

ACTIN IN *XENOPUS* OOCYTES

II. Intracellular Distribution and Polymerizability

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ABSTRACT

The largest oocytes of *Xenopus laevis* were broken open in the absence of shearing forces which might transfer actin from particulate to supernatant fractions. Particulate and postmitochondrial supernatant fractions were prepared by centrifugation. SDS-electrophoretic fractionation on polyacrylamide gels and quantitative scanning techniques were used to separate actin and to assay its amount in cellular fractions.

The actin has been identified in electrophoretograms by its molecular weight and its binding to DNase I. Oocytes contain 1.4–1.7 μg of actin per cell, of which up to 88% is recovered in the postmitochondrial supernate under a variety of conditions. In the soluble fraction, it represents about 8.8% of the total protein. Its concentration in native cytoplasm was directly assayed at 4.1 mg/ml. There is no detectable actin that can be transferred from the particulate to the soluble phase by neutral detergents or ionic conditions that would depolymerize muscle actin. Centrifugation of the soluble oocyte fractions showed that 75–95% of the actin can not be sedimented under forces that would pellet filamentous actin. Addition of potassium and magnesium to the cytoplasm, to concentrations that would polymerize muscle actin, does not increase the amount of sedimentable actin. Roughly one-third of the soluble actin is recovered from Sephadex columns at about the position of monomer. About two-thirds is in complexes of 100,000 daltons or greater.

KEY WORDS soluble
actin · oocytes · polymerizability

Actin has been identified in egg cells of echinoderms (23, 24, 32, 33) and amphibians (9, 15, 36). In this respect, egg cells seem to be similar to every other type of eukaryote cell that has been studied (for reviews, see references 1, 17, and 37). The only localizations of actin in eggs reported so far have demonstrated that at least some of the cellular actin exists in the cortex (15, 36) where it

may be involved in cleavage furrow formation (33, 36, 41).

By the use of probes that show the location of actin *in situ*, actin has been shown in a variety of cells to be organized into microfilaments, paracrystalline arrays, or "cables," globular structures or anastomosing networks (6, 11, 15, 26, 38, 39). In such studies, one gains the impression that cellular actin is largely organized into discrete structures. Some recent studies have indicated, however, that actins of nonmuscle cells may exist

in less structured states. For example, the periacrosomal vesicles of *Thyone* sperm contain actin in a highly concentrated, aggregated state (44). Endoplasm of *Physarum polycephalum* has been shown to contain nonsedimentable actin (22), and a careful search has revealed few filamentous structures (20, 21). Similarly, extracts of chick brain and fibroblasts have been shown to contain actin, of which only about 50% is sedimentable under conditions in which filamentous actin should have pelleted (5).

In this communication, we report on a series of studies concerning the localization, amounts, and sedimentable state of actin in the oocyte of *Xenopus laevis*. Because of the large size of this cell, we have been able to report results on a per cell basis and have made a direct estimation of the concentration of actin in native cytoplasm. We present evidence to show that most of the cellular actin is not bound to sedimentable structures and, although polymerizable in extracts (9), is itself not polymerized.

MATERIALS AND METHODS

Isolation of Oocytes

Mature females of *Xenopus laevis* were sacrificed by decapitation, and pieces of ovary were removed. Oocytes were freed from ovarian membranes by treatment in collagenase (Sigma Chemical Co., St. Louis, Mo., Type II) at 2 mg/ml in 0.1 M phosphate buffer, pH 7.4, for 20 min at 30°C with vigorous swirling (40). Isolated cells were washed in Ringer solution, and 90 undamaged, stage-6 oocytes (13) were selected under the dissecting microscope for each fractionation.

