JOURNAL OF BACTERIOLOGY, Dec. 1970, p. 1110-1118
Vol. 104, No. 3

Isolation and Characterization of an Extracellular Polysaccharide from *Physarum polycephalum*

J. JUSTIN McCORMICK, JUDITH C. BLOMQUIST, AND HAROLD P. RUSCH
McArdle Laboratory for Cancer Research, University of Wisconsin Medical School, Madison, Wisconsin 53706

Received for publication 9 September 1970

The myxomycetes are called slime molds because of the synthesis of copious amounts of extracellular material (slime) during parts of the life cycle. In *Physarum polycephalum*, small amounts of slime are produced during exponential growth of microplasmodia in shake flasks, but the amount of this slime increased 10- to 20-fold at 16 to 34 hr after microplasmodia were induced to form spherules by transferring them to salt solution. The slime obtained during both periods is the same; an acidic polysaccharide consisting of galactose, sulfate, and trace amounts of rhamnose. Analysis of the galactose-to-sulfate ratio gave a value of about 4 to 1. Infrared spectroscopy showed increased absorbance at 820 cm^{-1} characteristic of C-O-S vibrations. Electrophoresis on polyacrylamide gel revealed that the material moved as a single band which stained with Alcian Blue and periodic acid Shiff reagent. However, fractionation of identical material on Dowex columns and electrophoresis on cellulose acetate showed the slime to be made up of three major fractions. The polysaccharide appeared as an extracellular capsule closely adhering to the walls of the spherules. It could be separated from the wall by vigorous shaking. The increased synthesis of slime during spherulation was not blocked by cycloheximide, suggesting that new enzyme synthesis was not necessary for its formation.

Microplasmodia of *Physarum polycephalum*, which remain in spent medium in shake flasks, round up and make hard outer walls—a form called microsclerotia or spherules. Microplasmodia can also be induced to form spherules by transferring them to shake flasks containing salt solution (12) or to a medium containing mannitol (4). During spherule formation in spent medium or in salt solution, the solution in which the microplasmodia were shaken became extremely viscous. This viscous material, which can be separated by centrifugation, is a polysaccharide that could be precipitated by two volumes of ethanol. This report is about a method for the separation of this extracellular polysaccharide (slime) and about the nature of the material, the kinetics of its synthesis, and the effects of various metabolic inhibitors on its synthesis.

MATERIALS AND METHODS

Maintenance of cultures. Submerged stock cultures of *P. polycephalum*, subline M3c, were grown in shaken flasks at 22 C in 20 ml of semidefined medium plus hematin, as described by Daniel and Baldwin (5). Two-milliliter samples of microplasmodial suspensions were transferred to fresh medium at intervals of 44 to 48 hr, or 0.7-ml samples were transferred at intervals of 68 to 72 hr.

Spherules were prepared in one of two ways. (i) A full-grown culture of microplasmodia was allowed to shake in spent medium, or (ii) microplasmodia were transferred to salt solution about two-thirds of the way through exponential growth. Microplasmodia were centrifuged at 500 × *g* for 2 min to remove them from the growth medium; they were then washed once in 10 ml of salt solution and suspended in a volume of salt solution equal to the volume of growth medium they had been in. The salt solution was prepared by the method of Guttes and Guttes (12) as modified by Goodman et al. (11).

At selected intervals, 1-ml samples (unless otherwise specified) were removed from the shake flasks, centrifuged at 1,600 × *g* for 10 min, and stored at −20 C without removal of the supernatant. These samples were later used for determination of slime, glycogen, or free glucose.

Slime analysis. Before analysis, samples were thawed and centrifuged in the cold at 1,600 × *g* for 1 hr. The supernatant was removed, leaving a layer of slime overlying the pellet. This layer was carefully loosened, removed with a small spatula, and added to the supernatant. The pellet was then broken up and washed with 2 ml of 0.5% ethylenediaminetetraacetic acid (EDTA) and centrifuged for 10 min at 1,600 × *g*. The supernatant and slime were again removed and added to the supernatant from the first centrifugation. The pellet was retained for glycogen determinations.

