INCORPORATION OF SULFATE INTO THE CAPSULAR POLYSACCHARIDE OF THE RED ALGA *PORPHYRIDIUM*

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ABSTRACT

The accumulation of sulfate-^{35}S by *Porphyridium aerugineum* cells and subsequent appearance of solubilized capsular polysaccharide-^{35}S in the growth medium were examined The uptake of label by the cells was largely light dependent. Pulse-chase experiments using log phase cells revealed a rapid labeling of solubilized capsular polysaccharide, recovered from the medium as the cetylpyridinium chloride precipitate Polyacrylamide gel electrophoresis of the polysaccharide-^{35}S showed the sulfate to be firmly bound to an immobile fraction. Sephadex chromatography revealed the molecular weight of the polysaccharide to be in excess of 2×10^5. Acid hydrolysis of the polysaccharide-^{35}S released sulfate-^{35}S ion as evidenced by radioautography of thin layer chromatographs Preliminary electron microscope evidence suggests that the synthesis, movement, and deposition of the capsular polysaccharide on the cell surface are Golgi complex–mediated processes

INTRODUCTION

Whaley et al. (1972), Northcote (1971), and others have brought attention to the fact that many cellular functions are influenced by macromolecules which are associated with the cell surface. In most cases the Golgi apparatus and associated structures function in the synthesis, intracellular transport, and release of these macromolecules to the cell surface.

Porphyridium aerugineum is a unicellular red alga which deposits large quantities of polysaccharide on its surface The extracellular polysaccharide is intimately associated with the cell membrane (plasmalemma) and forms a mucilaginous capsule or "cell wall" (Fig 1). The polysaccharide is water soluble, and is continually replenished at the cell surface as it solubilizes into the medium. Therefore, the capsule must be considered a dynamic structure in that its turnover rate is high.

While working with this eukaryotic microorganism (Ramus, 1972), it was established that the polysaccharide is acidic (anionic) and of a large molecular weight. The constituent sugars were shown to be glucose, galactose, xylose, and several others not yet identified. One or more of the constituent sugars or sugar derivatives is sulfated. The kinetics of polysaccharide release from the cell was described for lag, log, stationary, and senescent phase cultures, in both the presence and absence of light Further, pulse-label experiments were conducted using $H^{14}CO_3^-$, showing ^{14}C absorption by the cell, ^{14}C fixation into the encapsulating polysaccharide, and its subsequent release into the medium.

Electron microscopy revealed that the polysaccharide is probably packaged in membranes within the cell (Fig. 2) and is released by fusion of the package and cell membrane. Since these cells have exceedingly well-developed Golgi complexes (Gantt et al., 1968), it appears likely that polysaccharide synthesis and transport in *Porphyridium* is a Golgi complex–mediated process,

and therefore of general significance to cell biology.

As mentioned previously, the kinetics of polysaccharide production were studied using $H^{14}CO_3^-$ in trace amounts (Ramus, 1972) It was thought that the use of a more specific label would improve studies of polysaccharide synthesis by increasing the resolution of labeling experiments. Since the cells are excreting primarily a polysaccharide product and because the polysaccharide is sulfated, it was felt that sulfate-^{35}S would be a reasonably good label with which to trace the synthesis, packaging, and release of polysaccharide. In addition, sulfate-^{35}S can be used to study the biochemistry of the incorporation of inorganic sulfate into the polysaccharide as sulfate ester.

The experiments described here are a necessary initial step in the pursuit of an understanding of the metabolic role of the sulfur in the synthesis, packaging, movement, and excretion of the extracellular polysaccharide. The first and last events, i.e. $^{35}SO_4^=$ accumulation by the cells and the appearance of ^{35}S-labeled polysaccharide in the medium, are discussed in this paper. Experiments designed to elucidate the intermediate metabolic pathway(s) of sulfate activation and incorporation into the polysaccharide are in progress.

MATERIALS AND METHODS

Axenic cultures of *Porphyridium aeruginеum* (Indiana University Culture Collection No. 755) are grown in "MCYII" liquid medium (Ramus, 1972) at 25°C and illuminated with CW fluorescent tubes (4000 ergs/cm²-sec) on a 16:8-hr light-dark cycle. Cells were kept in suspension by agitation on a gyrorotary shaker or by a stream of filtered air.

For observation with the electron microscope, cells were fixed in glutaraldehyde, embedded in a fibrin clot (Furtado, 1970), postfixed in OsO_4, and infiltrated with Epon 812, sectioned, and poststained with uranyl acetate and alkaline lead citrate (Ramus, 1972).

