DYNAMICS OF ANTIGENIC MEMBRANE SITES RELATING TO CELL AGGREGATION IN *DICTYOSTELIUM DISCOIDEUM*

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ABSTRACT

Membrane interaction in aggregating cells of *Dictyostelium discoideum* can be blocked by univalent antibodies directed against specific membrane sites. Using a quantitative technique for measuring cell association, two classes of target sites for blocking antibodies were distinguished and their developmental dynamics studied. One class of these sites is specific for aggregation-competent cells, their quantity rising from virtually 0-level during growth, with a steep increase shortly before cell aggregation. The serological activity of these structures is species specific; they are not detectable in a nonaggregating mutant, but present in a revertant undergoing normal morphogenesis. Patterns of cell assembly in the presence of antibodies show that selective blockage of these membrane sites abolishes the preference for end-to-end association which is typical for aggregating cells. A second class of target sites is present in comparable quantities in particle fractions from both growth-phase and aggregation-competent cells. Blockage of these sites leads to aggregation patterns in which the side-by-side contacts of aggregating cells are abolished. The target sites of aggregation-inhibiting antibodies are suggested to be identical or associated with the molecular units of the cell membrane that mediate cell-to-cell contacts during aggregation. The results indicate that in one cell, two independent classes of contact sites can be simultaneously active.

INTRODUCTION

The question of whether cell aggregation can be explained as the interaction of discrete molecular entities of the cell surface (1, 2, 3) has been studied by blocking different surface structures with univalent antibody fragments (F_{ab}) (4, 5). These fragments do not cause cell agglutination or membrane changes by cross-linking of cell surface antigens (6), and have a smaller molecular weight (50,000) than native antibodies. Blocking of certain membrane antigens by F_{ab} inhibits cell aggregation whereas blockage of others does not (5), suggesting that essential functions in cell aggregation can be traced to membrane struc- tures that are identical or associated with the binding sites for aggregation-inhibiting F_{ab}.

For these experiments, the cellular slime mold *Dictyostelium discoideum* has been used, because in the course of its development a multicellular system comparable to an animal tissue is built up from single cells by aggregation, and because non-aggregating mutants are available. During the growth phase this organism retains the single cell state, cells requiring several hours after the end of growth to become aggregation competent. During this preaggregation phase, cell shape and adhesive properties change (7), and new specific

No.	F_{ab}-species	Antiserum source	Antigen used for immunization	Absorption of F_{ab}
1	aggr-F_{ab}	Pool K 146 (5)	Particle fraction of aggregation-competent *D. discoideum* cells	None
2	gph-F_{ab}	K 355 (16)	Cell homogenate of growth-phase *D. discoideum* cells	None
3	cs-A-specific F_{ab}	Same as no. 1		aggr-F_{ab} was exhaustively absorbed with particles from lyophilized growth-phase cells as indicated in the legend
4	cs-B-specific F_{ab}	Same as no. 2		10 mg F_{ab} in 0.63 ml barbital buffer were absorbed with particles from 12.5 mg lyophilized aggregation-competent cells as described in the legend to Fig. 2
5	Nonimmune F_{ab}	Pool of preinjection sera	None	None

Absorption of aggr-F_{ab}. 2.5 ml particle suspension containing the yield from 250 mg lyophilized growth-phase cells were centrifuged at 22,000 g for 20 min at 4°C, and the sediment was mixed with 10 mg aggr-F_{ab} in 2.5 ml barbital buffer, pH 7.3. The mixture was incubated for 30 min at room temperature, for 60 min at 4°C, and centrifuged at 30,000 g for 20 min. This procedure was performed three to four times, and the final supernatant was dialyzed against distilled water and lyophilized.

cell membrane components become detectable with antibodies (8, 9).

As an operational term for the target sites of antibodies that block cell adhesion, we proposed the term "contact sites" (5). In this paper, the developmental dynamics of contact sites is studied using a quantitative technique for measuring cell agglutination (10). Our results indicate (*a*) that a new class of such membrane sites become detectable during a defined period of the preaggregation phase, and (*b*) that another class of these sites is present in both growth-phase and aggregation-competent cells. In the presence of F_{ab}, cells form different aggregation patterns, depending on the class of contact sites which are blocked.

METHODS

Culture of Organisms

D. discoideum strain v-12/M2 was grown in suspensions of *Escherichia coli* B/r (11). Growth-phase cells

were harvested at the end of the exponential phase, that is, 2–4 h before the time of complete exhaustion of bacteria (t_0), and aggregation-competent cells were harvested 5 h after t_0. For use as aggregation-competent cells in agglutination-inhibition assays, amoebae were washed three times and resuspended in 0.017 M phosphate buffer, pH 6.0, at t_0, adjusted to 1×10^7 cells/ml, and shaken for 5 h.

Aggr 50-1 and 50-2 are subclones of an UV-induced, nonaggregating mutant of M 2; and aggr 50-3 (rev) is a spontaneous revertant that aggregates and produces fruiting bodies. These strains were cultivated as the wild-type, except that aggr 50-1 and 50-2 were grown on *Salmonella minnesota* R 595 (26). Cells were harvested either 3 h before or 5 h after t_0 for comparison with growth-phase or aggregation-competent wild-type cells, respectively.

