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The Contribution of the Calcium-Dependent Interaction of Aggregation Factor Molecules to Recognition: A System Providing Additional Specificity Forces?

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Cells from the sponge Microciona prolifera display on their surfaces large but defined proteoglycan complexes (Microciona aggregation factor = MAF) that mediate species-specific cell aggregation by a process requiring high calcium ion concentrations. An analysis of MAF-MAF interactions based on binding studies of MAF to glutaraldehyde-fixed sponge cells and MAF-derivatized beads demonstrates that the requirement for high calcium concentrations can be overcome by extremely small amounts of certain polycations such as polybrene, polylysine, or histones. For measurements of the affinity of these substances to MAF, a method was adopted that partitions ^{125}I-labeled MAF between dextran and polyethyleneglycol in an aqueous two-phase polymer system depending on the net charge of the complex formed.

Since only polymers of positive charges affect binding and partitioning at low concentrations, large areas of interaction similar to those found in glycosaminoglycans are proposed for MAF. Through a multitude of appropriately spaced interaction sites, the rather weak selectivity of single charged sites could in such a system still provide strong enough specificities to explain species-specific cell sorting.

The biological significance of naturally occurring polycations as well as extracellular calcium includes their role in cell recognition, sorting out as well as the ordered and continual streaming movements of groups of cells seen in the mesohyl of live sponges.

Key words: cell recognition, sponges, aggregation factor, species specificity, proteoglycan, extracellular matrix

Species-specific reaggregation of sponge cells was studied as early as 1907 by Wilson [1]. Humphreys [2] showed that a molecule mediating reaggregation is released from the surface of Microciona prolifera cells in calcium- and magnesium-free seawater. This Microciona aggregation factor (MAF) was found to be a huge but well-defined proteoglycan complex with a high content of uronic acids and hexosamines [3, 4]. In the electron microscope it has a "sunburst" configuration with a central ring 800 Å in diameter and about 15 radiating arms, each about

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1,100 A in length. Mueller et al [5] provide in an accompanying paper an interesting description of a similar size aggregation complex from yet another marine sponge (Geodia cydonium).

MAF-depleted cells do not aggregate in the presence of calcium. Readdition of the aggregation factor restores the capability of the cells to aggregate. A surface site called baseplate, which can be liberated by mild hypotonic treatment [6], was postulated to be the binding site for MAF. The question arose whether the specificity for cell recognition among marine sponges was provided by the interaction between MAF molecules, by MAF-baseplate interaction, or by both. It could be shown [7] that MAF-cell surface interaction could be operationally separated from MAF-MAF interaction simply because the former was calcium independent, whereas the latter had the same dependence on calcium concentrations as did the sponge aggregation reaction (see Fig. 1)

Since the half-optimal calcium concentration for MAF-MAF interaction did not only coincide with that for MAF bead aggregation and sponge aggregation but also the normal calcium level of seawater, we postulated that calcium provides the ligative forces for sponge aggregation factor in vivo. The calcium-independent binding of MAF to the sponge cell surface could be shown to display a high species specificity. At first glance one would believe that this reaction would suffice

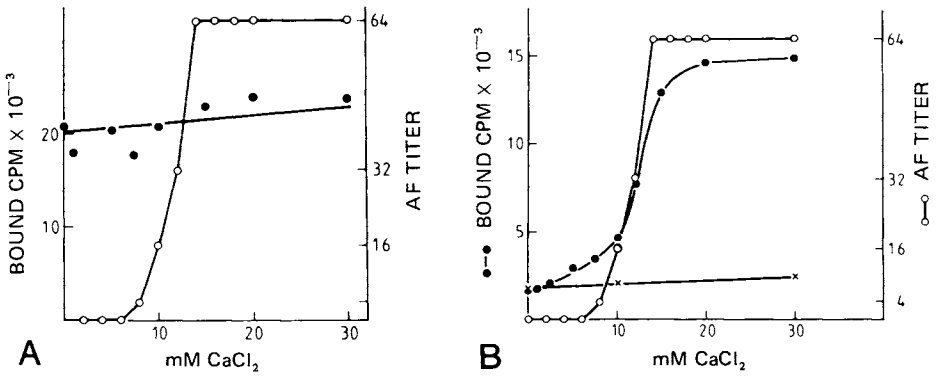

Fig. 1. A. Effect of Ca^{2+} on [^{125}I] AF binding to cells and on AF-mediated cell aggregation. ^{125}I-labeled aggregation factor (1 unit/ml, 2.1 × 10^6 Ci/mol) was incubated with 4 × 10^6 glutaraldehyde-fixed, chemically dissociated cells in CMFT with added $CaCl_2$ at the concentrations shown. After 20 min of incubation, binding of [^{125}I] AF to cells was determined as described under Materials and Methods. The Ca^{2+} dependence of AF-promoted cell aggregation was determined separately. Unlabeled AF (64 units/ ml) was serially diluted into wells containing 0.2 ml volumes of Ca^{2+}-supplemented CMFT at twice the Ca^{2+} concentrations shown. The assay was started by adding fixed, chemically dissociated cells in CMFT, and the end point at each Ca^{2+} concentration was read after 20 min. (○) Counts per minute of [^{125}I] AF bound; (●) AF titer [7]. B. Ca^{2+}-dependent binding of [^{125}I] AF to AF-conjugated beads. AF-coupled beads or BSA-coupled control beads (~ 10^6/mL) were incubated with ^{125}I-labeled aggregation factor (1 unit/ml, 2 × 10^6 Ci/mol) in CMFT supplemented with $CaCl_2$ at the concentrations shown. Following the 20 min-incubation in the standard assay, beads with bound [^{125}I] AF were separated by centrifugation and counted [see 7]. The Ca^{2+} dependence of AF-promoted cell aggregation, shown above in Figure 1a, is shown again in this figure. (●) [^{125}I] AF bound to AF beads; (X) [^{125}I] AF binding to BSA (control) beads; (○) AF-mediated cell aggregation (AF titer) in response to the respective Ca^{2+} concentrations (from [7]).