For $^{35}SO_4^=$ uptake experiments, cells from log phase cultures were collected by centrifugation (7000 *g*, 10 min), then resuspended in fresh medium to a density of 3×10^6 cells/ml and allowed to grow overnight. $Na_2{}^{35}SO_4$ was added (1.0 μCi/ml), and 50-ml aliquots of cells were dispensed to separate flasks. The flasks were left untreated, or wrapped in aluminum foil (dark control), or treated with 2.0 ml of neutralized formalin (fixed control). The cultures were placed on the shaker and sampled (three replicates) at intervals to 8 hr. Samples consisted of a 1.0 ml cell suspension applied to an HAWP (0.45 μ) Millipore filter with suction; the filters were washed with 20–30 ml of MCYII, dried *in vacuo* over silica gel, and counted. Even with repeated washing, filters retain some sulfate, therefore, corrections were made for nonspecific absorption by the filters.

Radioactivity was assayed ($Na_2{}^{35}SO_4$, 865 mCi/mmole; New England Nuclear Corp., Boston, Mass.) with an Intertechnique SL-30 liquid scintillation counter, using 5 ml toluene-Liquifluor (New England Nuclear Corp.) scintillation fluid in glass vials. Counting efficiency was determined by pipetting known quantities of a dilute $Na_2{}^{35}SO_4$ solution (0.1 μCi/ml; 2.2×10^3 dpm) directly onto an HAWP (0.45 μ) Millipore filter, and the filter was dried *in vacuo* over silica gel, then, the filter was placed in a scintillation vial. The degree of quenching due to toluene-extracted chlorophyll was calculated using the "channels-ratio method." Varying quantities of living cells were collected by suction on HAWP (0.45 μ) Millipore filters, and then varying quantities of a dilute $Na_2{}^{35}SO_4$ solution were added,as above. In all cases, corrections were made for the rapid decay ($t_{1/2}$ = 87 days) of the isotope.

The counting efficiency for ^{35}S, as expected, was inversely proportional to the amount of cellular material (both toluene soluble and insoluble) acting as quenching agent, especially chlorophyll. This relationship is linear over the range of cell densities encountered in these experiments ($0.1–4 \times 10^6$ cells/ml), and the counting efficiency ranged from 82 to 94%. The measured activity, even though near actual disintegration rates, was corrected accordingly.

For pulse-chase experiments it was necessary to define the conditions in which the cells would take up (absorb) labeled sulfate from the medium at the highest possible rate. Therefore, using cells starved for sulfate, sulfate-^{35}S uptake efficiency was determined as a function of varying amounts of cold carrier ($MgSO_4$). Here cells were washed with and allowed to grow in MCYII minus sulfate ion for 24 hr before the experiment. After this period of sulfate starvation, 50 ml of the cell suspension was transferred to each of six 125 ml flasks Each of these presterilized flasks contained 50 μCi of $Na_2{}^{35}SO_4$, but varying amounts of $MgSO_4$ (cold carrier) in MCYII. The "normal" concentration of sulfate ion in the MCYII medium is 4×10^{-4} M (0.1 mg of $MgSO_4 \cdot 7H_2O$/ml medium). The six experimental flasks contained the following concentrations of sulfate ion: 4×10^{-4} M, 2×10^{-4} M, 4×10^{-5} M, 4×10^{-6} M, 4×10^{-7} M, and minus sulfate. The cultures were sampled in triplicate as described above.

For experiments dealing with excretion of labeled polysaccharide into the medium, sulfate-starved cells were pulsed in 1 μCi $Na_2{}^{35}SO_4$/ml MCYII minus $MgSO_4$ for periods ranging from 15 min to 4 hr. At

the end of the pulse the cells were washed three times with MCYII at 5°C and resuspended in complete MCYII (chase) for the duration of the experiment. The solubilized polysaccharide was isolated from the medium and counted as follows. 12 ml of a cell suspension was centrifuged (15,000 *g* for 10 min), and the supernatant was then filtered through an HAWP (0.45 μ) Millipore filter to assure the removal of all cells. 10 ml of this filtered supernatant was placed in a test tube and heated to 40°C in a water bath, NaCl was added to 0.05 M, and cetylpyridinium chloride (CPC) was then added to a concentration of 0.1% to precipitate the polyanionic polysaccharide The tubes were agitated vigorously with a Vortex mixer, and the precipitated polysaccharide was collected on GSWP (0.22 μ) Millipore filters with suction The tubes were rinsed carefully three times with MCYII, and the washings were also applied to the filters. The filters were then dried and counted as described above. The CPC procedure for precipitation of the polysaccharide is quantitative (95% recovery), and is described in detail elsewhere (Ramus, 1972). It was found that some free sulfate was carried down with the CPC precipitate; therefore, supernatant samples were dialyzed against distilled H_2O for 48 hr before addition of CPC to remove free sulfate.

