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Biochemical and Functional Effects of Sulfate Restriction in the Marine Sponge, *Microciona prolifera*

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Abstract. The functional and biochemical consequences of sulfate restriction were studied in chemically dissociated *Microciona* sponge cells maintained in artificial seawater with or without SO_4^{2-}. In cells pre-treated to reduce preformed secretions, SO_4^{2-} deprivation reduced cell motility judged by the lack of aggregates in rotating or stationary cultures in comparison with controls. Microscopic examination showed that cells that customarily demonstrate cytoplasmic processes, such as filopodia and pseudopodia, exhibited marked decreases in these cellular processes when maintained in SO_4^{2-}-deprived artificial seawater. Uptake and incorporation of $^{35}SO_4^{2-}$ by disaggregated and pre-treated cells was higher under SO_4^{2-}-free conditions relative to controls; this effect was time dependent, rising to a maximum at 12 h, when a three- to seven-fold difference could be demonstrated. ^{3}H-leucine incorporation indicated that protein synthesis was similar in test and control populations. Comparative high voltage electrophoresis of supernatants containing $^{35}SO_4$ macromolecules from chemically dissociated cells indicated deficiencies of such $^{35}SO_4$ macromolecules if the rotated cells that released these secretions had been pre-treated in SO_4^{2-}-free artificial seawater.

The results of SO_4^{2-} restriction suggest that secretion of macromolecules or *Microciona* aggregation factor (MAF), and aggregation and locomotion of *Microciona* cells depend upon an adequate extracellular source of SO_4^{2-}, sulfate transport, and sulfation of macromolecules such as polysaccharides.

Introduction

Both vertebrate and in vertebrate cells require sulfated macromolecules on cell surface receptors and in intracellular fluid (Cassaro and Dietrich, 1977; Hogsett and Quantrano, 1978; Mulder, 1981; Klebe *et al.*, 1986; Mulder *et al.*, 1987). For example, mesenchymal migration of sea urchin embryos is blocked *in situ* in sulfate-deprived medium (Katow and Solursh, 1981), and cell motility and morphology in cell cultures have been influenced by sulfated glycosaminoglycans (Venkatasubramanian and Solursh, 1984). Blebbing has been observed on cell surfaces of sea urchin embryos maintained in sulfate-free seawater, but not the prolonged processes that accompany mesenchymal cell migration. In this instance it appeared that sulfate deprivation was capable of causing an inhibition of the formation of stable cell attachments to the basal lamina (Venkatasubramanian and Solursh, 1984; Akasaka *et al.*, 1980). The defect could be reversed by a 6-h pre-treatment in normal seawater.

Sulfate availability appears to be particularly important during early embryogenesis and differentiation in several species (Cassaro and Dietrich, 1977; Katow and Solursh, 1981; Lindahl, 1942; Immers and Runnstrom, 1965; Wenzl and Sumper, 1981). Particularly vital in this regard are the sulfated mucopolysaccharides; their presence correlates well with tissue-level organization and normal development (Wenzl and Sumper, 1981; Kinoshita and Saiga, 1979; Yamaguchi and Kinoshita, 1985).

This interesting background prompted us to address sulfation, using as a model *Microciona*, a relatively well studied marine sponge (Humphreys, 1963, 1967; Henkart *et al.*, 1973; Burger *et al.*, 1975; Jumblatt *et al.*, 1980; Misevic and Burger, 1986; Misevic *et al.*, 1987). These sponges are multicellular, but the relatively loose organization of embryonic and differentiated cells is easily disaggregated. If divalent cations are deleted from the

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supporting medium (seawater), a specific aggregation factor (AF)—a sulfated proteoglycan-like molecule—is released; this factor can then promote specific cell aggregation (Humphreys, 1963). AF contains two functional domains, one a cell binding portion, the other an AF interaction domain (Misevic and Burger, 1986). Cell aggregation by *Microciona* AF (MAF) appears to be based on multiple low affinity carbohydrate-carbohydrate interactions (Misevic *et al.*, 1987). The role of sulfate in such reactions is unknown, but other work shows that the migration and release from cells of proteoglycan-containing vesicles may be related to a high sulfate content (Albedi *et al.*, 1989; Takagi *et al.*, 1989). Monoclonal antibodies raised against sulfated proteoglycan from rat chondrocytes were used with immunoperoxidase electron microscopy to demonstrate relatively high concentrations of membrane-associated sulfated proteoglycans in cell processes and filaments from which matrix vesicles are presumably released into the surrounding medium (Takagi *et al.*, 1989).