to explain species-specific sorting out and aggregation of marine sponges, but it does not. Sponge cells can be separated from each other by mechanical means, which leaves the individual cell with a large amount of MAF on its surface. Since these cells already have their species-specific aggregation factor, and since they sort out, and in some cases aggregate even species specifically, some other explanations will have to be found. Two are given here without being followed up, since they will be quite difficult to test in an unambiguous way. The third one was subjected to some preliminary tests in this paper.

1. MAF, which is only able to bind species specifically in the absence of calcium, ie which has monovalent character in the absence of calcium, may become multivalent in the presence of calcium, and can now cross-link species-specific baseplate sites on opposite cells.
2. Baseplate type of molecules may occur in loose aggregates between cells and may mediate contacts between aggregation factor molecules of the same species on opposite cells.
3. As a working hypothesis, we would like to consider here the possibility that repetitive sequences of weak interactions between sponge factor molecules may build up respectable specificities and respectable forces contributing to the species-specific aggregation and sorting out phenomenon.

As a prerequisite for the possibility that weak forces mediated by calcium or polycations could govern MAF–MAF interactions, it has to be shown that the MAF–complex displays large areas suitable for interactions. Since we have only one aggregation factor of reasonable purity in our hands for the time being, we do have to wait with binding and sorting studies until we have two well-defined aggregation factors from different sponge species.

The large size of the MAF complex of about 21×10^6 daltons excludes the use of several standard techniques. By using ^{125}I-labeled MAF first in binding assays with MAF-coated beads and MAF-depleted, glutaraldehyde-fixed sponge cells, and second in an aqueous dextran/polyethyleneglycol two-phase system, we attempted to shed some light on the nature of MAF–MAF interaction. It could be shown that by adding polycations, both MAF binding to beads or cells and the partition of MAF in the two-phase system are affected at concentrations several magnitudes lower than the concentration of calcium (compared on the basis of charge concentrations) required for the same phenomena. We consider these effects as an indication for a type of binding involving large areas of MAF–MAF interaction exposing a multitude of negative charges, which have to be counterbalanced by calcium ions or a polycation with similar spacing of counterions.

MATERIALS AND METHODS

Sponges

Live specimens of Microciona prolifera were collected near Woods Hole, Massachusetts, by the supply department of the Marine Biological Laboratory. Sponges were maintained in tanks of running seawater as long as 1 week.

Buffers

Bicarbonate buffered artificial seawater (MBL-ASW) was prepared according to the Marine Biological Laboratory formula [8]. Calcium- and magnesium-free artificial seawater (CMF-ASW) was made as described by Humphreys [2].

Dissociated Sponge Cells

Suspensions of so-called chemically dissociated sponge cells were obtained by soaking sponge tissue fragments in cold bicarbonate buffered CMF-ASW, followed by gentle squeezing of the fragments through 25 μm mesh nylon cloth. Incubation in CMF-ASW for several hours on a rotary shaker in the cold resulted in the removal of most of the surface associated MAF.

Fixation of sponge cells with 1% glutaraldehyde was performed as described by Jumblatt et al [9].

Purification and Radioiodination of Microciona Aggregation Factor (MAF)

MAF was purified from the supernatant of chemically dissociated cells as described elsewhere [7]. Isolated MAF was kept at 4°C in 0.02 M Tris buffered CMF-ASW pH 7.2 containing 2 mM $CaCl_2$ (Ca-CMFT) and 0.05 NaN_3. One microgram protein (BSA standard) contained 1.2–1.7 units of MAF. By definition, one unit MAF/ml is the smallest amount giving the macroscopically visible cell aggregation under standard assay conditions [7].

Labeling with ^{125}Iodine (NEN, carrier free) was performed on ice. One thousand units (800 μg) of MAF were dissolved in 1.5 ml Ca-CMFT and mixed with 2–5 mCi $Na^{125}I$ in 4 μl and 37.5 μl 10 mM chloramine T (Eastman) in H_2O. The reaction was terminated after 5 minutes by addition of 37.5 μl 20 mM sodium metabisulfate. The mixture was passed through a Sepharose 4B column (8 ml), the void volume collected and purified by sedimentation in a sucrose gradient. Finally, the peak fraction was dialzyed against Ca-CMFT [7]. No loss of aggregation activity normalized to protein was detectable. The specific activity was approximately 3×10^4 Ci/mmole or 1.4 μCi/μg protein.