The amount of soluble extracellular polysaccharide was measured by the anthrone method, as modified by Ramus (1972), using isolated polysaccharide as a standard.

All determinations of cell densities were made by hemacytometer counts.

The preparation of polyacrylamide gels for electrophoresis of the polysaccharide closely followed the method of Peacock and Dingman, with the necessary modifications for their use with analytical columns (Holden et al., 1971). The 3% gels used were made in a Tris-ethylenediaminetetraacetate (EDTA)-borate buffer (pH 8.3) and contained sucrose (Holden et al., 1971). Approximately 50 μg of dialyzed CPC-isolated polysaccharide–^{14}C or ^{35}S (made up in electrophoresis buffer and sucrose) was layered onto each gel. Polysaccharide-^{14}C was obtained after a pulse of $NaH^{14}CO_3$ (Ramus, 1972). Power was applied at the rate of 2.5 ma/tube, and migration was allowed to continue for 8 hr at 10°C. After electrophoresis, gels were washed in distilled H_2O and then in 7% acetic acid, and stained for polysaccharide by the periodic acid–Schiff's reaction (PAS) (Holden et al., 1971) or with 0.1% alcian blue (Allied Chemical Corp., New York) in 7% acetic acid. As in the presence of the quarternary ammonium compound CPC, the acidic polysaccharide of *Porphyridium* precipitates in the presence of the cationic alcian stains, yielding a blue product in the presence of alcian blue, and a yellow product in the presence of alcian yellow. The precipitate can be solubilized in high concentrations of mono- or divalent cations (as 2 M Ca^{++}). Presumably, the anionic groups of the polysaccharide exchange the alcian molecules for the added cations. An attempt was made to use these principles as a basis for a quantitative assay, but the assay lacked sensitivity. Gels were scanned (in the case of PAS staining at 450 nm) in a Gilford gel scanner. 1 mm slices of the frozen gel were solubilized in Nuclear-Chicago solubilizer (Nuclear-Chicago, Des Plaines, Ill.) (1 ml) at 60°C in scintillation vials, toluene-Liquifluor was added directly, and the activity was counted.

Column chromatography was accomplished with Sephadex G-200 in a K 15/30 column (Pharmacia Fine Chemicals Inc., Piscataway, N.J). Sephadex and samples were made up in a 0.001 M acetic acid–0.001 M Na_2EDTA buffer. Void volume was determined with Blue Dextran 2000 (Pharmacia Fine Chemicals Inc.).

For thin layer chromatography, dialyzed ^{35}S-labeled polysaccharide was hydrolyzed for 90 min at 120°C in 2 M trifluoroacetic acid in an evacuated hydrolysis vial; the trifluoroacetic acid was evaporated, and the hydrolysate was resuspended in distilled water. The hydrolysate was chromatographed on Sil G-25 thin layer plates (Brinkmann Instruments, Inc., Westbury, N.Y.) using an ethyl acetate:methanol:acetic acid:water (60:15:15:10) migration solvent. Sugars were visualized by spraying the chromatograph with 5% aniline hydrogen pthalate in glacial acetic acid, and heating the chromatograph at 100°C until colored spots appeared. The thin layer chromatograph was radioautographed with medical X-ray film (Kodak Blue Brand, single coated). Spots in the silica gel localized with either spray reagent or radioautography were scraped from the glass plate, and the scrapings were placed in scintillation fluid for counting.

RESULTS

The results of the $^{35}SO_4^{=}$ uptake experiments are shown in Fig. 4. Under the conditions described (mild sulfate starvation, $MgSO_4$ concentration 4×10^{-4} M), the uptake of label into the cells was practically linear for the first 4 hr in the cells exposed to constant illumination. After 4 hr the rate of label accumulation decreased slowly, and by 24 hr (only the first 8 hr are shown in Fig 4) the organisms had approached sulfate saturation After 24 hr large quantities of free $^{35}SO_4^{=}$ remained in the medium. Cells grown in darkness (dark control) exhibited a slight accumulation of $^{35}SO_4^{=}$, but this accumulation of label was only 20% that of light-grown cells, indicating that the uptake of $SO_4^{=}$ is largely light dependent. Cells that were fixed with formalin (fixed control)

before the addition of $^{35}SO_4^=$ did not accumulate label, showing sulfate uptake in these experiments to be a biological process, and not chemical fixation or accumulation by diffusion.