In the studies to be described, the effects of seawater, with and without sulfate, upon subsequent aggregation of disaggregated *Microciona* sponge cells was examined, both in rotating and stationary cultures. The isolation of cells in a relatively simple culture medium, such as seawater, possesses advantages over such alternatives as perfusion, or *in vivo* methods, or the use of complex culture media. The level of sulfation can be controlled, all the relative enzyme systems are present in the cells, and the cellular uptake of labeled sulfur can be studied. Our primary purpose in the initial study was to observe the effects of sulfate deficiency, or restriction, upon cell process formation and locomotion and upon cellular aggregation. Using the conditions indicated by these observations, the incorporation of $^{35}SO_4$ was carried out, and correlations with intracellular-free sulfate and active sulfate (PAPS, *i.e.*, phosphoadenosine-5′-phosphosulfate) and sulfated macromolecules determined.

Materials and Methods

Sponges

Live specimens of *Microciona prolifera* were collected by members of the Supply Department of the Marine Biological Laboratory (Woods Hole, Massachusetts) during the months of July and August. Sponges were used on the day of collection or on the following day, but could be maintained in satisfactory condition for several days in the laboratory at ambient temperature in tanks of running seawater.

Buffers and artificial seawater preparations

Bicarbonate buffered artificial seawater (MBLSW) was made up according to the Marine Biological Laboratory formula (Humphreys, 1963; Cavanaugh, 1964). Calcium- and magnesium-free seawater (CMFSW) was prepared as described by Humphreys (1963). In aggregation assays, CMFSW was supplemented with 10 m*M* $CaCl_2$. Sulfate-free seawater was prepared as follows: in the case of MBLSW, magnesium chloride was substituted for $MgSO_4 - 7H_2O$; in CMFSW, sodium chloride was substituted for Na_2SO_4. The preparations were termed MBL $- SO_4$ and CMF $- SO_4$, respectively.

Dissociation of sponge cells

The chemical dissociation of *Microciona* cells (Humphreys, 1963) began with small lumps of tissue that were first rinsed to remove foreign material, and then blotted. Fragments (1–3 mm) were cut and placed in cold CMFSW in the ratio of 1 g/100 ml CMFSW. We dissociated the fragments by pressing them gently through no. 25 bolting cloth into a second volume of CMFSW. The resulting suspension contained about 2×10^7 cells/ml as estimated by hemocytometer counts. The suspension was spun in the centrifuge for 5 min at 2000 RPM and resuspended to make a concentration of 10^7 cells/ml. Small clumps were flushed gently with a Pasteur pipet and thereby readily broken up. The suspension was then rotated in CMFSW at 16°C for 6 h. The supernatant containing aggregation factor (AF) was removed, and the cells were washed and resuspended. The rotation was then repeated, first for 6 h in CMF $- SO_4$ and then for 24 h in MBL $- SO_4$. The cells were then divided into two aliquots: one was maintained in MBL $- SO_4$ for an additional 24 h, and the other was placed simultaneously in MBLSW for 24 h. The preconditioned cells from both aliquots were then pelleted and each aliquot resuspended in MBL $- SO_4$ and used in isotope labeling experiments.

Aggregation factor

AF was extracted and purified according to Humphreys (1963), as modified by Jumblatt *et al.*, (1980). Protein was estimated using the Bio-Rad colorimetric assay (Bradford, 1976).

Sponge cell aggregation assays (Humphreys, 1963; Jumblatt et al., *1980)*

Serial two-fold dilutions of AF were incubated for 20 min at 22°C with cells (10^7/ml) in the presence of $CaCl_2$. The cells were then visually inspected for evidence of aggregation.

Sponge cells in rotation-and petri dish cultures used to study cell motility and aggregation

Cells were prepared as described above, and batches were then adjusted to a concentration of 10^7/ml in the following buffered solutions: MBLSW, MBL $- SO_4$, and

CMFSW, CMF − SO_4. From each suspension, one aliquot was rotated in covered beakers for 24 h at 16°C. A second aliquot from each suspension was placed in glass petri dishes and maintained motionless at 22°C for 24 h. The presence of aggregates was then determined.

Microscopic studies

Following the incubation period, small aliquots of cultured preparations were mounted on glass slides, and overlaid with cover slips, which were sealed with resin to prevent evaporation. Some cell preparations were vitally stained with a 0.1% aqueous solution of Nile blue sulfate (Leith and Steinberg, 1972). The cells were examined by phase contrast and interference contrast microscopy with a Zeiss Axiophot microscope at magnifications of 100× and 400×.