Conjugation of MAF and BSA to Sephadex Beads

Superfine Sephadex G 25 beads (Pharmacia) were activated with CNBr as described by Cuatrecasas and Anfinson [10]. Then, 2.5 ml of packed beads was suspended in 5 ml CMF-ASW containing 2 mM $CaCl_2$, 0.1 M $NaHCO_3$ (pH 8.3), and 8 mg of MAF. Coupling was performed overnight at 4°C with gentle shaking. The reaction was stopped by washing with CaCMFT on a glass filter followed by incubation with 0.1 M glycin to saturate the remaining reactive groups. As a control, BSA-conjugated beads were prepared identically using 1 mg BSA/ml in place of the MAF.

^{125}I-MAF-Binding Assay

^{125}I-labeled MAF plus cells or beads were incubated together with different concentrations of cations under standard aggregation assay conditions [2, 11] in 16-mm wells (Falcon Multiwells, 3008), final volume 400 μl, on a rotary shaker. Following incubation, suspensions were transferred to the top of a cushion of 1 ml of 10% sucrose and 0.1% BSA in Ca-CMFT or MBL-ASW, respectively, in Eppendorf centrifuge tubes and centrifuged for 5 minutes at 1,000g. The supernatant was aspirated, and the tube containing the pellet of cells or beads was counted in a Packard Tri Carb Gamma Counter. To determine specific binding, the radioactivity in controls without cells or beads was subtracted from the experimental values.

Two Polymer Aqueous Phase Systems

The preparation of two-phase systems of dextran T500 Pharmacia (lot 4094) and polyethylene glycol 6000 (BDH Chemicals) was adapted from Albertson [12]. In short, the systems contained 5% (w/w) dextran, 4% (w/w) polyethylene glycol, and NaCl to make them isotonic to seawater. For charged systems NaCl was replaced by sodium phosphate (pH 7) or Na_2SO_4. To stabilize the pH in phosphate-free systems, 5 mM Tris (Sigma) pH 7 was added. After separation of the mixture into a dextran-rich bottom and a polyethylene glycol-rich top phase, 2 ml of both phases was put into 15-ml tubes, ^{125}I-labeled MAF, and eventually polycations or anions were added, and the entire mixture was well agitated. The phases are then permitted to settle. An aliquot of each phase was sampled and counted in a Packard Tri Carb Gamma Counter. All separations were performed at RT.

RESULTS

Binding of ^{125}-Labeled MAF to MAF Derivatized Beads

Calcium dependence of MAF–MAF interaction was shown by Jumblatt et al [7]. In Table I this dependence was corroborated with Sepharose superfine beads in order to increase the MAF-coated surface area and hence minimize a localized MAF enrichment, which could lead to the possibility of MAF self association. The MAF concentration was 0.02 units/ml (0.015 μg/ml), or 50 times lower than the minimal amount needed for macroscopically visible cell aggregation which by definition is 1 unit/ml. It was quite interesting to observe that the addition of only 10 μg/ml of the polycation polybrene resulted in a strong increase of MAF–MAF interaction and that this effect was absolutely independent of the presence of calcium. As a control, binding of MAF to BSA-derivatized beads was assessed. Both with and without calcium, affinity of MAF to BSA is very low. As opposed to MAF beads, the binding of MAF to BSA beads is only slightly affected by the polycation, thus ruling out unspecific charge effects.

Based on this strong effect by an artificial polycation it became of interest to check whether naturally occurring polycations would also exert an effect on MAF–MAF binding. The high content of basic amino acids in histones gives this class of proteins qualitatively similar charge characteristics as polylysine. The charge density is admittedly lower, but the charge spacing in polylysine is very

TABLE I. Binding of ^{125}I-Labeled MAF to MAF- and BSA-Derivatized Beads

	No calcium		10 mM calcium	
	cpm	%	cpm	%
MAF beads	124	7.3	1,688	100
BSA beads	42	2.5	67	4.0
Plain beads	6	0.4	93	5.5
MAF beads + 10 μg/ml Polybrene	1,408	83.4	1,368	81.1
BSA beads + 10 μg/ml Polybrene	318	18.8	87	5.1

MAF concentration in all experiments was 0.02 units/ml or about 0.015 μg/ml. Bead concentration was 4 × 10 beads/ml. The background of 452 cpm (no beads) was subtracted. The binding to MAF-derivatized beads in the presence of calcium was taken as 100%.

narrow and not optimal for interactions with MAF, as will be shown later. Figure 2 shows that histones also enable MAF molecules to bind to MAF-derivatized beads in the absence of calcium. The concentrations needed are very low. To achieve binding with calcium, concentrations more than 3 orders of magnitude higher (based on the amount of positive charges) are needed. The magnitude of the effect made us determine the concentration dependence of the effect of polycations, and, as a control, polyanions on MAF–MAF interaction. To enhance the surface available for binding of MAF, fixed Microciona prolifera cells were used. As recently shown by Jumblatt et al [7], these cells bind MAF onto their receptors in the absence of calcium. Any ^{125}I-MAF binding, in addition to the first layer, which is complete with about 2,500 cpm in this particular assay system used, is due to MAF–MAF interactions. Figure 3 shows that Polybrene increases MAF binding at concentrations below 1 μg/ml. For polylysine, higher concentrations are needed, whereas spermidine, having only three charges per molecule, does not exert any effect. Polyanions, like chondroitin sulfate or hyaluronic acid, do not affect MAF binding in the concentration range tested (Fig. 3). The fact that the base level of binding that is due to receptor–MAF interactions stays constant is indirect evidence that no negatively charged polyanionic MAF regions are involved in MAF binding to the cell surface. Earlier results [6] suggest a lectin-like interaction at this level. Control experiments in the presence of calcium with all four polyions (10 μg/ml) showed only slight differences compared to the calcium-free system. Polybrene without cells is not able to bring down any labeled radioactivity, and therefore excludes unspecific precipitation of MAF.