D. discoideum strain Ax-2 was cultivated axenically on yeast-extract/peptone medium supplemented with 18 g maltose/liter (12), if not stated otherwise. Growth-phase cells were harvested at a density of approximately 7×10^6/ml. To obtain aggregation-competent Ax-2 cells, growth-phase cells were washed and resuspended in phosphate buffer, adjusted to

E/E₀ axis showing graph: Mean number of cells per agglutinate (x-axis 1–10), with inset showing E/E₀ vs minutes after start of rotation (20–70), with 40rpm and 16rpm phases.
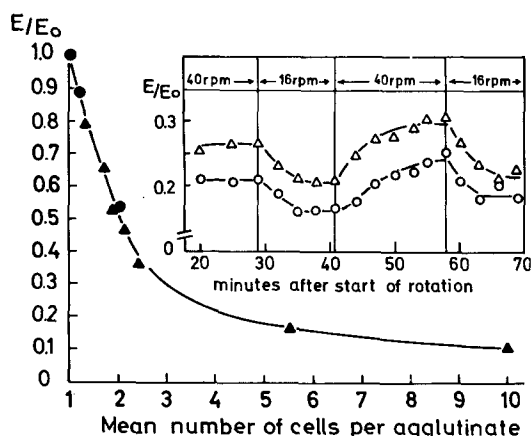
FIGURE 1 Optical density as a function of the mean number of cells per particle. The curve shows that the optical method reflects mean agglutinate size most sensitively in the range of one to three cells per agglutinate and becomes almost invariant against size changes if mean agglutinate size exceeds 5–10 cells per agglutinate. The data taken from two experiments (▲ and ●) were replotted from reference 10. *Inset:* Reversible increase and decrease of cell agglutination caused by altered shear. Growth-phase cells of strain M 2 were agglutinated in the presence of 5×10^{-5} M (○) and 2.5×10^{-4} M EDTA (△). To demonstrate reversibility of agglutination, shear was repeatedly altered by changing the speed of rotation from 16 rpm to 40 rpm, and vice versa. The reference for measurement of E_0 contained 2 mg/ml aggr-F_{ab} plus 1×10^{-2} M EDTA.

1×10^7 cells/ml, and shaken for 8–9 h. For some experiments, Ax-2 cells were grown on *E. coli* B/r as described for strain M 2, washed at t_0, and shaken for up to 9 h, that is, the time of full aggregation-competence.

Polysphondylium pallidum strain WS 320 was grown on *E. coli* B/r as described for *D. discoideum* but with continuous illumination, which is necessary in order to obtain aggregation-competent *P. pallidum* cells 9 h after t_0.

F_{ab} and Particle Preparation

F_{ab} was prepared as previously described (5) by papain digestion of purified rabbit IgG (13) and subsequent purification on CM-cellulose (14). Specifications of the F_{ab}-samples used are listed in Table I.

Particle fractions were prepared from lyophilized cell homogenates of either axenically grown Ax-2 cells, or M 2, aggr-50, and *P. pallidum* cells grown on bacteria, in essentially the same way as described in

reference 5. Particles were washed three times in barbital buffer, pH 7.3 (15; ionic strength reduced to $\mu m = 0.02$) before use for F_{ab}-absorption.

Assay of Agglutination-Inhibition

Cell agglutination and its inhibition by F_{ab} or chelating agents was measured at 40 rpm in a rotator containing up to 20 cuvettes of a shape which simultaneously allows application of standardized shear to, and measurement of the optical density of, suspended cells and agglutinates in a narrow beam of parallel light (10). Depth of the cuvettes was 2 mm and final cell concentration was 1×10^7/ml. Under these conditions, optical density represented essentially light scattering. To eliminate small variations between different cell suspensions, the quotient of the optical density of the sample (E) and that of a completely dissociated but otherwise identical reference (E_0), tested in the same run, was plotted. Measurements were taken when the optical density of the samples remained virtually constant, usually after 50–90 min of incubation (10). E/E_0 as a function of mean agglutinate size is shown in Fig. 1. For small- and medium-sized cell groups, agglutination proved to be reversible under our conditions (Fig. 1, inset).

Assay of Contact Sites by F_{ab}-Absorption

Agglutination-inhibition by absorbed F_{ab} was assayed as outlined in Fig. 2, using either growth-phase cells or aggregation-competent cells of *D. discoideum* strain M 2 as test cells. The cells were washed twice in phosphate buffer, once in barbital buffer, pH 7.3, and resuspended at a final density of 1×10^7/ml.

Pattern of Cell Assembly in Presence of F_{ab}

2×10^7 cells/ml were suspended in F_{ab}-solution, spread on cover slips, and incubated in moist chambers (11).