Sulfate incorporation studies using isotope-labeled, carrier-free sulfuric acid ($H_2{}^{35}SO_4$)

Radiolabeled $H_2{}^{35}SO_4$ (2 mCi/ml) was purchased from New England Nuclear. Aliquots of each preconditioned *Microciona* cell preparation in MBLSW and MBL − SO_4 were washed in MBL − SO_4, then calibrated to 10^7 cells/ml in sulfate-deficient artificial seawater, and incubated in rotating culture in medium containing 2 μCi/ml $H_2{}^{35}SO_4$. Replicate 1-ml aliquots of cells were placed on 25-mm diameter cellulose acetate filter discs (0.45 μm), beginning at 15 min, and at intervals thereafter up to 12 h. The dried discs were treated as follows: (a) for uptake studies, duplicate dried discs were each placed in a scintillation vial and 15 ml Aquosol-2 liquid scintillation fluid added; (b) for incorporation studies, filter discs were treated with 100% ethanol to precipitate proteins, washed twice in ethanol, dried, and treated as in (a). Counts were carried out in a Beckman LS6000 IC scintillation counter. The results are expressed as dpm/10^7 cells.

High voltage electrophoresis (HVE)

Channels (2″ wide) were pencilled on Whatman 3M filter paper (18 × 22″), which was then moistened with 1% sodium tetraborate pH 9.1. One aliquot could then be spotted on one channel for a total of nine assays on each sheet of moistened paper. Electrophoresis was carried out at 1 kV and 180 mAmp for 60 min; the current was then discontinued and the paper dried in a warm air oven. Each channel, containing one separated extract, was cut into one-inch strips and placed in vials to which was added Beckman Redi-Solv EP scintillation fluid for scintillation counting as described.

$^{35}SO_4$ Incorporation into secreted extracellular macromolecules

Microciona cell suspensions were pre-treated and chemically dissociated, as described. The suspension medium was either CMFSW or CMF − SO_4. To suspensions adjusted to a concentration of 10^7 cells/ml in a volume of 50 ml, was added 100 μCi of carrier-free $^{35}SO_4$; the suspensions were rotated for 12 h at 16°C. The supernatants were harvested, and concentrates were prepared and assayed for MAF (16). The MAF pellets were washed exhaustively, redissolved in a minimal volume of artificial seawater, adjusted to equal protein concentrations, and dialyzed overnight in electrophoresis buffer. Such preparations were assayed by HVE. Free $^{35}SO_4$ and PAP (^{35}S) were included in the assays as reference standards. Material that remained at the origin following electrophoresis was regarded as containing macromolecules that had incorporated $^{35}SO_4$. Results are expressed as dpm/mg protein.

Amino-acid incorporation using ^3H-leucine

Aliquots of cell preparations maintained in the presence or absence of sulfate were calibrated to 10^7 cells/ml and incubated in MBL − SO_4 in the presence of 100 μl of a 50 μCi/ml solution of ^3H-leucine (>300 mCi/mmol—New England Nuclear); aliquots were taken for counts at spaced times beginning at 2 min. The cells were treated with 100% ethanol as described above.

Results

Sponge cell aggregation

In contrast to cells suspended in sulfate-containing medium, aggregation was either partially or greatly retarded in samples suspended in sulfate-free seawater. This could be demonstrated as follows: (1) test cells that were prepared in the routine manner were rotated at 16°C in the presence of $CaCl_2$ along with supernatants derived from an equal number of chemically dissociated cells rotated in either CMFSW or CMF − SO_4. Supernatants prepared in sulfate free seawater (CMF − SO_4) proved relatively ineffective in aggregation assays when compared with the action of AF or supernatant derived from sponge cells that had been rotated in CMFSW (Fig. 1).

(2) AF prepared from fresh cells under normal conditions and concentrated, was tested in routine assays with chemically dissociated cells rotated in either CMFSW or CMF − SO_4. When cells which had been pre-treated in CMF − SO_4 were used in assay, the aggregation of sponge cells was reduced in comparison with cells that had been pre-treated in sulfate containing seawater (Fig. 2). A small rim of adherent cells that ordinarily collected at the liquid-air interface of the container was not observed in assays that included CMF − SO_4 treated cells.