Extracellular polysaccharide was extracted from the supernatant by a modification of the cetyl pyri-

dinium chloride (CPC) method of Scott (19). A 0.4-ml amount of a 10% CPC solution and a 0.5-ml volume of "heavy" celite prepared by the method of Schiller et al. (18) were added to each tube. The tubes were mixed well and centrifuged at 1,600 × *g* for 10 min at room temperature. When glucose determinations were made in the growth medium or salt solution, the supernatant was saved and tested with the Glucostat test (Worthington Biochemical Corp., Freehold, N.J.); otherwise, the supernatant was discarded.

The CPC-polysaccharide complex was washed twice with 0.04 M NaCl. Three milliliters of 1.2 M NaCl was added to each tube; the tubes were mixed and held overnight at room temperature. The tubes were centrifuged at room temperature at 1,600 × *g* for 10 min and the supernatants were retained. The celite pellet containing the CPC-slime complex was washed with 1.2 M NaCl until no hexose could be detected in the supernatant by means of the Dubois (8) phenol-sulfuric acid test. Supernatants from these washes were pooled and constituted the purified slime. For convenience and speed of extraction, 1.2 M NaCl was used, although 0.4 M NaCl will also solubilize the polysaccharide. The total slime present in this fraction was measured by the phenol-sulfuric acid test with galactose used as a standard.

When recovery of slime was desired, it was precipitated from this fraction by the addition of two volumes of 95% ethanol; the precipitate was washed in ethanol and dried in a vacuum oven at 45 C.

Glycogen analysis. The pellets to be analyzed for glycogen were suspended in 2 ml of distilled water and treated for 30 sec at a power setting of 6 with a Branson Sonifier (model LS-75) equipped with a microtip to release bound glycogen. Since sonic treatment does not rupture spherules, a French press (American Instrument Co., Silver Spring, Md.) was used on all preparations which contained spherules. Breakage was judged complete when no intact spherules could be found upon microscopic examination of the preparation. An equal volume of 30% KOH was added to each tube, and the contents were extracted by the Van Handel (22) procedure. Total glycogen was determined by the phenol-sulfuric acid test (8) with glucose as a standard or with the iodine method of Krisman (13). The methods gave equivalent results.

Chromatography and electrophoresis. The molecular species of the extracellular polysaccharide was determined on columns (22 by 1 cm) of Bio-Rad purified Dowex AG-1X2 (Cl), 100 to 200 mesh. A 10-mg sample of extracellular polysaccharide was applied to the column and eluted with a linear 0 to 1.5 M NaCl gradient with or without 8 M urea (16).

Electrophoresis of crude and purified extracellular polysaccharide was done with "Sepraphore III," cellulose acetate strips in a Gelman (Ann Arbor, Mich.) apparatus by the method of Pedrini and Pedrini-Mille (17). Electrophoresis was carried out at 250 v for 2 hr. A buffer of 0.05 M tris(hydroxymethyl)-aminomethane (Tris)–0.03 M boric acid–0.012 M EDTA (*p*H 8.5) was used, and Alcian Blue (0.5%), dissolved in 5% acetic acid, was used as a stain.

Gel electrophoresis was carried out by the method of Davis (6) with 5% polyacrylamide-gel columns (0.5 by 10.0 cm). Electrophoresis was performed for 60 min in 0.1 M Tris–glycine buffer (*p*H 8.3) with a current of 4 ma per gel. Staining was carried out with Amido Black (6) or with 1% Alcian Blue in 5% acetic acid for 4 hr (2). Excess stain was removed by frequent changes of 5% acetic acid. Some gels were then stained with periodic acid Shiff reagent by the method of Zacharius et al. (23).

Acid hydrolysis of the extracellular polysaccharide was carried out for 6, 12 and 18 hr in 1 N HCl or 6 N HCl at 100 C in a vacuum. HCl was removed by repeated evaporations at 45 C in a vacuum oven or with ion-exchange resins.

Chromatography was done on Whatman no. 1 paper or with MN-Polygram (Brinkman Instruments Inc., Westbury, N.Y.) cellulose thin-layer plates. Solvent systems used were butanol-pyridine-water (6:4:3, v/v), butanol-acetic acid-water (3:1:1, v/v), and pyridine-ethyl acetate-acetic acid-water (5:5:1:3, v/v). Ammoniacal silver nitrate, ninhydrin, and Galactostat (Worthington Biochemical Corp., Freehold, N.J.) were used as spray reagents to detect reducing sugars, amino compounds, and galactose, respectively.

