile extract, SB, which contained a reduced concentration of myosin, only solation of the gel resulted from the presence of agents causing contraction in S3. Contraction was reconstituted when *Dictyostelium* myosin was added back to SB. During Ca^{++}- or pH-elicited contraction, actin, myosin and the 95,000-dalton polypeptides were concentrated in the contracted extract. The same result was observed whether the contracted extract was collected by decanting or centrifugation, indicating that these proteins were specifically involved in the contraction of the extract. These results are consistent with the properties of contracting models reconstituted from purified macrophage proteins, where the presence of myosin and cofactor were required for optimal contraction of the model composed of actin and actin-binding protein (58).

The motile properties of extracts S2 and S3 prepared from *Dictyostelium* by high-speed centrifugation are in marked contrast to those of extracts prepared from *A. proteus* where high-speed centrifugation resulted in the loss of motility (62). This loss of motility was correlated with the removal of myosin from the extract (62) and is consistent with the solubility properties of myosin isolated from *A. proteus* (11, 12). These results suggest that the solubility properties of *Dictyostelium* myosin in the extracts are significantly different from those of *A. proteus* myosin (62, 63, 12). If *Dictyostelium* myosin is soluble in the extracts reported here, perhaps forming only small oligomers during contraction, it would explain the absence of obvious myosin thick filaments in the gelled or contracted extracts (Fig. 6). Further work is required to determine what effect myosin solubility differences might have on the formation of structure and on the mode of movement in cell extracts and in vivo (12).

The Relationship between Gelation and Contractility

As described above, conditions that solate the gel elicit contraction of *Dictyostelium* extracts containing myosin. This was true regardless of whether chemical (Ca^{++} or pH) or physical (pressure or mechanical stress) means were used to induce solution. The correspondence between solation and onset of contraction was apparent by comparing the time-course of solation of gelled SB in the presence of elevated concentrations of KCl, Ca^{++}, and high pH (Tables III, II, VI), and the onset of contraction in S3 in the presence of these agents. If the gel is composed of F-actin highly cross-linked by actin-binding proteins, then the interaction between actin and myosin might be

sterically restricted. If the gel is composed of a storage form of actin (71, 72) or actin-containing filaments like Mg polymer (25, 26), then the transformation of actin from the gelled state to free F-actin filaments would have to occur before actin-myosin interactions could produce force. In either case, only after the release of actin from the gelled state by dissociation of the actin-binding protein-actin interaction could contraction proceed (61–64). Therefore, gelation might constitute a regulatory mechanism for some nonmuscle cell contractions (63). The ability of physical agents to produce contraction via solation indicates that the regulation by gelation might be structural in nature. This hypothesis has been supported by the following observations: (*a*) the ATPase of S3 or SB containing myosin was stimulated by micromolar Ca^{++} that elicited solation; (*b*) the gelation activity was recovered in the supernate squeezed out during Ca^{++}-, pH-, and cold-induced contraction of S3, indicating that the components involved in maintaining actin in a gelled form were released from actin during solation and contraction; (*c*) the number of F-actin filaments distinguishable as free structures increased during contraction of myosin-containing extracts and during solation of the gel in the absence of myosin; and (*d*) the presence of a competition between actin-binding proteins and myosin for actin in the gel is suggested by the observation that increasing the *Dictyostelium* myosin or muscle HMM concentration in S3 or SB produces contraction of S3 and results in a large increase in the number of actin filaments in S3 and SB labeled with myosin in the solated extract (63).

Thus, the Ca^{++} regulation of contraction in these extracts might result from an inhibition of actin-myosin interaction by actin-binding proteins that are released in the presence of micromolar Ca^{++} or the other agents that induce solation (62–64). This might also explain the Ca^{++} regulation of contraction in a variety of nonmuscle cells (60, 24, 15, 28). If the gel-sol transformation is involved in the regulation of cell movement, it might account for the absence of high concentrations of troponin-tropomyosin-like proteins in nonmuscle cells since these proteins would not be required to regulate all of the cell actin at one time. Several investigators have demonstrated that desensitized actomyosin incubated in the presence of various nonmuscle cell extracts containing many uncharacterized components will undergo Ca^{++}-stimulated ATPase and turbidity increases (41, 73, 43) similar to those observed in the *Dictyostelium* extracts reported here. However, these investigators have interpreted such results as due to actin-linked regulation (tropomyosin-troponin like) not involving the structural transformation of actin.

Both the gel-sol transformation and the more conventional regulatory mechanisms may operate in various nonmuscle cells (63, 64). Contractility in regions of the cell in the gelled state might be regulated by Ca^{++} via solation, while regions of the cell containing free F-actin might be regulated by conventional mechanisms (14, 59). This possibility is plausible, since immunofluorescence studies have localized tropomyosin in regions of some cells containing bundles of F-actin free to interact with myosin (53), while in other regions believed to be undergoing or capable of undergoing dynamic movement no tropomyosin is detectable (33).

Hence, the gel might have at least three functions in vivo: (*a*) The gel might transmit tension generated by local contractions through an extended region of the cell; (*b*) the gel might prevent unorganized bulk contraction from spreading throughout the cell by incorporating a portion of the actin in a form (gel) that is incapable of interacting with myosin to produce sliding; and (*c*) the gel might determine the cell shape by forming part of the nonmotile cytoskeleton.

Is the Cytoskeleton In Vivo Equivalent to the Gelled Extract In Vitro?

One of the advantages of working with cell extracts when studying motility is that movement itself is assayed and agents used to probe movement in vivo (37, 32, 3, 22, 29, 74, 40, 61–63) can be studied directly on the extract under controlled conditions. Therefore, the properties of the cytoskeleton in vivo and gelled *Dictyostelium* extracts in vitro, derived by using physical (pressure, cold, and mechanical agitation) and chemical (cytochalasin B) probes, can be directly compared.

Studies investigating the effects of cold or pressure on intact amoebae have demonstrated that these agents produce solation of the cytoplasm, cell rounding and massive contraction of the cytoplasm (37, 32). Furthermore, the repeated mechanical stimulation of amoebae by shaking (3) or through local stimulation with a microneedle (29, 74, 40, 61) results in a drop in cytoplasmic consistency followed by contraction and rounding of the cell. Therefore, the solation and accompanying contraction of both cytoplasm in intact cells and

gelled *Dictyostelium* extracts in response to pressure, cold, and mechanical agitation indicate that the properties of the cytoplasmic gel in vivo are similar to those of gels formed in cell extracts and may result from similar molecular interactions (64).

Recently, cytochalasin B has been shown to inhibit gelation in several different cell-free extracts (76, 21, 51). It has been suggested that cytochalasin B dissociates actin from a high molecular weight actin-binding protein (76, 21). Cytochalasin B also solates gelled *Dictyostelium* extracts, and this solation is accompanied by contraction.

However, the effect of cytochalasin B on the intact cell is less definite. Cytochalasin B has been shown to inhibit some cell movements where microfilaments have been implicated in motility (7, 54; see reference 48 for review). However, cytochalasin B has been demonstrated to affect many cell processes (4). Recently Pollack and Rifkin (45) have shown that cytochalasin B will cause retraction of the lamellipodia of cultured cells, indicating that cytochalasin B may also induce contraction in vivo.

Future studies will concentrate on identifying the specific calcium-regulated actin-binding protein(s) and on characterizing the structural dynamics of actin and associated proteins during the gelation-solation-contraction cycle in vitro and in vivo.

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