Fractionation of Oocytes

A known number of oocytes were washed two times quickly in distilled water and placed in a round-bottomed watch glass. All glass surfaces which came into contact with homogenates or fractions were siliconized. The distilled water was removed with a fine-bore Pasteur pipette, and the cells were manually broken with watchmaker's forceps. This procedure was used to eliminate shearing stresses which might remove filamentous actin from larger structures. For each 90 oocytes, exactly 345 μ l of medium was added, with a Hamilton syringe (Hamilton Co., Reno, Nev.), and the components were mixed thoroughly with a fine-bore Pasteur pipette. The homogenates were then sealed with parafilm for incubation at the desired temperature.

Homogenizing medium contained 20 mM Tris-HCl, pH 7.4, 5 mM mercaptoethanol, and 5 mM MgCl₂. Variations of this medium are noted in the text and Tables.

For fractionation, the homogenates were transferred to thin centrifuge tubes of about 3 mm ID. These tubes had been volumetrically calibrated, using Hamilton syringes, so that linear measurements along the tube could be converted into volumetric measurements of fractions. The tubes were then centrifuged at 12,000 g for 20 min at 5°C. A sharply defined pellet could be expected to contain membranes, follicular residues, yolk, pigment, mitochondria, nuclei, and other particulates. This is called the P-12 fraction. An opalescent, postmitochondrial supernate topped the pellet and is referred to as the S-12 fraction. At the top was a thin layer of lipid material. After volumetric measurement of all fractions, the tube was scored and broken just under the lipid layer, allowing removal of the S-12 and P-12 fractions without lipid contamination.

Aliquots of the S-12 fraction were measured out and added to one-third volume of 3 \times concentrated sodium dodecyl sulfate (SDS) electrophoresis buffer. After removal of the S-12 fraction, the pellet was washed with homogenizing medium, pelleted again in the centrifuge, and all of the medium was removed from its surface. The entire pellet was then quantitatively transferred into exactly 3.60 ml of 1 \times SDS-electrophoresis buffer. After complete dissolution of S-12 and P-12 fractions in sealed tubes, they were immersed in a boiling water bath for 2 min, cooled and 10- μ l aliquots were subjected to SDS-polyacrylamide gel electrophoresis. By knowing the number of cells in each homogenate and the volume of each fraction, we were able to express the results as amounts per oocyte.

Electrophoretic Fractionation

Electrophoretic fractionation was accomplished on 18% polyacrylamide slab gels in 0.1% SDS (25). A 5% stacking gel was used, and the system was run at 30 mA for 4-5 h. This was sufficient to place the actin band about two-thirds to three-quarters of the way down the resolving gel. After fractionation, gels were stained in 0.2% Coomassie Brilliant Blue in acid methanol for 14-18 h with stirring and then destained in acid methanol for at least 48 h with stirring.

Quantitation of the Actin Bands on Polyacrylamide Gels

Highly purified actin from *Xenopus* skeletal muscle was lyophilized, weighed, and dissolved in 1 \times SDS buffer as an electrophoretic standard. Five aliquots of the standard at 0.4, 0.8, 1.2, 1.6, and 2.0 μ g were run on five slots of every gel as comparison standards. Each panel was cut from the slab gel after measuring the width of the actin bands with a microscope ocular reticle. All panels were then scanned with a Gilford scanning spectrophotometer at a wavelength of 595 nm. The light absorption of the actin peaks was determined by planimetric integration. The product of integration units times width units was taken as a measure of actin in the band