RESULTS

Measurement of Aggregation-Competence

To correlate molecular membrane changes with changes in cell adhesiveness, agitated suspension cultures were used to obtain synchronous development of cells. Under these conditions, suspended wild-type amoebae of *D. discoideum* reach a phase of maximal aggregation-competence several hours after exhaustion of food bacteria or, in axenic culture, after removal of growth medium (12, 17). Qualitatively, aggregation-competence
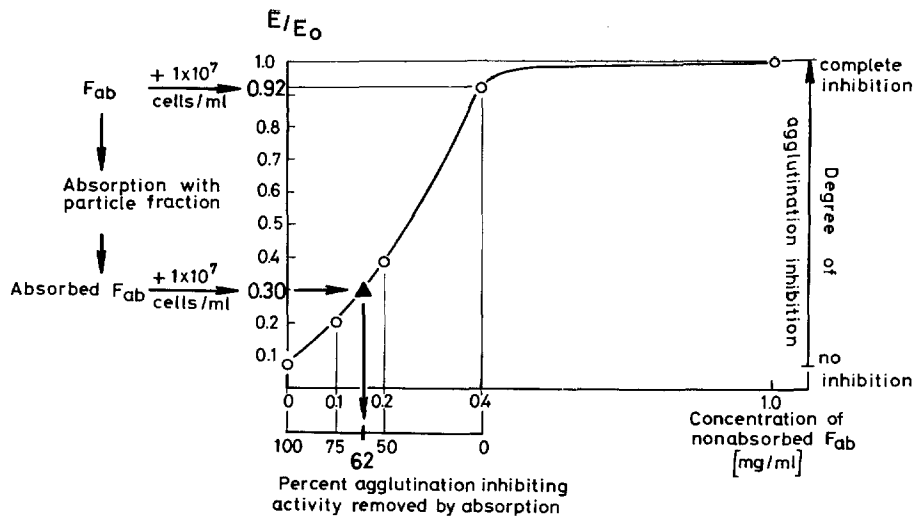
FIGURE 2 Assay for contact sites. Contact sites were assayed in particle fractions from cells according to the following program. In each experiment, serial dilutions of agglutination-inhibiting F_{ab} were run and used in the construction of a calibration curve relating E/E_0 as a measure of cell agglutination to F_{ab}-concentration. Such a curve is shown ($\bigcirc$). The left steep branch of the curve, where E/E_0 reflects F_{ab}-concentration most sensitively, was used in the assay. F_{ab} at a concentration which nearly completely inhibited agglutination was incubated for 30 min at room temperature and 60 min at 4°C with the 27,000 g sediment prepared from a defined quantity of lyophilized cells. The incubation mixture was then centrifuged for 10 min at 10,000 g. The residual agglutination-inhibiting activity of the supernatant was tested with cells from the same batch as used for testing the nonabsorbed F_{ab} samples. The point $\blacktriangle$ on the calibration curve indicates the concentration of nonabsorbed F_{ab} that inhibited agglutination to the same degree as the supernatant. Results are expressed as percent agglutination-inhibiting activity removed by absorption, as indicated by arrows.
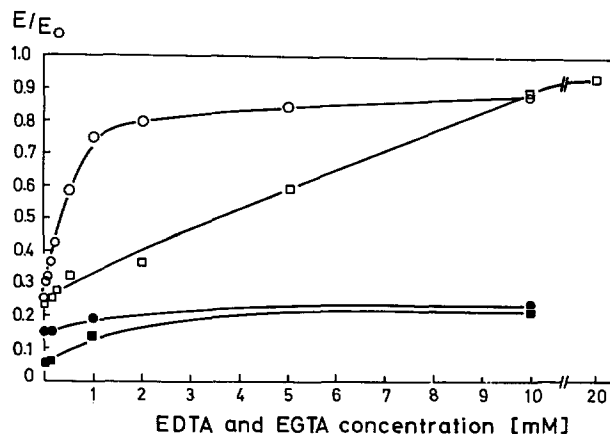

FIGURE 3 EDTA- and EGTA-stability of cell agglutination as a test for aggregation-competence. Ax-2 cells grown on bacteria were harvested either as growth-phase cells ($\bigcirc$, $\square$), or as aggregation-competent cells ($\blacksquare$, $\bullet$), and incubated in EDTA ($\bigcirc$, $\bullet$) or EGTA ($\square$, $\blacksquare$) for measurement of E/E_0. Medium: 0.017 M phosphate buffer, pH 6.0. Reference for E_0 as in Fig. 1.

can be evaluated by transferring cells onto a glass-water or air-water interphase, which allows them to spread and to migrate. Aggregation-competence is indicated by elongated, tightly

sticking cells which rapidly form cell chains and streamlike aggregates (7), whereas growth-phase cells remain single after spreading.

To obtain quantitative data on cell association,

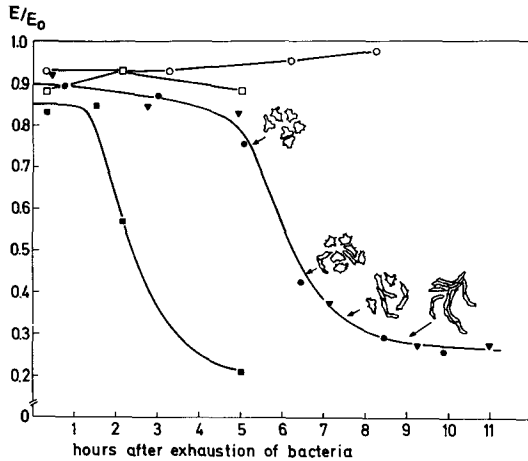E/E₀ rendered as E/E_0 on chart axis.

FIGURE 4 Development of aggregation-competence and inhibition of this process by EDTA. The capacity to form streams, which was taken as a qualitative criterion for aggregation-competence, commenced in strain M 2 at 3 h and in strain Ax-2 at 6–7 h after end of growth, and was fully developed in this strain at 8–9 h. This is illustrated by diagrams. The capacity for EDTA-stable cell agglutination developed parallel to that for stream formation, as shown for M 2 (■) and two cultures of Ax-2 (▼, ●). This process was inhibited by cultivation of M 2 (□) as well as Ax-2 cells (○) in 1×10^{-3} M EDTA. Cells were grown on bacteria, washed at end of growth, resuspended in 1×10^{-3} M EDTA or in medium without EDTA, respectively, and shaken. At intervals, samples were taken from these cultures, and the cells were washed and incubated in 1×10^{-2} M EDTA for measurement of E/E_0. Medium and reference were the same as in Fig. 3.

size of cell groups can be measured after exposure of cell suspensions to standardized shear (7, 10). Association of suspended cells will be referred to as "agglutination." It should be distinguished from "aggregation" of cells adhering to a surface. Aggregation of *D. discoideum* cells is the result of orientated cell movement and of cell-to-cell adhesion in competition with cell-to-substrate adhesion. Agglutination does not require cell motility; it is therefore independent of the chemotactic system that guides cell motion during aggregation.