Although all the above binding studies were made with low MAF concentrations, much below the level needed to get macroscopically visible cell aggregation, it cannot be excluded that at the surface of cells or beads heterogeneous high-density patches occur where the radioactive MAF can accumulate locally and lead to uncontrollable MAF self-association, which will obscure the affinities of single molecules of MAF. One way to solve this problem is to measure binding of charged substances to MAF by monitoring the influence of such molecules on the partition of labeled MAF in an aqueous two-phase system.

Influence of Polycations on the Phase Partitioning of MAF in a Dextran/Polyethylene Glycol System

The distribution of a macromolecular structure in an aqueous two-phase polymer system is influenced by the affinities of the structure to dextran and polyethylene glycol, and by charge effects that can be controlled by anions like phosphate or sulfate. These anions produce an electrostatic potential difference between the two phases [13]. Seventy-five percent of ^{125}I-labeled MAF is distributed in the lower (dextran) phase of a system containing only NaCl as a salt (Fig. 4; ie at 0 sulfate (S) and 0 phosphate (P)). In the presence of phosphate or sulfate, which by their slightly uneven phase distribution shift polyanions to the upper phase, MAF is brought to the polyethylene glycol phase (Fig. 4), thus proving its polyanionic characteristics at neutral pH.

Phase partition can also be used to quantify interactions between MAF and other molecules. If MAF interacts with molecules having a different partition coefficient, the partition of the labeled MAF complex should be different from the distribution of the individual compounds involved alone. Indeed, the addition of

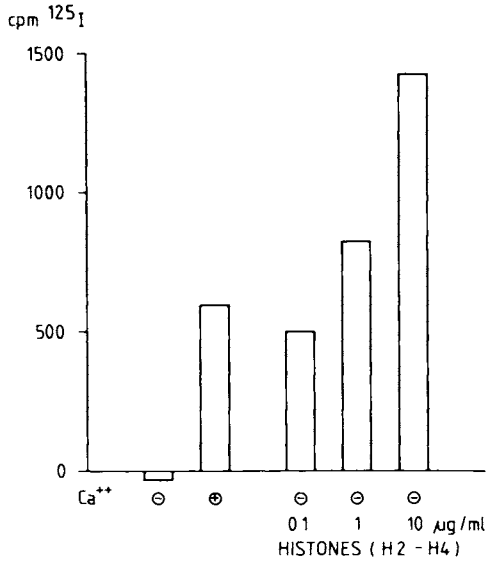

Fig. 2. Influence of histones on binding [^{125}I] labeled MAF to MAF-derivatized Sephadex superfine beads (MAF-SF beads). ⊖ No calcium; ⊕ 10 mM calcium. Concentrations: 0.02 units MAF/ml; 4 × 10^5 beads/ml. For more details see Methods.

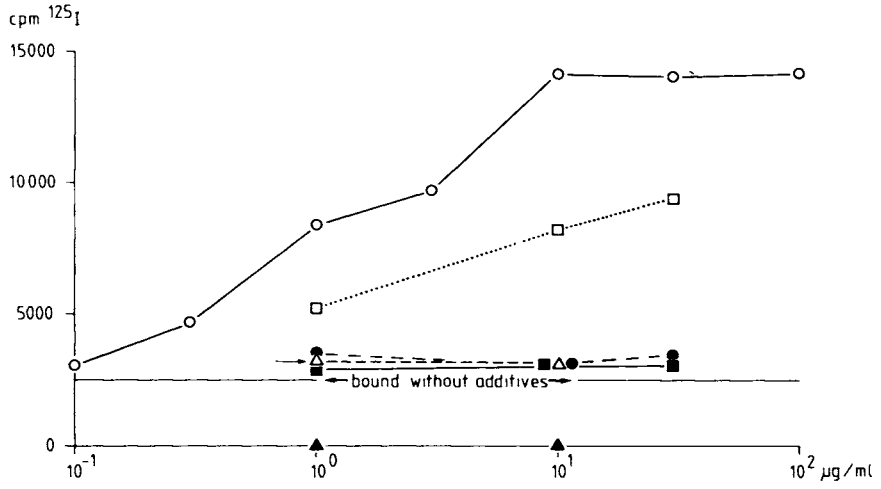

Fig. 3. Influence of different concentrations of polycations and polyanions on binding of MAF to glutaraldehyde fixed Microciona prolifera cells. Ordinate: [^{125}I] labeled MAF in cell pellet (cpm). Abscissa: concentration of polycations of polyanions (µg/ml). ○ — ○ Polybrene; □·····□ polylysine; △--△ spermidine; ●-•-● chondroitin sulfate; ■ — ■ hyaluronic acid; ▲ — ▲ polybrene without cells.

polylysine (Sigma Typ 1B, MW 70,000) reverses the partition of MAF totally (Fig. 4). The lowest amount of phosphate or sulfate tested brings all the MAF molecules into the lower phase. Histones (calf thymus, Calbiochem) used here as a more physiological polycation show a qualitatively similar interaction with MAF. Although a more highly charged system is needed to shift the radioactive MAF to the bottom phase, the overall behavior of histone/MAF complexes is reciprocal to MAF alone. This indicates that both polylysine and histones bind to such an extent to MAF that the negative charge of the MAF molecule is more than balanced, leading to a complex having a positive net charge.