Optimal conditions for the accumulation of labeled sulfate by the cells were determined as a function of competing cold carrier ($SO_4^=$). In the presence of varying amounts of cold carrier and a fixed amount of $^{35}SO_4^=$ (1 μCi/ml, specific activity = 865 mCi/mmole), the cells accumulated label as diagrammed in Fig 5. The initial rate of sulfate uptake was greater in reduced levels of cold carrier. Therefore, cells incubated in the normal MCYII medium (4×10^{-4} M $SO_4^=$) accumulated label at modest levels when compared to cells incubated in reduced levels of cold $SO_4^=$ (2×10^{-4} to no sulfate). It was concluded that the greatest accumulation of $^{35}SO_4^=$ by starved cells occurred in the absence of competing $SO_4^=$, and therefore all $^{35}SO_4^=$ pulses in pulse-chase experiments were conducted in the absence of cold carrier.

Pulse ($^{35}SO_4^=$)–chase ($SO_4^=$) experiments were conducted to determine the combined rate at which label was accumulated by log phase cells, and subsequently excreted into the medium as polysaccharide-^{35}S. Starved cells were pulsed, washed repeatedly in complete medium at 10°C, resuspended in complete medium, and cells and medium were sampled immediately for a zero-time analysis. Fig. 6 shows the accumulation of excreted polysaccharide-^{35}S in the medium and the change in rate of excretion after a 15 min pulse of $^{35}SO_4^=$. The rate at which the label appears fixed into solubilized capsular polysaccharide increases dramatically to approximately 2.5 hr after the pulse, then declines. By 6 hr the rate had declined to a low steady-state level. From Fig. 6 it is seen that isotope fixation after a 15 min pulse begins almost immediately and lasts approximately 4 hr, demonstrating that the available sulfate in the cells is fixed rapidly into the polysaccharide, and that the pathway leading to fixation is quite direct.

In the above experiment it was impossible to calculate the actual specific activity of the excreted polysaccharide, because the quantity of polysaccharide in the medium during the initial stages of the experiment was below the sensitivity of the anthrone assay. This is in part due to the rigorous washings after the pulse to remove free $^{35}SO_4^=$, a procedure which also removes most of the polysaccharide adhering to the cell surface. In addition, these experiments were conducted with log phase cells, where the amount of capsular mucilage is already at a minimum (Ramus, 1972). However, cells with a minimum of encapsulating mucilage are most desirable for these pulse-chase experiments. Here, the polysaccharide on the cell surface is immediately available for solubilization as it is deposited, a factor which increases the resolution of the experiment. Nevertheless, data concerning the specific activity of the excreted polysaccharide were desirable. Therefore, a longer term pulse-label experiment was conducted, one in which specific activity could be measured because polysaccharide levels would be in the sensitivity range of the anthrone assay (greater than 10 μg/ml). Therefore, a 4 hr $^{35}SO_4^=$ pulse was followed by a 168 hr (1 wk) chase.

Throughout the course of this experiment the accumulation of total polysaccharide in the medium (as measured by the anthrone assay) was approximately linear (Table I), while the $^{35}SO_4^=$ appearing in this polysaccharide fraction increased rapidly at first, then gradually leveled (Table I) The specific activity of the excreted polysaccharide increased to 50 hr, then slowly declined (Fig. 8) The rate of change in specific activity of the solubilized polysaccharide peaked at about 20 hr after the pulse, and then declined rapidly during the remainder of the experiment (Fig. 8). These data reassure us that whether in a short (15 min) or long (4 hr) term pulse-label experiment, the label appears in the medium as a burst which represents a radical change in the specific activity of the excreted polysaccharide. Further, only one

TABLE I

$^{35}SO_4^=$ Pulse (4 hr; 1 μCi/ml; 865 mCi/mmole)—Chase

hr after pulse	cpm × 10^{-5}/ml cells	μg/ml polysaccharide	cpm × 10^{-3}/ml medium
0	7.68	Below assay	4
2	7.46	Below assay	12
18	6.65	12.6	62
26	6.50	14.8	91
42	6.26	16.5	115
50	5.91	16.5	136
66	5.71	20.0	144
72	5.64	22.6	152
100	5.23	29.1	185
138	4.64	33.8	200
145	4.61	39.5	210
168	4.38	46.5	215