The distinction between agglutination and aggregation is necessary because growth-phase cells agglutinate but do not aggregate into streams when dispersed on a substrate. The association of aggregation-competent cells into streams depends on an EDTA-stable type of cell adhesion which is absent in growth-phase cells, as shown by the

EDTA-sensitivity of their agglutination (7). Fig. 3 shows that EDTA- as well as EGTA-stability of agglutination distinguishes aggregation-competent cells from growth-phase ones. In the present study we identify suspended cells as aggregation competent by their ability to agglutinate in the presence of 10^{-2} M EDTA.

Cell Differentiation to Aggregation-Competence

EDTA-stable cell agglutination as an indicator of cell differentiation was measured in cells of strains Ax-2 and M 2 harvested at different times after the end of growth. A steep increase of EDTA stability occurred immediately before the qualitative criteria of aggregation-competence became detectable (Fig. 4). No increase was, however, measured when cells were continuously incubated in EDTA beginning with the end of growth (Fig. 4). This demonstrates that although association of aggregation-competent cells is EDTA stable, differentiation from the growth-phase state to aggregation-competence is not, and explains reports (18, 19) according to which cell aggrega-
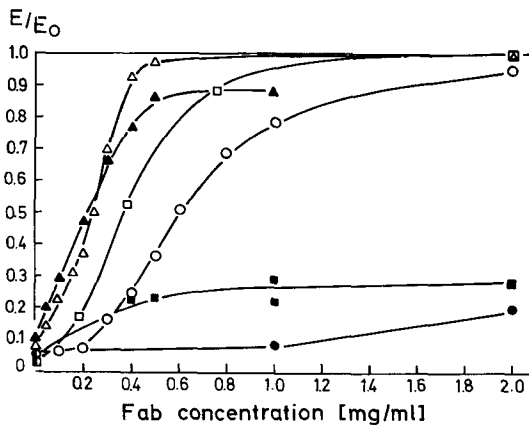

FIGURE 5 Agglutination-inhibition by aggr-F_{ab}, and by aggr-F_{ab} absorbed with particles from growth-phase cells. Agglutination of aggregation-competent cells by aggr-F_{ab} was completely inhibited in the presence (△) as well as in the absence (□) of 10^{-2} M EDTA. Inhibition by aggr-F_{ab} absorbed with particles from growth-phase cells was nearly complete in the presence (▲) but not in the absence (■) of 10^{-2} M EDTA. Agglutination of growth-phase cells was completely inhibited by aggr-F_{ab} (○). Absorbed aggr-F_{ab} no longer significantly inhibited agglutination of these cells (●). The references for E_0 contained 2 mg/ml and 4 mg/ml aggr-F_{ab} for complete dissociation of aggregation-competent and growth-phase cells, respectively.
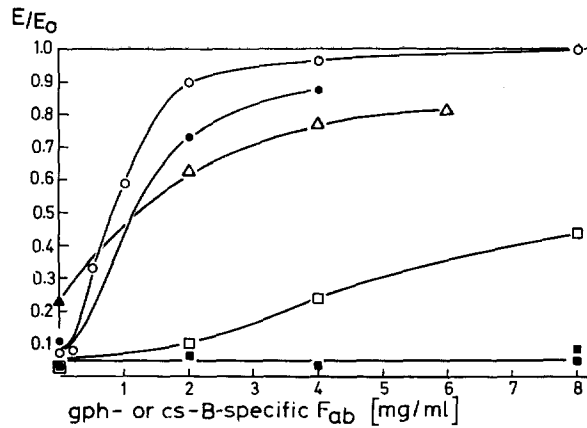
FIGURE 6 Agglutination-inhibition by gph-F_{ab} and cs-B-specific F_{ab}, and by a combination of the latter with cs-A-specific F_{ab}. F_{ab} directed against growth-phase cells (gph-F_{ab}) partially inhibited agglutination of aggregation-competent cells (□). This activity became nondetectable after absorption of the F_{ab} with a small quantity of particles from aggregation-competent cells (■). To demonstrate that the bulk of cs-B-specific F_{ab} species was still present in the absorbed preparation, agglutination-inhibition of growth-phase cells by this preparation (●) was compared with that of nonabsorbed gph-F_{ab} (○). Contribution of cs-B-specific F_{ab} to agglutination-inhibition of aggregation-competent cells was tested by adding absorbed gph-F_{ab} to 0.5 mg/ml absorbed aggr-F_{ab}, a concentration that saturates cs-A of the test cells (△). The zero point on the abscissa (▲) represents a sample containing absorbed aggr-F_{ab} only. References for E_0 as in Fig. 5.
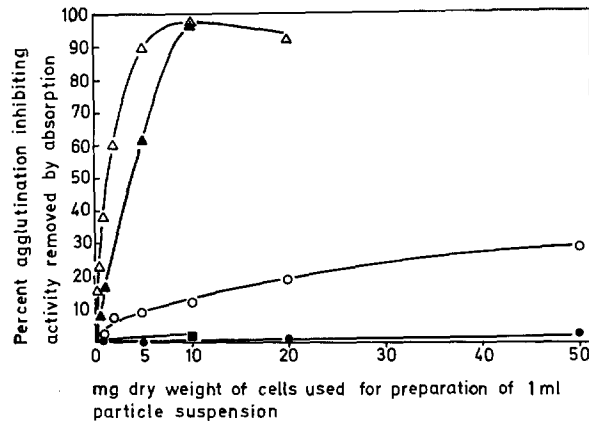