Radioactive labeling. To label slime with ^{14}C, microplasmodia were inoculated into growth medium containing 0.1 μCi of glucose-*UL*-^{14}C per ml. Slime labeled with ^{35}S was prepared by adding microplasmodia to salt solution containing 1.0 μCi of ^{35}S-sulfate per ml or by growing microplasmodia in medium containing ^{35}S-sulfate at the same concentration and then transferring them to nonradioactive salt solution for transformation to spherules. D-Glucose-*UL*-^{14}C (240 mCi/mmole) was purchased from Schwarz BioResearch Inc., Orangeburg, N.Y. ^{35}S-labeled sulfate (carrier free) was purchased from Amersham/Searle, Des Plaines, Ill. To count radioactive fractions, 0.1 ml of a sample was added to 10 ml of "Scintisol" (Isolab Incorporated, Akron, Ohio), and radioactivity was determined on a Packard Tri-Carb liquid scintillation spectrometer.

Other procedures. Infrared spectrophotometry was done on a Beckman IR 10 infrared spectrophotometer. Slime was prepared for infrared analysis by making a pellet from 1.5 mg of purified slime and 200 mg of KBr with a dye and press. For sulfate analysis, the EDTA method of Lloyd et al. (14) was used.

Chemicals. β-Galactosidase was purchased from Sigma Chemical Co., St. Louis, Mo. Actinomycin D was a gift of K. Beyer of Merck, Sharp and Dohme.

RESULTS

Purification of polysaccharide. After removal of spherules from the salt solution by centrifugation, the viscous supernatant was reacted with the detergent, CPC. At this point, the solution was approximately as viscous as water, and a yellow-white "clump" of CPC-polysaccharide complex floated in the solution; this was centrifuged into celite which had been added to the tube. Figure 1 shows that the CPC-polysaccharide complex dissociated in 0.4 M NaCl, but not in 0.04 M NaCl.

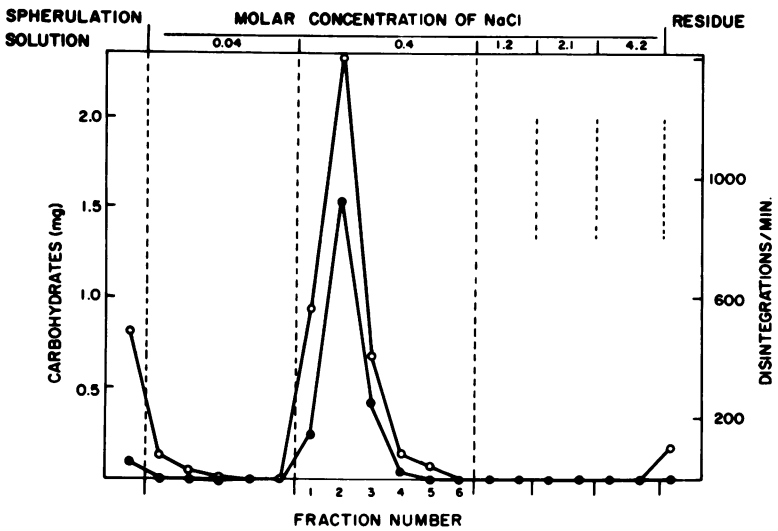

FIG. 1. *Elution pattern of slime from the CPC-slime complex by stepwise addition of increasing molarity of NaCl.* ●, *Colorimetric determinations by the phenol-sulfuric acid method;* ○, *radioactivity determinations. The phenol-sulfuric acid-positive material in the spherulation solution was determined to be glucose by the Glucostat test.* ^{14}*C-glucose was used as the source of the label.*

Radioactivity could be incorporated into the phenol-sulfuric acid-positive material by labeling with ^{14}C-glucose (0.10 μCi/ml) during the logarithmic phase of growth of microplasmodia. The phenol-sulfuric acid-positive material that remained in the spherulation solution (Fig. 1) after CPC precipitation was shown to be glucose by the Glucostat test.