Petri dish cultures

Chemically dissociated sponge cells were rotated in changes of CMFSW and washed with CMF − SO_4. Ali-

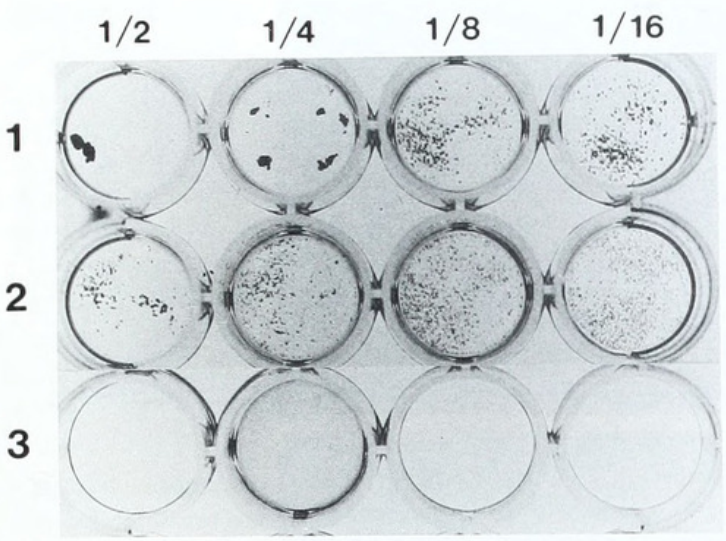

Figure 1. Results of aggregation assays using supernatants derived from chemically disaggregated cells rotated in sulfate-free artificial seawater. Fresh normally processed *Microciona* cells and $CaCl_2$ added. Photograph depicts results at 1 h. Wells from left to right in first two rows (1 and 2) depict serial dilutions of supernatant preparations. *Microciona* cells and $CaCl_2$ in CMFSW are in bottom-most (3) well (2nd from left). Reading from the top: Row 1, assay contains supernatant (MAF) from CMFSW cells; Row 2, contains supernatant from CMF − SO_4 cells. Aggregation is impaired in presence of CMF − SO_4 supernatant and dilutions.

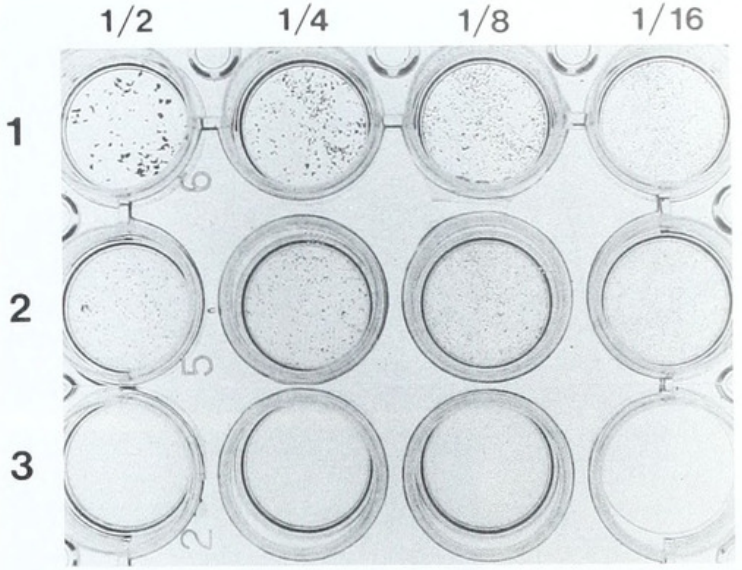

Figure 2. Aggregation assays using *Microciona* cells prepared from suspensions rotated in CMFSW or CMF − SO_4. Assays were carried out in calcified dilutions of MAF prepared as described (12, 16). Photograph depicts results at 1 h. Wells from left to right in rows 1 and 2 depict serial dilutions of MAF in CMFSW with $CaCl_2$. Wells at bottom contain samples of each cell preparation in calcified CMFSW. Reading from the top from left to right: first row, assay contains cells pre-treated in CMFSW; second row, cells pre-treated in CMF − SO_4. Aggregation is impaired in cells which had been pre-treated in CMF − SO_4.

quots of cells were then placed in even suspension in the following media: CMFSW, CMF − SO_4, MBLSW, MBL − SO_4. Each suspension was gently pipetted with a Pasteur pipet and counted; the count in each suspension was adjusted to 10^7/ml. Thirty ml of suspension were then poured into large glass petri dishes and gently pipetted to assure an even distribution of cells. The dishes were then covered and were permitted to remain undisturbed for 24 h at 22°C. Inspection at the end of this period revealed aggregates of varying sizes in samples that had been suspended in MBLSW and CMFSW. Aggregation was not observed in cell suspensions lacking sulfate (Fig. 3).