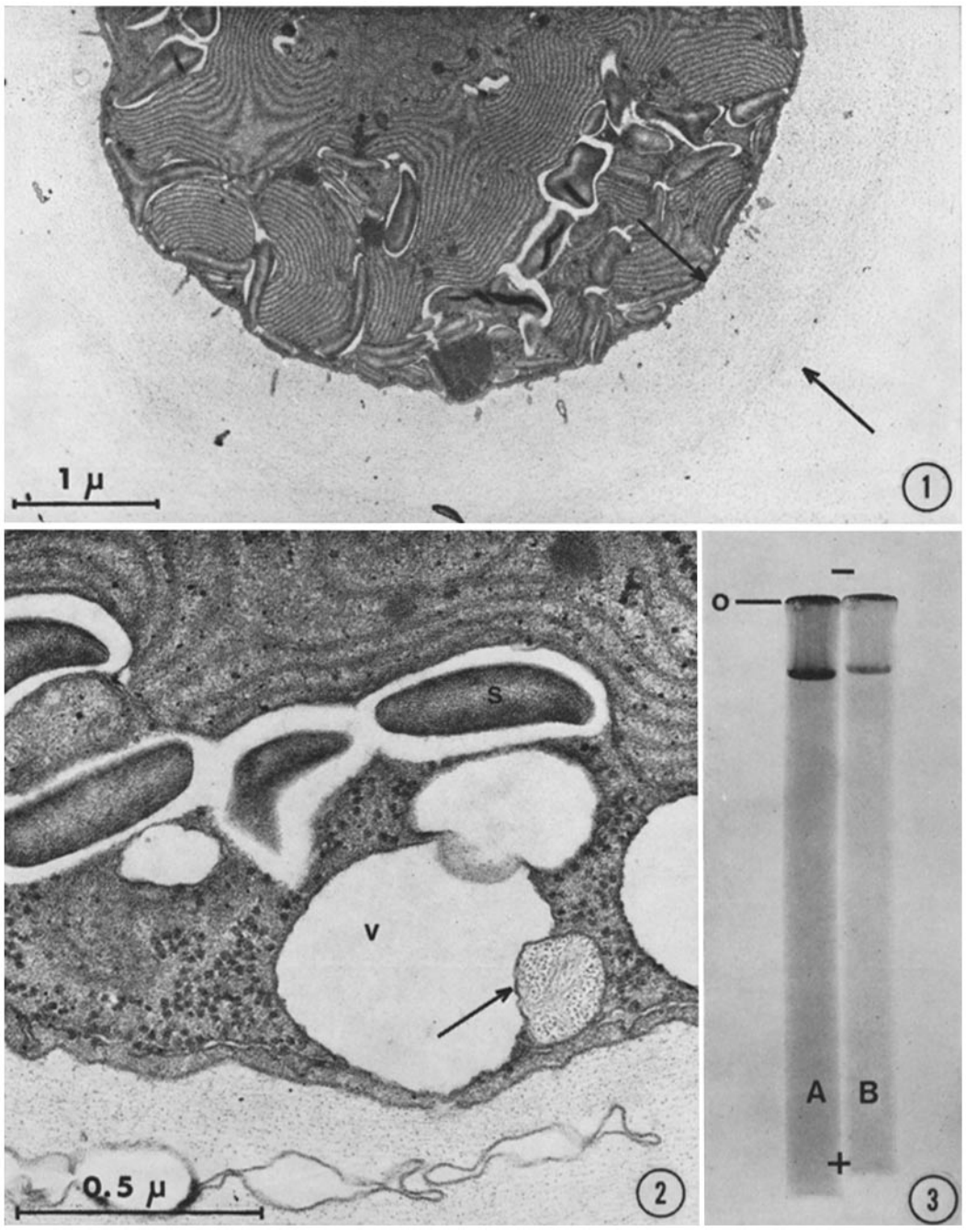
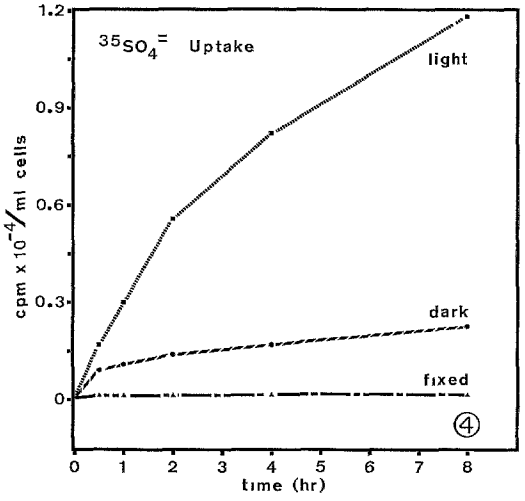
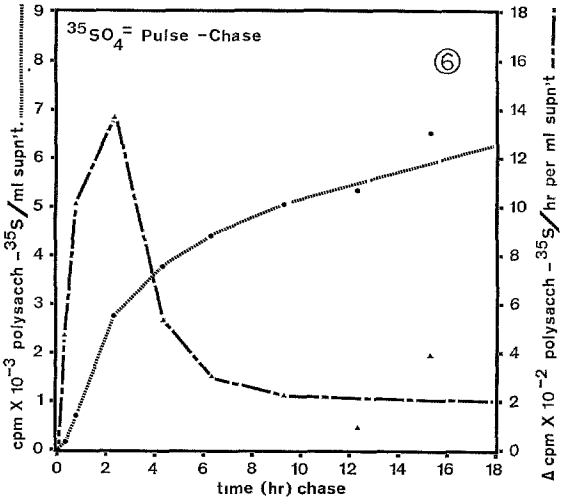