Polybrene, showing the strongest effects in binding studies, had a more complex effect. Over the entire concentration range of phosphate and sulfate tested, Polybrene decreased affinity differences of the MAF complexes between the two phases compared to MAF alone and brought the partition coefficient near 1 (Fig. 4). Although it is at first glance surprising that an agent mediating MAF-MAF interactions should show such a behavior, the unexpected partition results can be explained by assuming that Polybrene binds to MAF with a high affinity, and that it neutralizes the negative charges without leading to a complex with a net positive charge. Only such more or less neutral complexes lacking repellent forces are able to associate and to form a MAF-matrix.

Concentration Range of Polycations and Calcium Showing Effects on MAF Partition

Unfortunately, the binding of calcium to MAF in a charged partition system cannot be measured directly, owing to the low solubility of $CaSO_4$ and $CaHPO_4$. However, since partition of MAF is also influenced by cations in a system containing only NaCl (left end of the curves in Fig. 4), it is possible to quantify calcium binding to single MAF molecules in such an uncharged system. Figure 5 shows that only above a concentration of 10 mM calcium is the partition of MAF influenced. This finding agrees well with the results from the binding assays [7], showing half-maximal MAF-MAF interaction only at and above a calcium concentration of 10 mM. Polylysine and histones exert their effects at much lower concentrations. At 0.1 μg/ml for polylysine and at 1 μg/ml for histones, the partition of MAF is strongly affected (Fig. 5). At higher concentrations the MAF-polycation complex shows a partition similar to the polycations alone, thus indicating again that the net charge of these complexes is highly positive. Polybrene displays effects at still lower concentrations (0.01 μg/ml). Nevertheless, the effects over the whole concentrations range tested are never as pronounced as with polylysine. This result is further support for the notion that, although Polybrene binds with high affinity to MAF, the smaller charge density of this polycation prevents the complexes formed to become heavily positively charged.

A comparison of the concentrations of positive charges in solutions containing 30 mM calcium or 0.1 μg/ml polylysine or 0.1 μg/ml Polybrene — which all exert the same effect on MAF partition — shows a charge concentration of 60 mM for calcium but only 0.78 μM for the polycations. Cooperativity between the charged groups of the polycations is the most probable explanation, since the single cation binding site of MAF shows a fairly high specificity for calcium. Magnesium, for example, cannot substitute for calcium. However, we have to assume a reciprocal arrangement of negative charges of glucuronic and/or aspartic acid on the side of the MAF molecules corresponding to the Polybrene charge spacing.

Influence of Polybrene on Cell Aggregation and Tissue Spreading

In the living system Polybrene enhances MAF-mediated aggregation of dissociated sponge cells in the absence of calcium. The effect is rather transitory and observed only during the first minutes of the assay.

Additional processes needed to stabilize the aggregate and involving the action of live cells [14] seem to need calcium also. In the presence of calcium, Polybrene (20 μg/ml) has no apparent effect on aggregation and rounding up of the aggregates. However, if after 12 hours on the rotary shaker the aggregates are allowed to stand and settle, attachment to the dish, fusion of the single balls of

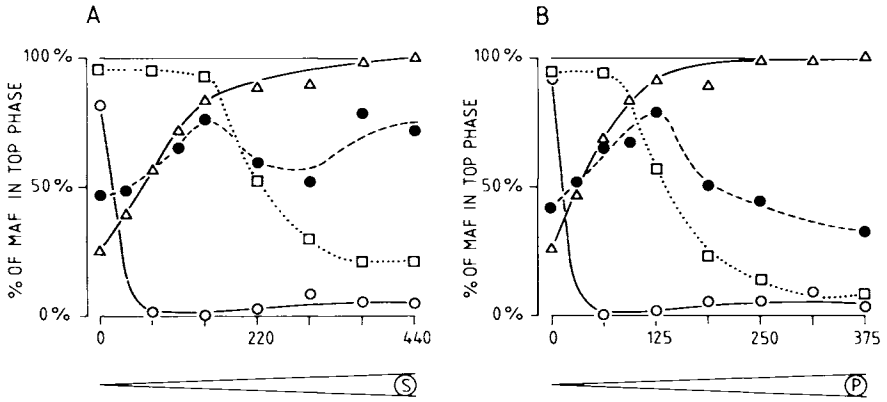

Fig. 4. Influence of polycations on MAF partition in dextran/polyethylene glycol aqueous phase systems. Concentrations: MAF, 0.02 units/ml; polycations, 10 μg/ml; salts isotonic to seawater; pH 7. From left to right NaCl is increasingly replaced by sulfate (A) or phosphate (B), making the systems more charged. Ordinate: % of radioactivity (^{125}I-MAF) in upper phase. Abscissa: A, sulfate (mM); B, phosphate (mM). △--△ MAF; ○—○ MAF + polylysine; □····□ MAF + histones; ●--● MAF + Polybrene.