Microscopic studies

The most striking observation was a relative lack of cellular blebbing or of filopodia or pseudopodia in cell preparations suspended in sulfate-free seawater. In contrast, we commonly observed, in suspensions containing sulfate, slender filopodia that sometimes extended a long distance from single cells or from aggregates (Fig. 4A–D). Other, more substantial processes were suggestive of a cell elongating in the direction of a second cell using a single pseudopod as a means of locomotion. We observed occasional single cells, or cell clumps, from which multiple filopodia emerged, creating a stellate or radiating pattern (Fig. 4B). All of these appeared to be natural events in the presence of sulfate. They were greatly reduced or lacking in single cells and aggregates when sulfate was absent, whether Ca^{2+} and Mg^{2+} were present (Fig. 4E–F). Our general impression was that processes were most often seen in relatively agranular cells of intermediate to large size.

Incorporation of $^{35}SO_4$

The results of $^{35}SO_4$ uptake into *Microciona* cells are shown in Figure 5. Each sampling time point depicts the average of duplicate tests, and the values are expressed as

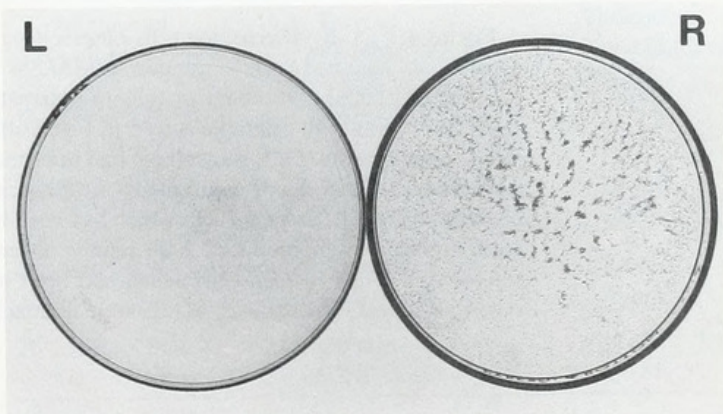

Figure 3. Aggregation of *Microciona* cells maintained in stationary culture in petri dishes. Readings are at 24 h under conditions as described in the text. Aggregation is observed when cells are maintained in MBLSW (right) but not when cells are in MBL − SO_4 (left).