FIGURE 7 Assay of cs-A in particles from growth-phase and aggregation-competent cells, and species-specificity of these sites. 0.8 mg aggr-F_{ab} (△, ○) or cs-A-specific F_{ab} (▲, ●) in 1 ml was absorbed with particles from Ax-2 cells harvested either during growth (○, ●) or at aggregation-competence (△, ▲). To test cross-reactivity of cs-A with *P. pallidum* antigens, cs-A-specific F_{ab} was absorbed in the same way with particles from aggregation-competent *P. pallidum* cells (■). Agglutination-inhibition of all F_{ab} samples was tested with aggregation-competent M 2 cells in the presence of 10^{-2} M EDTA.

tion in *D. discoideum* is completely inhibited by EDTA or EGTA.

Two Classes of Contact Sites in Aggregation-Competent Cells

EVIDENCE FOR A CLASS OF SITES WHICH ARE SHARED BY GROWTH-PHASE AND AG- GREGATION-COMPETENT CELLS: CONTACT SITES B (CS-B): F_{ab} directed against aggregation-competent cells (aggr-F_{ab}) completely inhibited agglutination of these cells as well as that of growth-phase cells (Fig. 5). After exhaustive absorption of this F_{ab} with particle preparations from growth-phase cells (Table 1), agglutination of those cells was no longer significantly inhibited.

The same absorbed F_{ab} inhibited agglutination of aggregation-competent cells only partially, the degree of inhibition remaining constant with increasing F_{ab} concentration (Fig. 5). This shows that one class of contact sites is shared by growth-phase and aggregation-competent cells. These sites will be referred to as cs-B.

Agglutination of aggregation-competent cells was completely inhibited by absorbed aggr-F_{ab} if EDTA was added to the cell suspension (Fig. 5). This shows that EDTA inactivates cs-B, and agrees with the finding that agglutination of growth-phase cells, which by definition is due to cs-B, is inhibited by EDTA (Fig. 3).

EVIDENCE FOR A CLASS OF SITES WHICH ARE RESTRICTED TO AGGREGATION-COMPETENT CELLS: CONTACT SITES A (CS-A): The sites blocked by absorbed aggr-F_{ab} represent a second class of contact sites, referred to as cs-A. They are responsible for the EDTA-stable type of cell agglutination characteristic for aggregation-competent cells. The exhaustively absorbed aggr-F_{ab} is used as a cs-A-specific F_{ab} preparation. The finding that absorption of aggr-F_{ab} with particles from growth-phase cells does not remove cs-A specificity, demonstrates that these cells contain no cs-A or only negligible quantities. Spurious amounts must be present, however, since growth-phase cells elicited a weak antibody response against cs-A. F_{ab} from an antiserum prepared by immunizing rabbits with growth-phase cells (gph-F_{ab}) did not only completely inhibit agglutination of these cells, but also considerably that of aggregation-competent cells (Fig. 6). Much of the activity against the latter was due to cs-A-specific F_{ab} as shown by absorption of the gph-F_{ab} with a small quantity of particles from aggregation-competent cells. The cs-B-specific F_{ab} prepared in this way still inhibited agglutination of growth-phase cells almost completely (Fig. 6), indicating again that cs-A did not significantly contribute to the adhesion of these cells. The activity of the same F_{ab} against aggregation-competent cells, however, had been rendered nondetectable by our technique (Fig. 6).

In conclusion, agglutination-inhibition by F_{ab} and EDTA showed that growth-phase cells contain only cs-B which are inactivated by EDTA. In addition to cs-B, aggregation-competent cells contain cs-A, which are responsible for an EDTA-stable type of cell association. F_{ab} preparations

were obtained by absorption which specifically blocked either cs-A or cs-B. These specific F_{ab} preparations can be used to check the above conclusion. If aggregation-competent cells agglutinate by the combined activity of cs-A and cs-B, then cs-A-specific F_{ab} which inhibits agglutination of these cells only to a small extent should be supplemented by cs-B-specific F_{ab}, resulting in complete agglutination-inhibition.
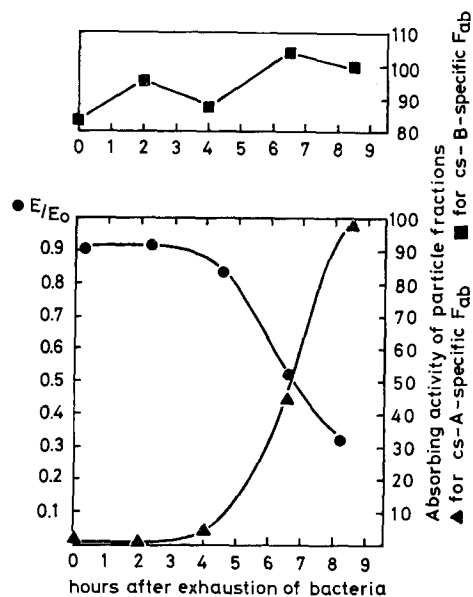