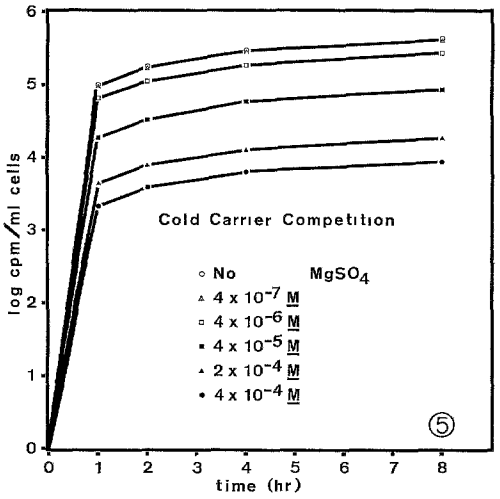
FIGURE 1 Section of stationary phase cell. Note fibrillar nature (arrows) of encapsulating polysaccharide. × 20,000.

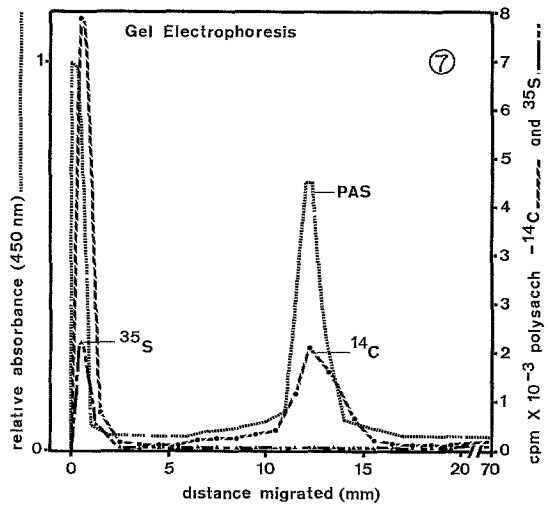
FIGURE 2 Membrane-bounded fibrillar material in cytoplasm (arrow), presumably a Golgi-derived vesicle transporting polysaccharide to the periphery of the cell. (*v*) vacuole; (*s*) starch × 70,000.

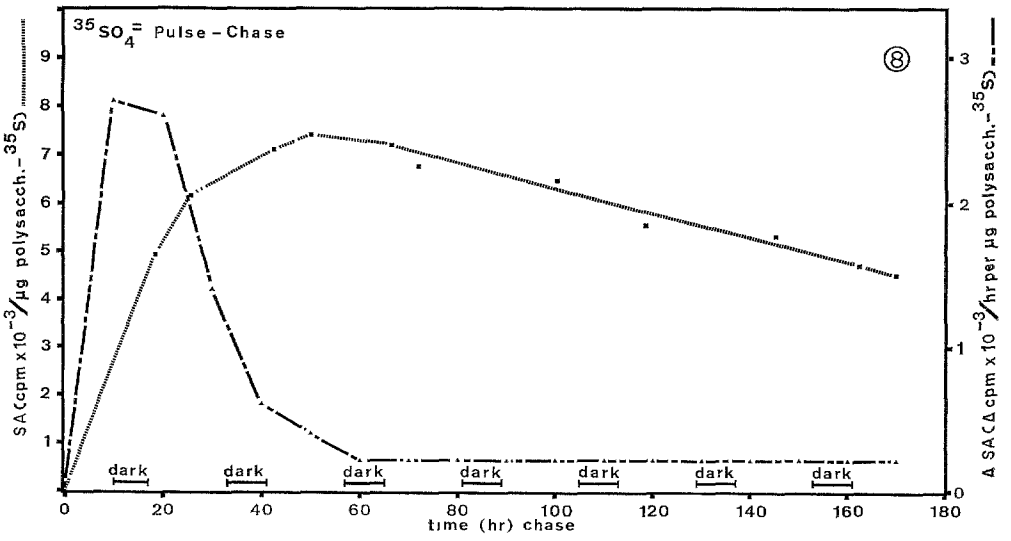
FIGURE 3 Electrophoresis of polysaccharide on 3% polyacrylamide gels. Gel (*A*) stained for polysaccharide by PAS technique, and (*B*) stained with alcian blue (*o*) origin. × 1.











burst appears, representing the original pulse The increasing level of radioactivity in the polysaccharide was accompanied by a concomitant drop in the level of radioactivity in the cell samples (Table I). At the end of the experiment 65% of the label that had disappeared from the cells could be accounted for in the excreted polysaccharide, i.e., (counts per minute gained in polysaccharide per milliliter of medium)/(counts per minute lost per milliliter of cells) = 0.65 × 100. The rest of the label (35%) probably leaked from the cells as free sulfate, or was excreted as low molecular weight metabolites as amino acids (Ramus, 1972).