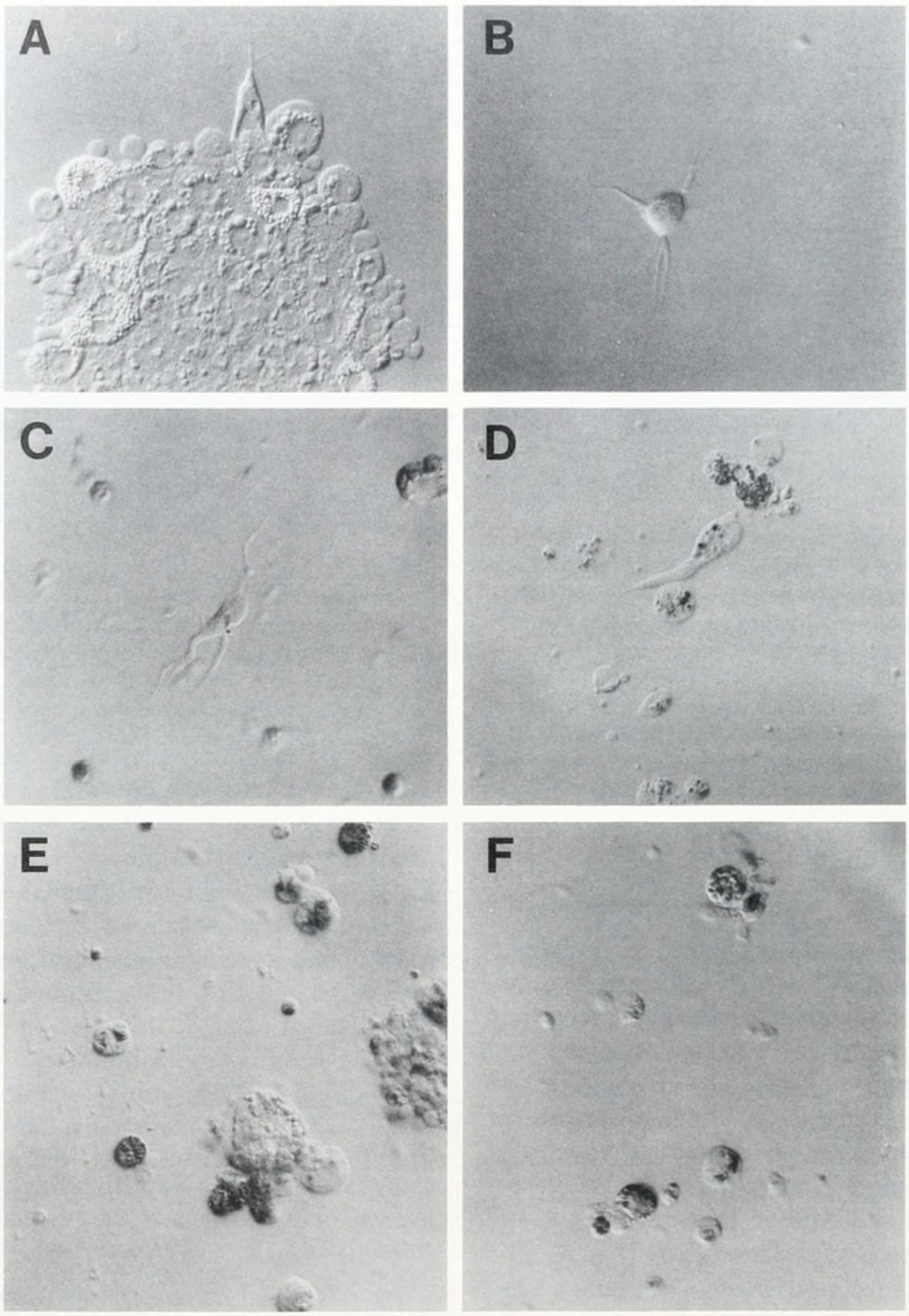

Figure 4. (A–B) *Microciona* cells observed by interference contrast microscopy 400× magnification. Cells shown here had been in rotation in MBLSW as described in text. Filopodia or pseudopodia, some of considerable length, are noted in cells in aggregates as well as single cells. Multiple filopodia produce a stellate appearance in cell or cells seen in Figure 4B. (C–D) Interference contrast study of *Microciona* cells 400× magnification. Cells shown here had been maintained stationary in petri dish cultures in MBLSW as noted in text. Filopodia or pseudopodia are frequent in single cells and in small aggregates. (E) Interference contrast study of *Microciona* cells which had been in rotation in MBL − SO_4 as described in text. Typically, small aggregates were produced in the relative absence of filopodia or pseudopodia. (F) Interference contrast microscopy of *Microciona* cells which had been maintained stationary in MBL − SO_4 in petri dishes as described in text. The majority of cells were unattached, lacking processes, or adherent in very small clusters.

dpm per 10^7 cells. The highest uptake of $^{35}SO_4$ occurred in cell samples pre-treated in sulfate-free medium; in comparison, cells pre-treated in sulfate-containing medium demonstrated considerably lower levels of $^{35}SO_4$ uptake beginning minutes after the addition of $^{35}SO_4$. In replicate experiments with sponge derived from two collecting stations, cells in MBL − SO_4 showed progressive increases in $^{35}SO_4$ uptake and incorporation up to 12 h,

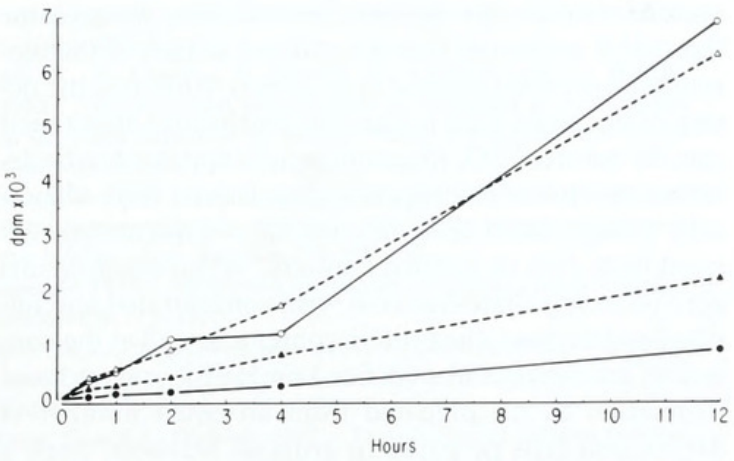

Figure 5. Uptake of $^{35}SO_4$ by *Microciona* cells. Pre-treated *Microciona* cells were distributed into flasks containing (a) MBLSW, (b) MBL − SO_4 at a concentration of 10^7 cells/ml and placed in rotation for 24 h. Centrifuged pellets from each flask were washed with MBL − SO_4 and suspended in MBL − SO_4; 5 μl $H_2{}^{35}SO_4$ (carrier free) was added to each flask and rotated at 16°C. Radioactivity of duplicate 1-ml aliquots was monitored at intervals up to 12 h. The solid lines depict uptake of $^{35}SO_4$ by cells pre-treated in MBL − SO_4. The broken lines represent cells pre-treated in MBLSW. Circles and triangles signify experiments carried out on cells from sponge obtained at two different collecting stations.