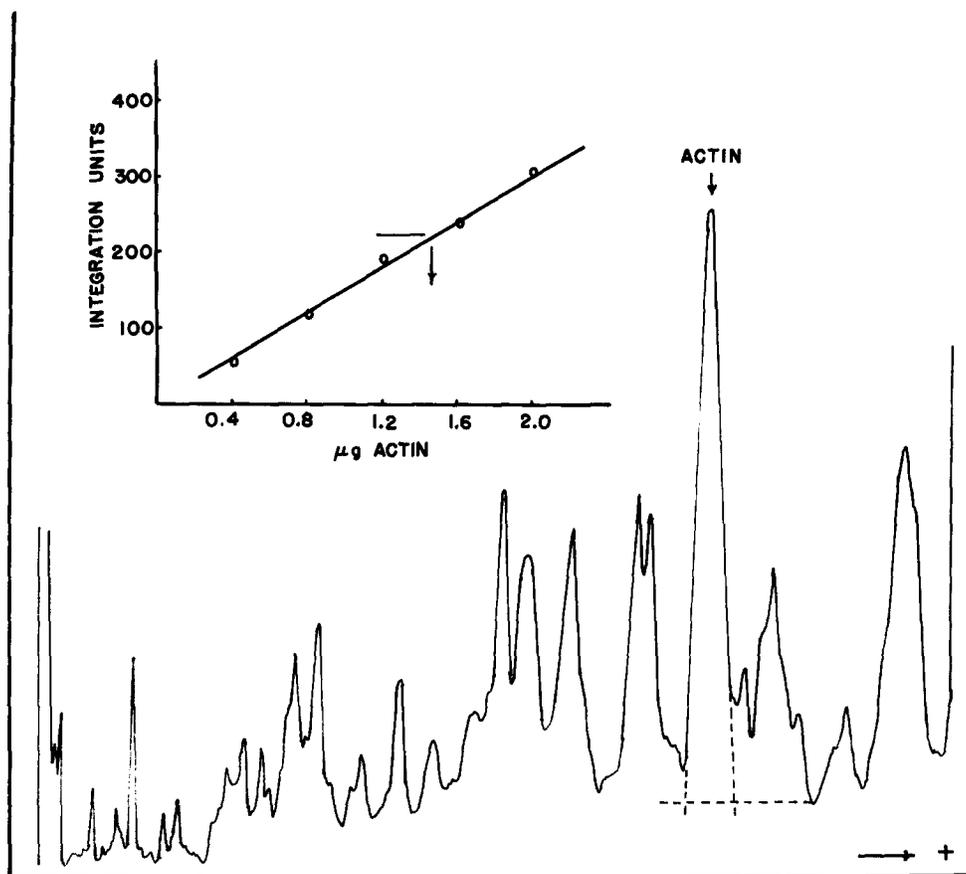


FIGURE 1 A densitometric scan of the S-12 fraction of stage-6 oocytes, extracted in the presence of 2 mM magnesium ion and electrophoresed on an 18% polyacrylamide gel in the presence of SDS. Migration of proteins was left to right, with proteins of lower molecular weight lost from the right end of the gel. The actin band is indicated, and the area actually integrated is demarcated by the dotted lines. The inset shows the standard curve obtained by scanning the series of muscle actin standards run on different slots of the same gel.

and compared with the actin standard series (Fig. 1). The uncertainty about the true baseline resulted in a quantitative uncertainty of about $\pm 10\%$.

Affinity Chromatography using *DNase I-Agarose*

Chromatographically purified deoxyribonuclease I from bovine pancreas (Sigma) was immobilized on Bio-Gel A-15 m agarose beads (Bio-Rad Laboratories, Richmond, Calif.). A 2.0-ml column containing 0.6 mg of enzyme was packed in a 5-ml plastic syringe. 0.4 ml of oocyte extract containing 8 OD_{280} units was applied to the column. Oocyte proteins were eluted with increasing concentrations of guanidine-HCl. Eluted fractions were assayed for optical density at 280 nm. The peak fractions were collected, dialyzed against distilled water, lyophilized, and dissolved in SDS-electrophoresis buffer for electrophoretic analysis. The procedure was that of

Lazarides and Lindberg (27).