FIGURE 8 Developmental dynamics of cs-A and cs-B in Ax-2. Particle-bound cs-A (▲) and cs-B (■) were assayed by F_{ab}-binding, and cs-A activity in living cells was tested by measuring EDTA-stable cell agglutination (●). For preparation of particles, Ax-2 cells were harvested at intervals during their differentiation to aggregation-competence, washed, and lyophilized. cs-A were assayed by absorbing 0.8 mg cs-A-specific F_{ab} in 1 ml with particles prepared from 10 mg dry cells and testing agglutination-inhibition of the absorbed F_{ab} with aggregation-competent cells in 10^{-2} M EDTA. cs-B were determined by absorption of 2 mg aggr-F_{ab} in 1 ml with particles from 5 mg dry cells and testing agglutination-inhibition with growth-phase cells. The quantity of cs-A and cs-B has been expressed as percent absorbing activity of reference particles from aggregation-competent cells. The cs-A data were calculated by using the calibration curve (▲) shown in Fig. 7 for linearization. The cs-B data were calculated from a calibration curve for Ax-2 particles, similar to the M 2 curve (△) shown in Fig. 10. The capacity to form EDTA-stable cell associations was tested as in Fig. 4, except that axenically grown Ax-2 cells were used.
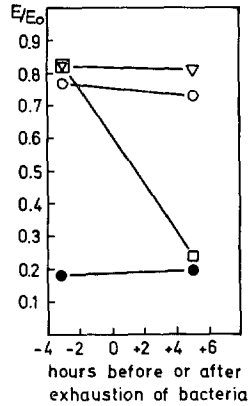
FIGURE 9 Absence of EDTA-stable and presence of EDTA-sensitive cell adhesion in the aggregation-deficient mutant aggr 50. Activity of cs-A was tested by measuring EDTA-stable cell agglutination in two morphologically deficient clones, aggr 50-1 (○) and 50-2 (▽), and in the revertant aggr 50-3 (rev) (□) that undergoes normal morphogenesis. Only the revertant developed the capacity for EDTA-stable cell agglutination. Agglutination in the absence of EDTA is shown for aggr 50-1 (●) to demonstrate activity of cs-B in the mutant.
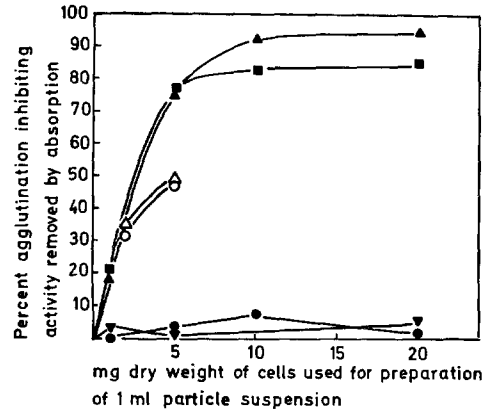
FIGURE 10 Nondetectability by F_{ab}-binding of cs-A, and detectability of cs-B in mutant aggr 50. For assay of cs-A, cells of aggr 50-1 (●), 50-2 (▼), 50-3 (rev) (■), and M 2 (▲) were harvested 5 h after exhaustion of bacteria, and particles from these cells were used for absorption of cs-A-specific F_{ab} in the same way as shown in Fig. 7 for Ax-2. For assay of cs-B, 2 mg/ml aggr-F_{ab} were absorbed with particles from aggr 50-1 (○) and from aggregation-competent M 2 cells (△) and tested with growth-phase cells.

Fig. 6 shows that agglutination-inhibition by the F_{ab} mixture approximates this point.

Developmental Dynamics of cs-A

To obtain quantitative data on contact sites during the course of development, particle fractions were prepared from cells harvested at subsequent stages of differentiation to aggregation-competence. F_{ab} was absorbed with these particles and the remaining agglutination-inhibiting activity was retitrated with living test cells. A specific test system for cs-A consisted of cs-A-specific F_{ab}, EDTA, and aggregation-competent test cells. EDTA was used because in the test cells it largely paralyzed adhesion due to cs-B.

cs-A-specific F_{ab} increased selectivity for cs-A to a greater extent than that obtained with nonabsorbed aggr-F_{ab} (Fig. 7).

The assay system was checked by absorption of F_{ab} with increasing quantities of particles from either growth-phase or aggregation-competent cells. With the latter, F_{ab}-absorption increased with particle concentration up to nearly 100% (Fig. 7). Growth-phase cells did not reduce the agglutination-inhibiting activity of cs-A-specific F_{ab} (Fig. 7). This proves (a) that the test system is cs-A specific, (b) that cs-A are not detectable in particles from growth-phase cells and, (c) that particles prepared from aggregation-competent cells contain the complete set of target sites for agglutination-inhibiting F_{ab}-species.