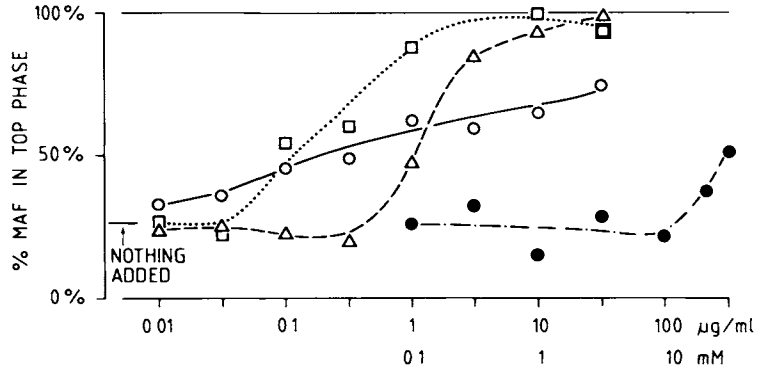

Fig. 5. Influence of polycation and calcium concentration on partition of ^{125}I-MAF in a dextran/polyethylene glycol system. Ordinate: % of [^{125}I]-labeled MAF in upper phase. Abscissa: Polycation concentration in μg/ml calcium concentration in mM. □····□ Polylysine; △--△ histones; ○—○ polybrene; ●—•—● calcium.

cells, and spreading proceeds very rapidly in a control culture, but is totally inhibited in the presence of 2 μg/ml Polybrene (Fig. 6). This inhibition of fusion and spreading is fully retained over several days (Fig. 6f). Survival of cells seems not to be impaired. At present we can only speculate about possible effects of Polybrene on such a multistep event like cell aggregate fusion or spreading.

DISCUSSION

MAF has been shown to contain two different classes of binding sites, one for the interaction with the cell surface (MAF–baseplate interaction), and a second class for the calcium-dependent MAF–MAF polymerization [7]. We report here

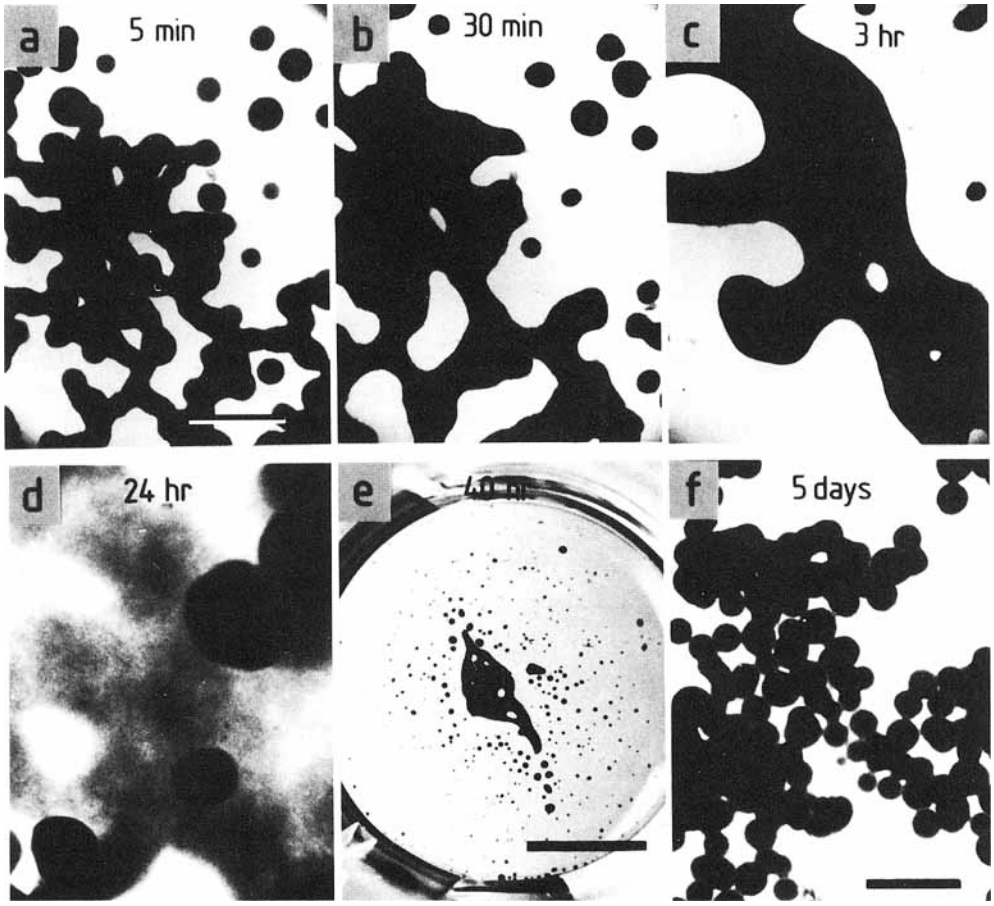

Fig. 6. Fusion and spreading of Microciona prolifera cell aggregates and the effects of Polybrene. Dissociated cells were kept for 18 hours on a rotary shaker leading to small rounded aggregates similar to (f). Minutes and hours indicate time since termination of shaking which allows fusion, attachment to the dish, and, later on, spreading. a, b, c, d) Control culture; bar 250 μ m. e) Overview of control culture; bar 5 mm. f) Well containing 2 μ g/ml Polybrene and showing neither fusion nor spreading 5 days after termination of shaking. Bar 250 μ m.