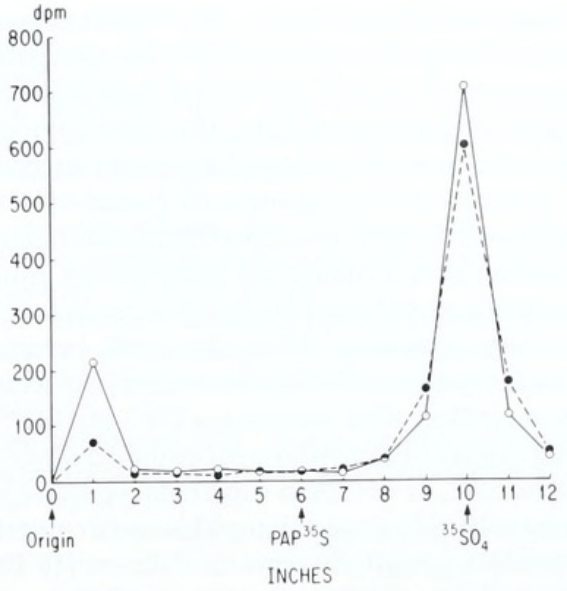

Figure 6. High voltage electrophoresis of $^{35}SO_4$ macromolecules in supernatant preparations derived from rotated chemically dissociated *Microciona* cells with incorporated carrier free $^{35}SO_4$. 75 μl placed at origin. Conditions: 1% sodium tetraborate pH 9.1, 1 KV, 180 mA, 1 h. CMFSW □---□; CMF − SO_4 ■---■

in contrast to controls. At 12 h, values for $^{35}SO_4$ were three- to eight-fold higher in sulfate deprived cells than in controls (6925 and 6603 dpm in test samples versus 882 and 2075 dpm in controls). Incorporation of $^{35}SO_4$ into macromolecules was calculated to be 70–85% of $^{35}SO_4$ uptake at the 12-h sampling time. In recent separate experiments with extended sampling times, the differential in counts between cells pre-treated with MBLSW and with MBL − SO_4 remained for up to 5 days, at which time sampling was discontinued. In samples of cells pre-treated with MBL − SO_4, elevated counts exhibited some fluctuations, but remained at high levels during the period of sampling. Cells were collected for extracts that will be analyzed by HVE for the distribution of $^{35}SO_4$ macromolecules. This study will be reported in a separate publication.

$^{35}SO_4$ Incorporation into secreted extracellular macromolecules

The protein yields in the extracellular secretion from 5×10^8 *Microciona* cells were as follows: from CMFSW cells, 1.12 mg; and from CMF − SO_4 cells, 0.37 mg. The yield of $^{35}SO_4$ macromolecules revealed by HVE after subtraction of background values was: for CMFSWMAF, 190 dpm/50 μg protein; for CMF − SO_4 supernatant, 35 dpm/50 μg protein (Fig. 6).

Incorporation of ^{3}H-leucine

Incorporation of ^{3}H-leucine into protein by *Microciona* cells in sulfate-free seawater was comparable to that of cells rotated in sulfate-containing seawater; aliquots from specimens obtained at two collecting stations were examined. In all instances, incorporation was prompt, with mild to moderate increases over the testing period (Table I).

Discussion

A role for sulfated polysaccharide recognition in sponge cell aggregation was suggested by Coombe *et al.* (1987) based upon an analysis of endogenous polysaccharide

Table I

^{3}H-leucine incorporation into Microciona *cells maintained under different conditions of sulfate availability*

Conditions of culture	Time of sampling	^{3}H-Leucine incorporated (dpm)
1a. MBL + SO_4	30 min	17111*
	2 h	20586
1b. MBL − SO_4	30 min	19149
	2 h	25644
2a. MBL + SO_4	30 min	13688
	2 h	21646
2b. MBL − SO_4	30 min	12140
	2 h	19781

* Average of duplicate ethanol precipitated samples after subtraction of background values.

10^7 cells per sample.

Specimens 1 and 2 were obtained at two different collecting stations.

from sponge cell cholate lysates. The extract possessed a high content of sulfate and inhibited the aggregation of intact sponge cells, as did the sulfated compounds polyvinyl sulfate and dextran sulfate. The latter compounds, coupled to erythrocytes, rendered the erythrocytes agglutinable in the presence of sponge cell lysates.