FIGURE 11 Cell assembly in presence of cs-A-specific F_{ab}, cs-B-specific F_{ab}, or EDTA. Aggregation-competent M 2 cells suspended in F_{ab}, were spread on cover slips and photographed after 2–3 h of incubation in moist chambers. (A) Control: Stream formation in 8 mg/ml nonimmune F_{ab}. Terminal cell contacts as well as side-by-side contacts are formed. (B) Complete inhibition of cell agglutination in 4 mg/ml aggr-F_{ab}. Being unabsorbed, this F_{ab} blocks both cs-A and cs-B. (C) Terminal cell contacts in 8 mg/ml cs-B-specific F_{ab}. Cells are assembled tip-to-tip into rosettes, cell chains, and reticulate patterns. (D) Terminal cell contacts in 1×10^{-2} M EDTA. Patterns are similar to those in C. Cell endings are often stretched into thin processes, indicating strength of adherence at circumscribed areas of the cell surface. (E) Irregular, or side-by-side arrangement of cells in 8 mg/ml cs-A-specific F_{ab}. Only loose cell groups are formed.
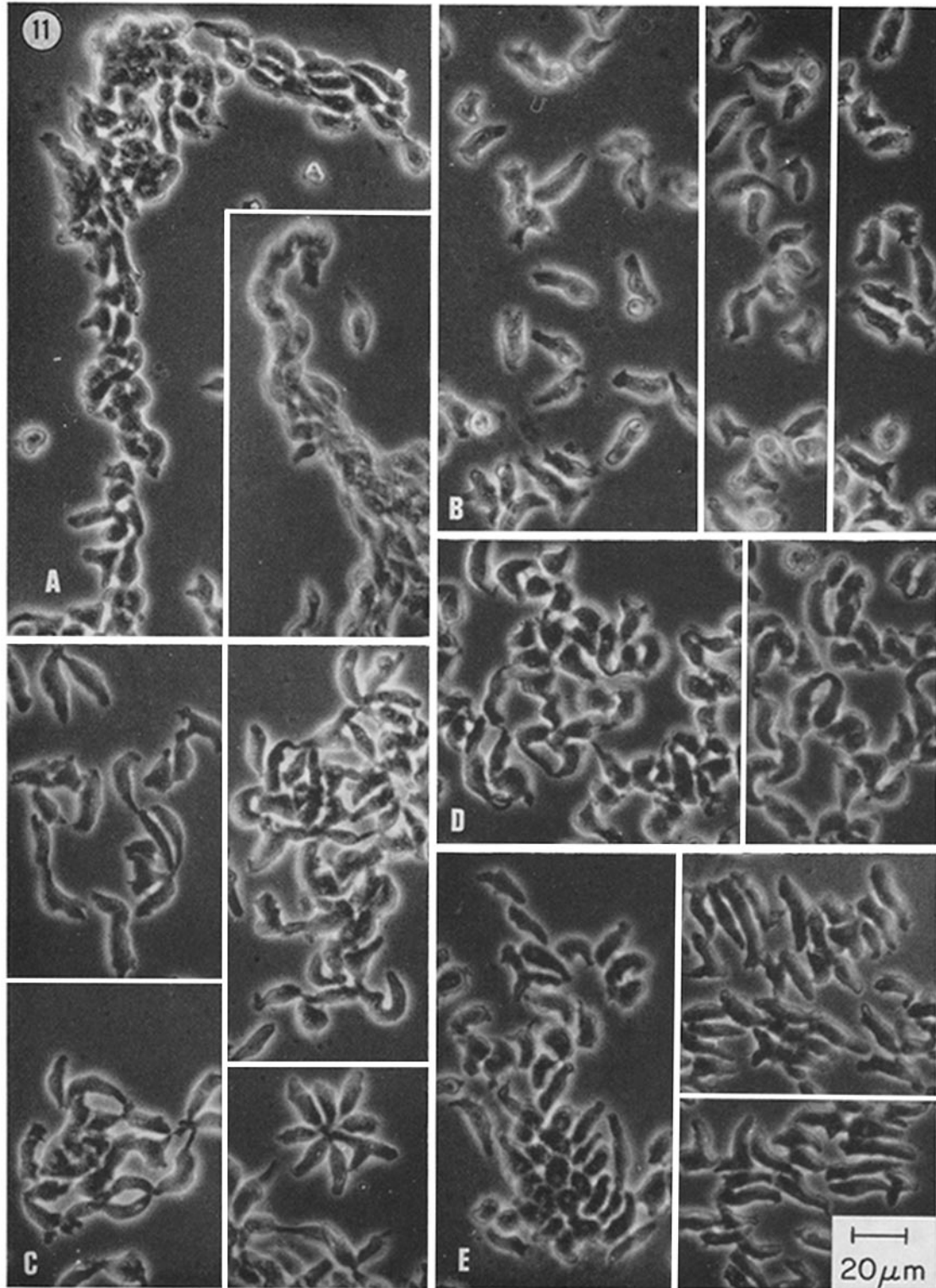
The increase of particle-bound cs-A during cell development parallels that for EDTA-stable cell adhesion, both rising sharply just before full aggregation-competence (Fig. 8). This demonstrates that commencement of aggregation is preceded by a period of intense membrane transformation, characterized either by insertion of cs-A as new constituents into the membrane, or by exposure or activation of these sites.

Species specificity of cs-A was demonstrated by absorption with particles from aggregation-competent cells of *P. pallidum* (Fig. 7), a species that does not coaggregate with *D. discoideum* (20).

Persistence of cs-B during Cell Development

The finding that cs-B are present in particles from both growth-phase and aggregation-competent cells, does not exclude quantitative changes of these sites during development. To quantitate cs-B, growth-phase cells were used as test cells. Because these cells contain practically no cs-A, only cs-B-specific F_{ab} inhibits their agglutination. Therefore, any F_{ab}-preparation containing cs-B-specific F_{ab} can be used in agglutination-inhibition tests. We have used aggr-F_{ab} for absorption to assay cs-B in particles.