evidence that large areas of interaction are involved in the latter binding type, and that calcium can be substituted by very low amounts of certain polycations. Since the molecular weight of the MAF molecule of 21×10^6 daltons does exclude analysis by standard polyacrylamide electrophoresis or isoelectric focusing, an aqueous two-polymer phase system composed of dextran and polyethylenglycol was used to determine affinities of cations to MAF and the net charge of the complex formed. The partition and binding studies suggest that the polycation Polybrene, which displays a lower density of positive charges than, for example, polylysine, fits best to neutralize and and cross-link MAF, which is heavily charged owing to glucuronic acid and aspartate residues. The fact that fewer charges of Polybrene are needed as compared to calcium to bind to MAF or to mediate MAF–MAF interaction suggests cooperative effects. Only large regions of MAF molecules with a certain kind of repetitive arrangement of negative charges can provide the basis for such an effect. The inability of divalent cations like Mg^{++}, Sr^{++}, Ba^{++}, Cr^{++}, and others to substitute for Ca^{++} and to promote cell aggregation [15] is further evidence for the specific make-up of the charge binding sites on the MAF molecule needing the specific cation calcium or appropriately spaced polycations. Similar specificities of interactions were recently found by Turley and Roth [16], who observed the agglutination of hyaluronic acid derivatized beads with chondroitin-6-sulfate derivatized beads. The interaction is quite specific, chondroitin-4-sulfate being not able to substitute for chondroitin-6-sulfate, and appears to occur between the carbohydrate chains. Mechanisms suggested for such binding between sugar chains are aggregated helices and/or interactions of specialized regions leading to "egg box" structures [17].

We postulated a few years ago that a multitude of weak interactions may be the basis of strong and specific cellular interactions [18]. At that time we were concerned with interactions between MAF and the baseplate. It was shown recently, however, that large molecules like glycosaminoglycans with highly repetitive sequences can, through a multitude of weak interactions based on evenly spaced charges or dipoles, provide the force for the sorting out of cells [19].

Müller et al [5], who were able to enhance reaggregation of Geodia cydonium cells not only with polylysine but also with spermidine and putrescine, suggest that the cations bind between aggregation factor and aggregation receptor. In our system, MAF–cell interaction is clearly calcium-independent [7], and spermidine, which enhances the stickiness of most cells, does not influence MAF binding to the cell receptor (baseplate) and it does not influence MAF self-association. At this stage, it is not yet possible to compare these results obtained with different sponge species and, probably more important, different assays.

The mechanism of binding between MAF molecules is not known. Calcium and polycations could form bridges between different molecules (Fig. 7), or they could act only by neutralizing the net charges of MAF structures, thus allowing nonionic interactions of fitting regions to be either homologous or heterologous. If the rigidity of primary aggregates of cells formed in the presence of Polybrene is due to impaired "sliding" of MAF structures past each other, the first hypothesis has to be favored.

The demonstration by Steinberg [20] and his group, Magnani et al [21], as well as by Takeichi et al [22] of two separable mechanisms of adhesion in mammalian cells, a calcium-dependent and a calcium independent-one, which both are

able to segregate heterotypic cells, provides another example that highly specific lock key interactions between cells may not necessarily be the only force in cell adhesion and sorting. All the evidence from other systems for the existence of low specificity mechanisms for cell-cell interaction suggests the working hypothesis that similar interactions may play a role in sponge cell interaction and MAF-MAF binding. Although the calcium-independent MAF-cell interaction is highly species specific, it does not yet lead to aggregation. Two explanations for species-specific sponge cell aggregation using the lock and key mechanism are provided in the introduction. The data provided here allow the suggestion of a third explanation summarized in the following working hypothesis.

Macromolecules having evenly spaced dipoles, charges, or otherwise effective groups tend to sort out and to enrich themselves in distinct phases. Slight differences in the spacing of the glucuronic acids present or other potential interaction sites between the aggregation factors of different sponge species could result in a calcium-dependent aqueous two-polymer phase system displaying preferential affinities between cells. Although the single interaction in such a system can at best be only partially selective and by no means specific, the display of a multitude of such factors on the surface of adjacent cells can result in big energy differences between homologous and heterologous cell-cell interactions. This applies to any poor affinity link with a high valency [18]. Calculations on mammalian cells made by Edwards [19] showed that such mechanisms as were postulated a while ago by Steinberg [20] could explain sorting in cellular systems. To what degree they involve carbohydrate and protein or protein-protein interactions is an open question. All three kinds may be used in different systems.