Column Chromatography

A fractionation of the soluble components of oocyte extracts was performed on a 2.0×33.0 cm Sephadex G-100 column. The column was equilibrated and eluted with a buffer containing 20 mM Tris-HCl pH 7.4 + 2 mM β -mercaptoethanol + 2 mM $MgCl_2$ + 10^{-5} M $CaCl_2$ + 10^{-5} M ATP. The fractionation was done at $4^\circ C$ at 10–12 ml/h. Fractions of 3.2 ml were collected and precipitated with cold 5% trichloroacetic acid. The precipitates were centrifuged down, washed with ether, dried, and dissolved in SDS-electrophoresis buffer for electrophoretic analysis.

RESULTS

Electrophoretic fractionation of the soluble components of *Xenopus* oocytes showed a prominent

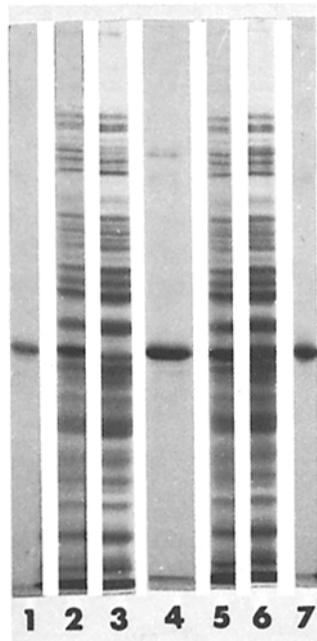


FIGURE 2 Electrophoretograms produced in acrylamide gels in the presence of SDS. The bands are stained with Coomassie Blue. The origin is near the top, the dye front near the bottom. Panels 1 and 7 are purified *Xenopus* muscle actin. Panels 2 and 6 are unchromatographed samples of the S-12 fraction of oocytes. Panel 5 is the material eluted from an agarose column after applying the S-12 fraction seen in panel 6. Panel 3 is the material eluted from an agarose/DNase I column after applying the S-12 fraction seen in panel 2. Panel 4 is the material eluted from the agarose/DNase I column by 3 M guanidinium chloride after the material seen in panel 3 was eluted and the column washed (see Materials and Methods).

band at about 43,000 daltons which exactly co-migrated with actin from *Xenopus* skeletal muscle (Fig. 2). It was possible to establish the identity of actin in these extracts by inducing gelation of soluble components (9) by procedures similar to those used by Kane (23) for sea urchin eggs. Actin has been identified as a major component of the gel by several criteria (10). In addition, we noted that removal of the actin gel in extracts also resulted in the removal of most of the band in electrophoretograms which co-migrated with muscle actin. It was clear that the co-migrating band in fact contained actin.

We wished to assay actin quantitatively by photometric scanning of the actin band on electrophoretograms, but needed to know whether other nonactin proteins were included in the band. To

investigate this point, we took advantage of the ability of pancreatic deoxyribonuclease I (DNase I) to bind actin with high selectivity (27). An oocyte extract was prepared and centrifuged at 85,000 g for 3 h. An aliquot of the supernate was passed through a DNase I-agarose column as described in Materials and Methods. As a control, an equal aliquot was passed through an identical column which contained no enzyme. The results are presented in Fig. 2. Note the quantitative removal of stainable material from the actin location in panel 3. Material co-migrating with muscle actin, eluted from the DNase I column by 3 M guanidine-HCl, is seen in panel 4. Elution of the control agarose column with 3 M guanidine-HCl produced no detectable protein bands. We have concluded that all of the stainable material at the actin location in electrophoretograms is actin.

We became interested in learning how much actin is present in oocytes and where it is located. Stage-6 oocytes were broken without shearing forces in a buffer consisting of 20 mM Tris-HCl, pH 7.4 ± 5 mM mercaptoethanol + 5 mM MgCl₂ (TMM) and were centrifuged at 12,000 g for 20 min at 5°C. The amount of actin in the S-12 fraction was then determined as described in Materials and Methods. The results can be seen in Table I. In well-washed P-12 fractions, no actin was detectable. Nevertheless, on the basis of the lower limit of detectability of actin in our system, it is possible that up to 0.48 μg/cell are present in the P-12 fraction which could not be seen in the presence of the dominating yolk proteins. We do know that there are 0.12 μg in the germinal