Particles from cells harvested at different stages between growth-phase and aggregation-competence revealed no significant change in their cs-B-content (Fig. 8). This emphasizes that cs-A are regulated independently from cs-B during development.

Assay of cs-A and cs-B in an Aggregation-Deficient Mutant

Mutant aggr 50 formed only loose rounded cell groups on agar plates, without any indication of streamlike cell association. In suspension culture this mutant did not acquire EDTA-stable cell agglutination (Fig. 9), indicating a defect in cs-A. This was confirmed by F_{ab}-absorption, which revealed no significant amounts of cs-A in particles from two mutant clones (Fig. 10). In a revertant, selected by its capacity to aggregate and to form fruiting bodies, both EDTA-stable cell adhesion and absorption of cs-A-specific F_{ab} corresponded to the wild-type (Figs. 9 and 10).

In the absence of EDTA, aggr 50 cells agglutinate (Fig. 9), suggesting cs-B to be active in this mutant. Accordingly, no significant difference between wild-type and aggr 50 cells was found

when particle fractions were assayed serologically for cs-B (Fig. 10). These results further support the idea of two independent classes of membrane components engaged in cell adhesion.

Patterns of Cell Assembly in Presence of Different F_{ab}-Species

To determine if both cs-A and cs-B are relevant for wild-type aggregation patterns, cell aggregation was observed in the presence of either cs-A-specific or cs-B-specific F_{ab}. In contrast to aggr-F_{ab}, which inhibited cell aggregation completely (Fig. 11 B), blockage of only one class of contact sites allowed the formation of cell assemblies that showed selective aspects of normal cell aggregation (Fig. 11 A).

cs-B-specific F_{ab} suppressed cell adhesion over the entire cell surface except at the tip and rear ends where cells adhered strongly to each other (Fig. 11 C). A preference for end-to-end contacts was also observed when cells were incubated with EDTA instead of cs-B-specific F_{ab} (Fig. 11 D), as expected from the EDTA-sensitivity of cs-B.

With cs-A-specific F_{ab}, cells formed only loose associations adhering to each other at any part of their surface: side-by-side or with irregular orientation (Fig. 11 E).

DISCUSSION

Previous studies have shown that the action of univalent antibody fragments (F_{ab}) on the surface of aggregating *Dictyostelium* cells is specific in two respects: (a) with F_{ab} of a given antiserum it is possible to block intercellular adhesion completely while leaving the receptor system for chemotactic signals intact, (b) antibodies against certain carbohydrate antigens, although binding to the cell surface, do not inhibit aggregation (5). These results indicate that F_{ab} must bind to specific molecules of the cell surface in order to block cell adhesion, and, being bound to those structures, selectively blocks this function. We referred to the target antigens of antibodies inhibiting cell-to-cell adhesion as "contact sites." This term is not intended to imply a definite mechanism for the action of these sites in binding cells together; nevertheless we prefer the idea that contact sites act as cell-to-cell receptors or cell ligands (1, 2, 21).

The present quantitative study of contact sites revealed two classes of these sites differing with respect to developmental dynamics and pattern

of cell assembly caused by them. The principal result is that one class of these sites (cs-A) is incorporated into the membrane, or exposed or activated there, shortly before commencement of aggregation-competence, whereas the quantity of the other sites (cs-B) remains virtually constant during development.

Species-specificity and developmental dynamics of cs-A resemble that of a particle-bound antigen studied by Sonneborn et al. (9). Possibly this antigen is identical with cs-A, although for identification it would be necessary to exclude other membrane components, e.g. a cyclic-AMP-phosphodiesterase, the activity of which follows a similar developmental program (17, 22). Tyler (1) and Weiss (2) have postulated pairs of complementary cell surface structures which by interaction attach tissue cells to each other. An interesting version of this receptor-antireceptor concept is the hypothesis of Roseman (23), who attributes this function to the interaction of surface-bound glycosyltransferases with glycosyl-acceptors of oligosaccharide nature (24, 25). cs-A and cs-B do not correspond to the postulated pairs of complementary sites because they can be selectively blocked, and consequently function independently of each other. It remains open, however, whether cs-A and cs-B each consist of two different molecular species representing complementary structures in the sense of receptors and antireceptors, or whether these contact sites interact with complementary structures as yet undetected.

Our results do not establish that the binding sites for agglutination-inhibiting antibodies are identical with, rather than topographically or functionally linked to, membrane sites that function in cell adhesion. The strict correlation during development between EDTA-stable and EDTA-labile cell associations on one hand and detectability of cs-A and cs-B on the other, argues in favor of an identity. This is emphasized by the finding that in mutant aggr 50 the absence of stream formation is correlated with the absence of cs-A.

Taking identity as granted, cs-A are involved in polar cell adhesions, causing the cells to aggregate in chains. It remains open whether incorporation of cs-A into the membrane is spatially differentiated, or whether these sites become preferentially activated at the actual cell poles. The polar cell adhesions caused by cs-A are of principal morphogenetic relevance, since the

formation of long streams of cells, which in typical aggregates are radially orientated around centers, depends on them.

cs-B assist cs-A in stream formation by laterally binding cell chains together. The contact sites are then molecular determinants for supercellular pattern formation. In order to understand more complicated multicellular fabrics, it is important to realize that a cell can simultaneously carry different contact sites capable of functioning independently of each other.

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