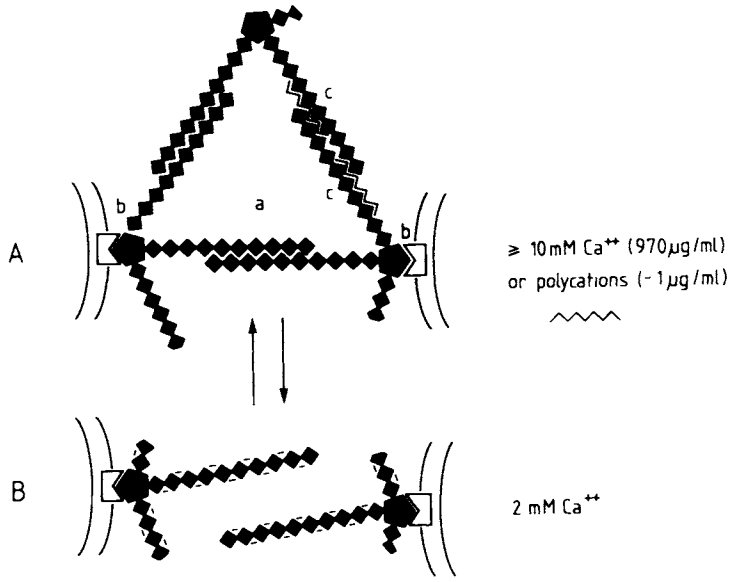

Fig. 7. Model showing proposed MAF-MAF interactions. A) At high calcium concentration strong MAF-MAF interactions (a) lead to MAF polymerization and cell aggregation. Minute amounts of polycations can substitute for calcium (c). B) MAF reversibly inactivated at low calcium concentration.

The extremely low amounts of certain polycations needed to display strong effects in vitro makes it worthwhile to speculate on possible functions of related molecules in the in vivo situation. Continually occurring injuries of the sponge surface by mechanical means (waves, animals) could lead to a release of histones from damaged cells in the traumatized region, bringing about polymerization of MAF molecules to a rigid jelly. On the other hand, polycations and probably even the modulation of the calcium concentration in the extracellular matrix, which has not been considered so far, may be used to regulate the fluidity of the mesohyl. Although all major cell types of Microciona prolifera bind MAF [23], the cells between the epithelial layers are intensely moving through the MAF matrix. Periodically, this movement is interrupted by short contractions, called "Starrungszustand" (= "in rigid state") by Ankel [24] and reviewed by Rasmont [25].

The strong effect Polybrene displays on fusion and spreading of cell aggregates could in this light be explained by the more rigid dovetailing of MAF molecules, preventing the sliding of interacting regions of two adjacent MAF complexes past each other while weaker calcium interactions still permit gliding of two cells past each other. Figure 7 depicts a model based on the proposals put forward in the present paper. The experimental data for such a mechanism are at present largely circumstantial, and remain to be supported by complementary experiments with purified aggregation factors from other sponge species.

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REFERENCES

1. Wilson HV: J Exp Zool 5:245, 1907.
2. Humphreys T: Dev Biol 8:27, 1963.
3. Cauldwell C, Henkart P, Humphreys T: Biochemistry 12:3051, 1973.
4. Henkart PS, Humphreys T: Biochemistry 12:3045, 1973.
5. Müller WEG, Zahn RK, Kurelic B, Müller I: Exp Cell Res 113:409, 1978.
6. Weinbaum G, Burger MM: Nature (Lond) 244:510, 1973.
7. Jumblatt J, Schlup V, Burger MM: Biochemistry 19:1038, 1980.
8. Cavenough GM: "Formula and Methods of the Marine Biological Laboratory," 6th ed. Woods Hole, Massachusetts: Marine Biological Laboratory, 1956, p 67.
9. Jumblatt J, Weinbaum G, Turner R, Ballmer K, Burger MM: In Bradshaw K, Frazier U, Merrel R, Gottlieb D, Hogue-Angeletti R (eds): "Surface Membrane Receptors." New York: Plenum Press, 1977, pp 73–86.
10. Cuatrecasas P, Anfinson CR: Meth Enzymol 22:345, 1971.
11. Burger MM, Jumblatt J: In Lash JW, Burger MM (eds): "Cell and Tissue Interactions." New York: Raven Press, 1977, pp 155–172.
12. Albertson PA: Endeavour 1:69, 1977.
13. Reithermann R, Flanagan SA, Barondes SH: Biochim Biophys Acta 297:193, 1973.
14. Burkart W, Jumblatt J, Simpson TL, Burger MM: In Levi C, Boury-Esnault N (eds): "Sponge Biology." Paris: Colloques Internationaux du CNRS No. 291, 1979, pp 239–246.
15. Rice D, Humphreys T: Biol Bull 155:462, 1978.
16. Turley EA, Roth S: Nature 283:268, 1980.

17. Muir H, Hardingham TE: MTP Int Rev Sci Biochem Ser 15:153, 1975.
18. Burger MM: In Karkinen M, Saxén L, Weiss L (eds): "Cell Interaction in Differentiation." New York: Academic Press, 1977, pp 357–376.
19. Edwards PAW: Nature (Lond) 271:248, 1978.
20. Steinberg MS: J Exp Zool 173:395, 1970.
21. Magnani JL, Thomas WA, Steinberg MS: Dev Biol 96, 1981.
22. Takeichi M, Ozaki HS, Tokunaga K, Okada TS: Dev Biol 70:195, 1979.
23. Burger MM, Burkart W, Weinbaum G, Jumblatt J: In Curtis ASG (ed): "Cell-Cell Recognition." Cambridge: Cambridge University Press, 1978, pp 1–24.
24. Ankel WE: Zool Anz 28:426, 1965.
25. Rasmont R: In Levi C, Boury-Esnault N (eds): "Sponge Biology." Paris: Colloques Internationaux du CNRS No. 291, 1979, p 2129.