TABLE I
Amounts of Actin in Stage-6 Oocytes of *Xenopus laevis* Expressed as Micrograms/Oocyte

Particle-free cytoplasm (S-12)*	Cytoplasmic particulates and nucleus and cortex (P-12)†	Total per oocyte
<i>M</i> ± <i>SEM</i>		
1.24 ± 0.15	0.48§	1.72§
<i>n</i> = 7		

* *n* represents both the number of determinations and the number of different animals.

† In other experiments, we have found that a protein co-migrating with actin in both nucleus and cytoplasm is retained in an insoluble form under the conditions employed for these fractionations.

§ This estimate is based on the lower limit of detectability of actin and represents the maximum possible value.

TABLE II
Effect of 1% Triton X-100 on the Yield of Actin in the S-12 Fraction of Oocytes

Frog no.	Without Triton	With Triton
	<i>μg of actin/oocyte</i>	
7	0.79	0.72
13	1.16	1.25
Average	0.97	0.98

vesicle and a smaller amount in the cortex of a protein which co-migrates with actin. In the extraction medium used, we have found that the actinlike proteins of the nucleus and cortex remain insoluble and therefore are undoubtedly present in the P-12 fraction. We conclude that 72–88% of the cell's actin is to be found in the S-12 fraction under conditions in which filamentous or aggregated actin would not be sheared off of sedimentable cell structures.

Under the conditions of extraction employed (TMM), the amount of actin in the S-12 was compared with the total amount of protein in that fraction. Using the Lowry procedure (29) with a bovine serum albumin standard, we found actin to represent 8.8 ± 0.14 percent of the total protein.

On the basis of the localization of actin in other cells by *in situ* methods, we expected to find actin in polymerized states, perhaps bound to some of the many membrane systems to be found in the cytoplasm of amphibian oocytes. The apparent concentrations of sodium and potassium ions of these cells (8, 34) would strongly favor polymerization of muscle actin. On the basis of this expectation, we tried many extracting media designed to depolymerize actin or release it from membrane linkage in the P-12 fraction. At a concentration of 1%, Triton X-100 will completely remove the plasma membrane of oocytes. Yet, the inclusion of Triton X-100 in the homogenizing medium does not detectably increase the yield of actin in the S-12 fraction (Table II). Similarly, when the homogenizing medium was designed to depolymerize actin and when the homogenates were incubated for 40–120 min, or when the extraction temperature was raised to 20°C or sucrose added, the yield of actin in the S-12 fraction was not detectably increased (Table III). Aside from the small amounts of actinlike proteins in the nucleus and cortex, there is no evidence for actin in the particulate structures of the P-12 fraction. We conclude that most of the oocyte actin exists in the soluble phase.

We next attempted to find out whether the actin of the S-12 fraction is in polymerized or aggregated form. To establish this point, we performed some experiments in which the S-12 fraction was centrifuged for 3 h at 83,000 g at 2°C. Under these conditions, filamentous or extensively aggregated actin would be expected to pellet. The extracting medium was varied widely. The results of analyses of the actin in the supernate and pellet are shown in Table IV. There was some apparent variability in the amount of sedimentable actin from animal to animal, but the conclusion is inescapable that between 75 and 95% of the soluble actin of the oocyte is neither polymerized extensively nor aggregated into sedimentable structures. Sucrose seemed to facilitate the sedimentability of the soluble actin. This is consistent with the observation that gelation of extracts from *Xenopus* oocytes requires high sucrose concentrations (9).