Since a number of similar polysaccharides could go into solution between 0.04 M and 0.4 M NaCl, the peak shown in Fig. 1 may not represent a single compound. To examine this point further, crude extracellular polysaccharide or CPC-purified polysaccharide was dissolved in 8 M urea and run on a polyacrylamide-gel electrophoresis or applied to columns of Dowex AG-1X2 and eluted with a linear salt gradient as described above. Figure 2 shows the profile of the gel electrophoresis. All of the material travels as a single slow-migrating band, which stains with Alcian Blue and with periodic acid Schiff reagent but not with Amido Black. On occasion, slime preparations contained another acidic polysaccharide which moved near the front on gel electrophoresis. This material is a glycoprotein containing galactose and is now under study. All analytical results reported here were done on slime found to travel as a single slow-migrating band on gel electrophoresis. When the slime was eluted from a Dowex column, however, three major peaks (A, B, and C) and occasionally up to three minor peaks were seen (Fig. 3).

To determine whether the extracellular polysaccharide synthesized in small quantities during growth and that synthesized in large quantities during spherule formation were the same, the supernatant solution obtained from six shake flasks of microplasmodia in early logarithmic growth was purified by extraction with CPC. Dowex column chromatography showed that this material was identical to the polysaccharide synthesized during spherule formation. Indication for a number of molecular species was shown by electrophoresis on "Sepraphore III." Electrophoresis of the crude slime gave the wide band seen at the top of Fig. 4, whereas electrophoresis of fractions A, B, and C from the Dowex columns gave the respective patterns shown in Fig. 4. The wide band seen with the unfractionated slime, therefore, is composed of fractions A, B, and C. The close similarity of these fractions did not allow them to be separated by electrophoresis of the crude material.

Characterization of the polysaccharide. To investigate the source of the negative charge of the polysaccharide, sulfate with a ^{35}S label was added to the microplasmodia, in a logarithmic phase of growth or when they were transferred to salt solution. Radioactive sulfur was associated with the polysaccharide synthesized during spherule formation. To determine whether this association represents incorporation of ^{35}S or mechanical entrapment, the CPC-purified slime was precipitated with ethanol and centrifuged, and the precipitate was redissolved in 1.2 M NaCl. Each time the material was dissolved, samples were

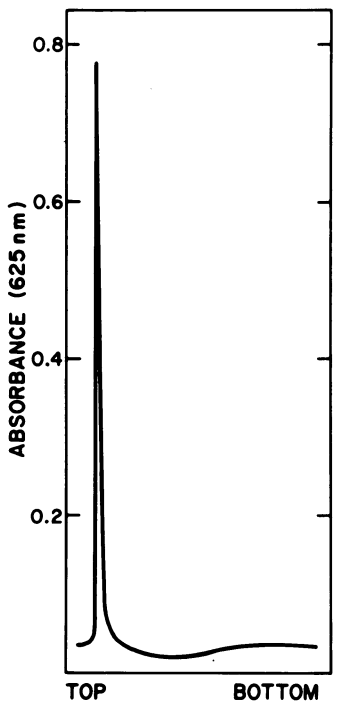

FIG. 2. *Profile of slime run on polyacrylamide-gel electrophoresis and stained with 1% Alcian Blue and with periodic acid Shiff reagent. The slime moved as a single homogeneous band—no other bands were seen.*

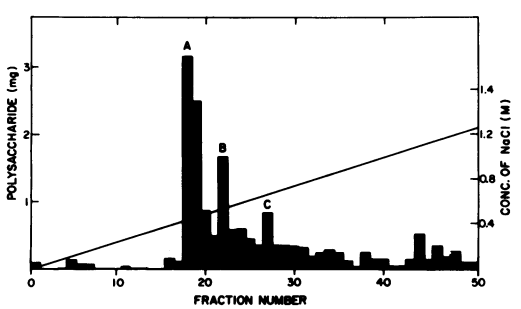

FIG. 3. *Elution profile of Physarum slime from a Dowex AG 1X2 column with a continuous, linear NaCl gradient containing 8 M urea. Fractions A, B, and C are the three major peaks found in all preparations.*

removed for determination of radioactivity and galactose content. The specific activity remained constant in experiment A (Table 1), in which the label was added at the time the microplasmodia were transferred to salt solution, and in experiment B, in which the label was added at the time the microplasmodia were inoculated into the growth medium. In experiment B, the microplasmodia were washed free of growth medium after 48 hr of growth and transferred to salt solution. The ^{35}S-sulfate was thus taken up by the microplasmodia during growth and used for slime synthesis during starvation. Since the specific activity of the ^{35}S remained constant, it appeared that it was a constituent of the polysaccharide, probably in the form of sulfate groups. Direct sulfate analysis of the polysaccharide was undertaken as described above; the results are shown in Table 2. The galactose and sulfate concentrations were measured for three inde-