In the present studies, pre-treated chemically dissociated cells maintained in a sulfate-free environment exhibited greatly reduced motility and marked changes in functional behavior. Aggregation of *Microciona* cells became impaired under these conditions, particularly in stationary cultures, regardless of the presence of Ca^{2+} and Mg^{2+}. The effect of a sulfate-free environment could be observed in *Microciona* cells, as well as in supernatants derived from cells under sulfate-free conditions. This was demonstrated in controlled aggregation assays, as follows. (1) The capability of AF derived from cells in CMFSW was compared with that of supernatants derived from cells chemically dissociated in CMF − SO_4 with healthy *Microciona* cells and $CaCl_2$ being employed in the assays; and (2) cells prepared in sulfate-free artificial seawater were tested in standard assays with AF prepared in the usual manner from chemically dissociated cells. Of special note was the finding that aggregates were absent or greatly reduced in size when cells under these conditions were maintained in stationary cultures. Random collision in rotation cultures probably accounted for the small aggregations noted, but even in this circumstance, aggregation in the absence of sulfate was modest when compared with *Microciona* cells maintained in sulfate.

The most obvious morphologic change in individual cells maintained in sulfate-free medium was a reduction in cell processes such as filopodia and pseudopodia. The relative lack of such processes most likely contributed to the inability of these cells to form normal contacts, especially when in stationary cultures. In the presence of sulfate, the primary activity seemed to reside in medium- to large-sized cells, cells which were relatively agranular, some of which were reminiscent of choanocytes (Kuhns *et al.,* 1980). It remains to be established whether certain cell types in *Microciona,* such as larval cells, possess special components that are unusually sensitive to sulfate deprivation, and that are necessary for secretion or cellular migration, as is the case in the sea urchin embryo.

The secretion of sulfated polysaccharide appears to be necessary to maintain these functions, as established earlier by Immers and Runnstrom (1965), and this form of secretion is diminished or sulfate-poor in parallel with sulfate restriction. However, there is no direct evidence from our work that sulfated polysaccharides were specifically affected by changes in seawater sulfate content. The evidence presented relates to the flow of sulfate into cells, and the biosynthesis of sulfated macromolecules as important factors in cell locomotion and cell adhesion for reasons that are yet unclear. Nevertheless, we presume that MAF molecules become sulfated as part of the biosynthetic process (Misevic *et al.,* 1987). Although the design of this study does not enable conclusions about their specific nature, $^{35}SO_4$ macromolecules appeared to be deficient in *Microciona* supernatants derived from chemically disaggregated cells, in contrast to supernatants derived from cells in sulfated seawater. When supernatants derived from sulfate-free cells were concentrated and purified as described, the protein content, as well as the content of macromolecules (defined on HVE), were reduced in relation to AF prepared from an equal number of *Microciona* cells prepared in artificial seawater. Such a deficit may be caused by a defective transport of secretory vesicles to the cell surface in the absence of sulfate.

We suspect, from our results, that sulfation of macromolecules such as polysaccharides may be crucial in the trans-golgi transport of vesicles and their secretion into the extracellular matrix. These results, coupled with findings that sulfate depleted cells can greatly augment $^{35}SO_4$ incorporation relative to controls, suggest a mechanism whereby extracellular sulfate deficiency can alter the sulfation process, perhaps by influencing membrane composition and function. Note in this context that amino acid uptake and incorporation was similar in test and control cells as judged by experiments using ^{3}H-leucine.

Our studies of sulfate depleted cells suggest that the sulfate assimilatory pathway is altered when sulfate becomes rate limiting. Regulation and transport in such a system has been explained by the existence of a specific membrane permease in two bacterial species, *Salmonella typhimurium* and *Anacystis nidulans* (Green *et al.,* 1989); the permease genes have been cloned and, from these, a polypeptide structure of a putative membrane component determined. Homologies with message derived from sulfate-restricted *Microciona* sponge might be sought using probes derived from these bacteria. Studies in sulfate-deficient wheat and barley roots have also defined a sulfate transporter that was sensitive to DIDS, an inhibitor of anion transport (Clarkson and Saker, 1989). A sulfate permease, if defined in our system, would encourage further studies to define ways in which cells recognize sulfate levels and transduce this signal into altered sulfate transport, increased biosynthesis of sulfated glycoconjugates and altered cell locomotion. Fine structure differences in MAF derived from sulfate-deprived versus normal cells may prove important in defining extracellular prompting mechanisms which initiate or modulate such changes (Brunner, 1977).

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