Since the soluble actin in extracts of these cells

TABLE III
Yield of Actin in the S-12 Fraction of Oocytes in Various Media

Animal	Isolation medium contained:*			
	5 mM Mg ⁺⁺ , 0°C	5 mM Mg ⁺⁺ , 20°C	No Ca ⁺⁺ , no Mg ⁺⁺ , 3 mM EDTA, 0°C	No Ca ⁺⁺ , no Mg ⁺⁺ , 0.9 M sucrose, 0°C
	<i>μg/oocyte</i>	<i>μg/oocyte</i>	<i>μg/oocyte</i>	<i>μg/oocyte</i>
1	1.70	—	1.20	—
2	0.86	—	0.76	—
3	1.07	—	1.53	—
4	0.99	—	1.72	—
5	1.55	—	1.03	—
6	1.66	1.69	—	1.37
Average	1.27	—	1.30	—

* All media contained, in addition, Tris-HCl, pH 7.4 + 5 mM mercaptoethanol.

TABLE IV
Amounts of Sedimentable and Nonsedimentable Actin in the S-12 Fraction of Oocytes

Extraction buffer contained*	Supernate	Pellet
	<i>μg actin/oocyte (% total)</i>	
5 mM Mg ⁺⁺	1.49 (94)	0.10 (6)
5 mM Mg ⁺⁺	0.97 (77)	0.29 (23)
100 mM K ⁺	1.03 (97)	0.03 (3)
5 mM Mg ⁺⁺ + Triton X-100	1.02 (85)	0.18 (15)
0.9 M sucrose	1.37 (56)	1.07 (44)

* All media contained, in addition, Tris-HCl, pH 7.4 + 5 mM mercaptoethanol.

can polymerize into fibrous gels in vitro (9), we wondered whether the actin concentration in the cytoplasm was too low for effective polymerization in vivo. To check on this point, we performed two experiments to assay the actin concentration in native, yolk-free cytoplasm. Cells were packed in a thin centrifuge tube at 2,000 g and all of the medium was aspirated off the top of the tightly packed cells. The "dry" cells were then crushed, and their contents were stratified by centrifugation at 25,000 g. Measured aliquots of yolk-free cytoplasm were assayed for actin. The results are presented in Table V. At 4 mg/ml, purified *Xenopus* muscle actin will readily polymerize in vitro (10).

The apparent potassium concentrations, recorded for amphibian oocytes (8, 34), are sufficient to induce polymerization of muscle actin. We wondered whether the actual concentration in oocytes was really below the threshold for actin polymerization because of some sort of ion sequestration. To check this possibility, we did an experiment in which the yolk-free cytoplasm of oocytes from a single female was assayed for actin and then treated in three different ways. One-third of the cytoplasm was diluted with two volumes of buffer containing 100 mM KCl and 2 mM MgCl₂. One-third was diluted with two volumes of buffer containing no KCl and no divalent cations but containing 5 mM EDTA. The last third was injected with a solution whose volume was only 10% of the cytoplasmic volume but whose ionic concentration brought the ionic concentration of the total cytoplasm to 100 mM KCl and 2 mM MgCl₂.

After incubation of all three samples for 65 min at 21°C, the third undiluted sample was diluted with two volumes of buffer containing 100 mM KCl and 2 mM MgCl₂. All three diluted samples were then centrifuged for 3 h at 85,000 g at 2°C. Actin in all supernates was assayed and compared with the actin in the original, undiluted cytoplasm. The results are summarized in Table VI. Injection

TABLE V
Concentration of Actin in Particle-Free
Cytoplasm

Frog no.	mg actin/ml Cytoplasm*
1	4.0 ± 0.27
2	4.2 ± 0.24

* M ± SEM for three replicate determinations.

TABLE VI
Sedimentable and Nonsedimentable Actin in Oocyte Supernates after Various Ionic Treatments

Treatment	Nonsedimentable	Sedimentable
	% Total actin in yolk-free cytoplasm	
Cytoplasm diluted with 100 mM KCl and 2 mM MgCl ₂	63	37
Cytoplasm diluted with 5 mM EDTA	105	0
Particle-free cytoplasm made 100 mM with KCl and 2 mM with MgCl ₂	72	28

of sufficient potassium and magnesium to induce polymerization of purified muscle actin, and an actin concentration of around 3.6 mg/ml, did not result in additional polymerization during the course of the incubation.