Evidence for the presence of sulfate ester in the polysaccharide comes from several quarters, in addition to the experiments described above. Previously (Ramus, 1972), it was shown that polysaccharide precipitated (after dialysis) from the growth medium by the CPC/ethanol isolation protocol contained 7 8% sulfate by weight, based on the barium chloranilate assay of the acid hydrolysate Further, the polysaccharide is polyanionic (a conclusion based on its CPC precipitability) due mostly to sulfate ester.

If this anionic polysaccharide were placed in an electrophoretic field, any free sulfate should rapidly migrate from the polysaccharide, and conversely, the fixed sulfate should remain associated with the polysaccharide Therefore, polysaccharides-^{14}C and ^{35}S precipitated from dialyzed growth medium was resolubilized and migrated on 3% polyacrylamide gels The gels were then stained for polysaccharide (PAS and alcian blue), scanned in visible light, sliced into 1 mm segments, and counted. As seen in Figs. 3 and 7, a component of the PAS-positive and alcian blue binding material migrated into the gel, while the other component(s) stayed at or near the origin. About 75% of the ^{14}C label stayed at the origin (Fig. 7), while 25% of it co-migrated with the band. The coincidence of PAS reactivity, alcian blue stainability, and ^{14}C activity, combined with CPC precipitation recovery data (Ramus, 1972), is taken as proof for the presence of the polysaccharide on the gel. ^{35}S activity was found at or near the origin, but not in the migrated band. The fact that ^{35}S was still present after electrophoresis, that it coincided with ^{14}C activity and polysaccharide-specific staining reactions, is taken as evidence for fixation of the ^{35}S into the polysaccharide, undoubtedly as the sulfate ester The component which migrated appears to be sulfate free, indicating that the polysaccharide is heterogeneous in structure.

Sephadex G-200 gel filtration of polysaccharides-^{35}S and ^{14}C resulted in their exclusion from the column in the void volume. This indicates that the molecular weight of the polysaccharide is in excess of 2×10^5.

Evidence was needed to demonstrate that the sulfur present in the polysaccharide was bound as a sulfate ester Therefore, an acid hydrolysate of the polysaccharide-^{35}S was chromatographed on a silica gel, thin layer chromatograph (TLC) $Na_2{}^{35}SO_4$ was applied as a control. The only radioactivity in the hydrolysate co-migrated with the free sulfate control (Table II), indicating that the ^{35}S was present as acid-hydrolyzable sulfate.

FIGURE 4 Accumulation of sulfate-^{35}S by light-grown, dark-grown, and formalin-fixed cells. Approximately 3×10^6 cells/ml, log phase, 1 μCi $Na_2{}^{35}SO_4$/ml medium

FIGURE 5 Accumulation of sulfate-^{35}S by cells in the presence of varying concentrations of $MgSO_4$ (cold carrier) Approximately 3×10^6 cells/ml, log phase, 1 μCi $Na_2{}^{35}SO_4$ (carrier free)/ml medium.

FIGURE 6 Excretion of solubilized capsular polysaccharide-^{35}S. 15 min pulse, 1 μCi $^{35}SO_4^=$/ml, approximately 3×10^6 cells/ml, log phase cells, sulfate starved for 24 hr before pulse Graph shows appearance of label in CPC-precipitable fraction (counts per minute $\times 10^{-3}$ polysaccharide-^{35}S per milliliter of supernatant) and change in rate of appearance (Δ counts per minute $\times 10^{-2}$ polysaccharide-^{35}S per hour per milliliter of supernatant).

FIGURE 7 Electrophoresis of labeled polysaccharide on 3% polyacrylamide gels. Shown is correlation between PAS reactivity (relative absorbance at 450 nm), ^{14}C activity and ^{35}S activity.

FIGURE 8 Excretion of solubilized capsular polysaccharide-^{35}S. 4 hr pulse, 1 μCi $^{35}SO_4^=$/ml, approximately 3×10^6 cells/ml, log phase cells starved for 24 hr before pulse. Graph shows specific activity of the polysaccharide-^{35}S solubilized into the medium (counts per minute $\times 10^{-3}$ per microgram polysaccharide-^{35}S) and the rate of change of the specific activity (Δ counts per minute $\times 10^{-3}$ per hour per microgram polysaccharide-^{35}S).

TABLE II

TLC of Acid Hydrolyzed Polysaccharide-^{35}S

Spot	Sugar	R_f	cpm	Means of localization
1	—	0.04	4939	Radioautograph
2	Unknown	0.34	27	Reagent
3	Galactose	0 57	10	Reagent
4	Glucose	0.62	0	Reagent
5	Fucose(?)	0.69	1	Reagent
6	Xylose	0.74	12	Reagent
7	Unknown	0.79	16	Reagent
8	Unknown	0.86	11	Reagent
Control	$Na_2{}^{35}SO_4$	0.07	22916	Radioautograph

DISCUSSION

Porphyridium aerugineum represents a new and promising microorganism for the study of both biosynthesis of sulfated polysaccharides by red algae and Golgi complex–mediated processes in cells in general. *Porphyridium* is easily manipulated in axenic batch culture. The physiological state of cell populations (lag, log, stationary phases) can be regulated, and synchrony of the division cycles can be imposed by rigid photoregimes. The possibility exists for obtaining regulatory or structural mutants, resulting in impaired ability to produce a capsule. The Golgi complex is well developed, and appears to play a central role in the synthesis, transport, and deposition of polysaccharide on the cell surface. *Porphyridium* has only one capsular polysaccharide fraction (as opposed to the crystalline, semicrystalline, and amorphous fractions in other plants; see Northcote, 1971 for review), which minimizes ambiguity in biosynthetic studies. Finally, the surface area-to-volume ratio of *Porphyridium* is large (cells seldom exceed 8 μ in diameter), which also facilitates the study of surface phenomena.

Sulfated polysaccharides are present on the surfaces of all red algal cells (Percival, 1970), generally as amorphous mucilaginous filler between microfibrillar (mostly cellulosic) elements of the cell wall. Agar and carrageenan are the best known of these compounds, and are used extensively by manufacturers as colloidal stabilizers. Their role in the biology of the red algae is at present unknown.

To our knowledge, only one other similar investigation has been conducted on the incorporation of sulfate into the polysaccharide of a red alga, specifically the sulfation of carrageenan by *Chondrus crispus* (Irish moss) (Loewus et al., 1971). Here, sulfate uptake by immersed pieces of this multicellular seaweed was measured by disappearance of $^{35}SO_4^{=}$ from the incubation medium, in contrast with direct measurement of label picked up by *Porphyridium* cells. Cold carrier competition data are similar in both cases. With *Chondrus*, pulse labeling of the sulfated polysaccharide was not reported, presumably because of the difficulties involved in recovering polysaccharide quantitatively, although incorporation was ably demonstrated. Loewus et al. (1971) demonstrated the presence of ^{35}S in λ- and k-fractions of the carrageenan by elution on a Sephadex G-100 gel permeation column, and subsequent correlation of radioactivity with anthrone reactivity. *Porphyridium* extracellular polysaccharides were excluded from Sephadex G-200; therefore, polyacrylamide gel electrophoresis was used to demonstrate that ^{35}S was bound to the polysaccharide. The ^{35}S label was acid hydrolyzable, indicating that the sulfur is present in the polysaccharide as a sulfate ester.

The experiments described here demonstrate that *Porphyridium* cells incubated in medium containing $^{35}SO_4^{=}$ will accumulate label, then excrete $^{35}SO_4^{=}$-labeled polysaccharide in amounts that can be monitored easily. Pulse-chase experiments showed that sulfate fixation into the capsular polysaccharide is rapid and direct. Hence, the system should prove amenable to experiments designed to delineate the metabolic pathway(s) for incorporation of sulfate into the capsular polysaccharide. In other algae studied (Hodson et al., 1968), the sulfate ion is first activated

enzymatically in the presence of adenosine triphosphate to form adenosine-3′-phosphate-5′-phosphosulfate (PAPS). PAPS is then available to the cell for use as an activated sulfate donor. Experiments are presently in progress to ascertain whether the sulfate-activating enzymes and PAPS are indeed present in *Porphyridium*, and if so, whether they are involved in the synthesis of the sulfated polysaccharide

The Golgi complex appears to be the structural compartment within the cell which mediates the synthesis, intracellular transport, and release of some macromolecules to the surfaces of differentiating cells (see Whaley et al., 1972 and Northcote, 1971 for review) Our preliminary evidence suggests that the production of capsular polysaccharide by *Porphyridium* is also a Golgi complex–mediated process. Golgi complexes were seen to produce vesicles laden with fibrillar material similar to the polysaccharide on the cell surface (Gantt et al , 1968; Ramus, 1972). Therefore, to fully elucidate the role of the Golgi complex in this system, we are attempting to demonstrate the sequential movement of sulfate-^{35}S through membrane-bounded structural compartments within the cell, first by electron microscope radioautography (Jamieson and Palade, 1971), and then by isolation of polysaccharide synthetase particles (Ray et al , 1969).

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