Several reasons can be advanced to explain why most of the soluble actin in oocytes is not polymerized. One obvious possibility is that soluble actin is complexed as small aggregates or to other molecules so that polymerization is inhibited. It is conceivable that cycles of polymerization and depolymerization could be facilitated or controlled through such complexes. We were curious about whether the nonsedimentable actin of the S-12 fractions exists as actin monomer or as complexed entities.

To investigate this point, we prepared an S-12 fraction in the presence of 2 mM MgCl₂, calcium, and ATP. Any sedimentable actin was removed by high-speed centrifugation. The high-speed supernate was then fractionated on a Sephadex G-100 column, as described in Materials and Methods. Results are shown in Fig. 3. Actin was found both at the approximate position of monomer and also prominently in the void volume in complexes greater than 100,000 daltons in size.

DISCUSSION

The actin recovered in supernates of *Xenopus* oocytes can be called actin by four criteria. It comigrates with *Xenopus* muscle actin in SDS electrophoresis. It can be polymerized in vitro into filamentous, cross-linked gels (9). The gels made in vitro cross-react strongly with rabbit antibodies made against purified rabbit muscle actin. The oocyte soluble actin is quantitatively bound by

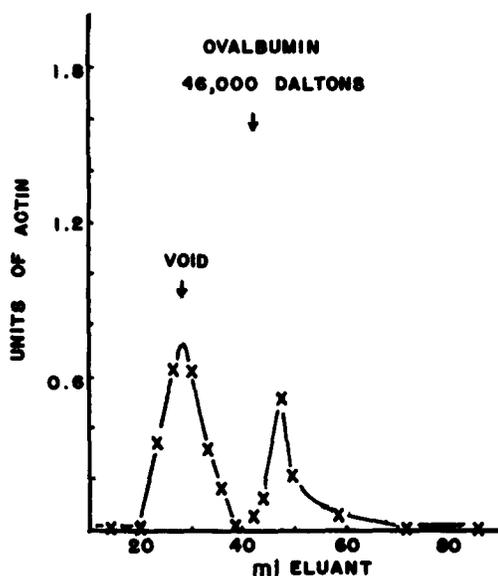


FIGURE 3 A plot of the emergence of oocyte actin from a Sephadex G-100 column at neutral pH and in the presence of calcium, magnesium, ATP, and mercaptoethanol. The location of the void peak was determined with blue dextran. The elution position of purified ovalbumin is shown as a molecular weight marker. Units of actin are given in arbitrary units on a semiquantitative scale.

DNase I (see reference 27 for details of specificity).

Despite these similarities, however, it is not possible to claim that oocyte actin is identical to muscle actin. In bovine tissues, it has been shown (30) that actins from cardiac muscle and brain have different primary structures and are different from actin from skeletal muscle. Similarly, in human cardiac muscle, blood platelets, and uterus, actins have been found that differ by slight changes in primary structure (14) or amino acid composition (2). Nonmuscle actins have been distinguished from muscle actin and from one another in other cell types as well, where differences observed could be due to differences in primary structure or to amino acid modifications (16, 18, 42, 43). From such studies in other cells, we must entertain the possibility that soluble oocyte actin could be identical to or different from *Xenopus* skeletal muscle actin. It seems likely that there is more than one type of actin included in our determinations and that the oocyte actins are different from muscle actin (see reference 17).

Why is the bulk of oocyte actin in a nonpolymerized state? In the presence of sufficient potas-

sium and magnesium ions, and at concentrations of actin sufficient to support polymerization of muscle actin in vitro, the soluble actin remains largely nonsedimentable. The fact that the soluble actin may be polymerized in vitro (9) in the presence of sucrose indicates that it is fully capable of polymerization. Two general reasons can be advanced to explain why the soluble actin of oocytes remains mostly unpolymerized: it could resemble muscle actin in its polymerization properties but be inhibited by intracellular conditions, or it could be uninhibited but differ from muscle actin in its fundamental polymerization characteristics.

We attempted to determine whether soluble oocyte actin could be recovered as an identifiable complex under reasonably physiological conditions. With the use of a Sephadex G-100 column, the results were not definitive. Some of the soluble actin emerged at the position of monomer \pm about 2,000 daltons, but more came out in the void volume with an aggregate weight of over 100,000 daltons. This finding could be interpreted to mean that most of the soluble actin was complexed to an inhibitor of polymerization. We suspect, however, that the actin in the void volume represents actin in either homocomplexes or "stuck" to other proteins in fortuitous or specific interactions (see reference 3). It could be relevant that slightly denatured actin (35) or actin with nucleotide removed (7) reversibly forms oligomers.

If this tentative conclusion is correct, then perhaps soluble oocyte actin has different polymerization characteristics than muscle actin. Such differences have in fact been described with a variety of nonmuscle actins (for a review, see reference 37). In human blood platelets, an actin has been found that remains polymerized under conditions that would depolymerize muscle actin, while a second form is depolymerized under ionic conditions that would polymerize muscle actin (16).

Actin in very high concentration exists in an unpolymerized state in the periacrosomal vesicles of *Thyone* sperm. It is more difficult to polymerize this actin by ionic means than to polymerize muscle actin (44). In chick brains, Bray and Thomas have identified an actin that requires a higher concentration than muscle actin in order to polymerize extensively in vitro. The authors concluded that, at an intracellular actin concentration of 3-5 mg/ml, much of this actin would be expected to remain unpolymerized even under ionic

conditions favoring muscle actin polymerization (5).

Because of these observations on other cellular systems, we presently favor the idea that oocyte actin is mostly unpolymerized because its threshold of polymerization is higher than that of muscle actin. Such a condition could be due to an altered primary structure, to amino acid modifications, or possibly, to altered requirements for polymerization initiation.

In this study, actin was found to represent about 8.8% of the total proteins of the soluble fraction of oocytes in the presence of magnesium ion. It is difficult to make direct comparisons between this value and those measured in other cells because of variability in the way in which the results are expressed. Nevertheless, in sea urchin eggs, actin constitutes 8–10% of total egg protein, including yolk (32). The embryonic chick fibroblast contains actin which is about 8.5% of total cellular protein (4). In developing chick brains, actin is about 8–10%, and in adult chicken brains about 6% of the total soluble proteins (35). These estimates may be compared to those for skeletal muscle in which actin makes up 17–34% of the total proteins (19). We conclude that actin does not seem to be accumulated or “stockpiled” in egg cells, relative to other proteins, to a greater extent than in other nonmuscle cells.

The unusual size of the amphibian oocyte has allowed us to make the first direct estimate of the concentration of actin in undiluted cytoplasm. The value of 4.1 mg/ml may be compared to the concentration of actin in chick fibroblasts. A value of about 2 mg/ml was estimated, based on a reasonable guess about cell volume (4).

Our findings in *Xenopus* oocytes indicate that cellular actins exist in both polymerized and non-polymerized states, perhaps in dynamic equilibrium (see also references 21 and 22). Functional needs for cables, gelated structures such as cortex (15, 36) or nucleoplasm (31), or other structural forms may well result in recruitment of soluble actin. The dynamic relationship between soluble monomers and formed structures is well known in several other cellular systems (e.g., references 12 and 28). Actin, however, seems to be unusual in the diversity of the structures and functions in which it is involved. It will be of interest in future research to find out how much of the diversity of function is due to modifications of the actin molecule and how much to specific polymerization-initiating and binding components.

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