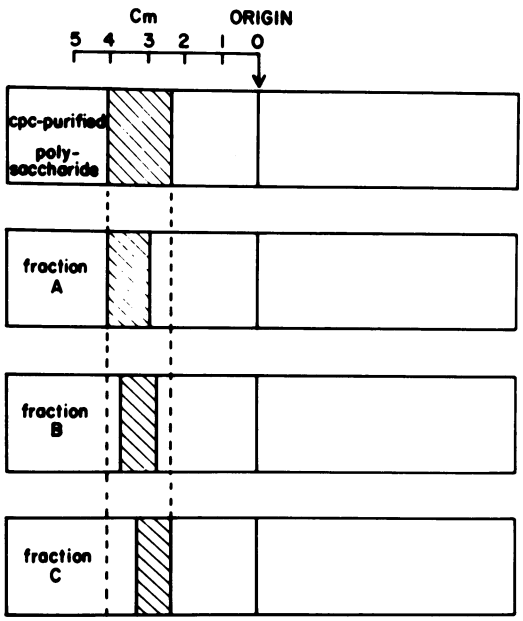

FIG. 4. *Electrophoretic pattern of Physarum slime run on "Sepraphore III" and stained with 0.5% Alcian blue. Fractions A, B, and C were obtained by precipitating these fractions with ethanol from the Dowex column eluant. Cathode is to the right.*

TABLE 1. *Incorporation of ^{35}S sulfate into Physarum polycephalum*

Expt A[a]	Specific activity[b]
1st Precipitation	7,852
2nd Precipitation	7,830
3rd Precipitation	8,170
Expt B[c]	
1st Precipitation	7,258
2nd Precipitation	8,058
3rd Precipitation	7,755

[a] ^{35}S (1 μCi/ml) added to salt solution.
[b] Results expressed as counts per minute per milligram of galactose.
[c] ^{35}S (1 μCi/ml) added to growth medium.

TABLE 2. *Analysis of 10-mg samples of extracellular polysaccharide for galactose and sulfate*

Determination	Sample		
	1	2	3
Galactose[a] (mg)	8.02	8.20	8.50
Sulfate (mg)	1.04	1.06	1.02
Recovery (%)	91	93	95
Molar ratio of galactose-sulfate	4.2/1	4.2/1	4.5/1

[a] Phenol-sulfuric acid-positive material with galactose as the standard.

pendent samples of the material giving galactose-to-sulfate ratios of 4.2, 4.2, and 4.5.

To determine the number of anionic groups per repeating unit of polymer, fractions A, B, and C, obtained from Dowex column chromatography, were reacted with a carbocyanine dye by the method of Edstrom (9). The absorption maximum of the dye-polysaccharide complex has a characteristic value which is dependent upon the ratio of repeating units to anionic groups. The maxima for fractions A, B, and C were between 640 and 645 nm (Fig. 5). From Edstrom's data, this suggests a ratio of one anionic group to each three repeating units. All three fractions appear to have about the same maximum, although there are small but reproducible differences between the fractions (A always peaks at a wave number slightly higher than B, and B slightly higher than C).

Chromatography of acid hydrolysates of polysaccharide prepared as described above yielded a single ammoniacal silver nitrate-positive spot which moved with the same R_F as galactose in the three solvent systems. This spot also gave a positive reaction with Galactostat (used as a spray reagent). No ninhydrin-positive spots were found. When chromatograms were heavily loaded, trace amounts of a carbohydrate that moved with the same R_F as rhamnose were also found (W. Grant, *unpublished data*). Chromatography of acid hydrolysates of polysaccharide labeled with ^{14}C yielded only one radioactive spot which migrated in the same manner as galactose. Hydrolysates prepared with 1 N HCl for 12 hr were analyzed by the phenol-sulfuric acid test with galactose as a standard and with Galactostat. These tests gave identical values for the amount of galactose and confirmed the absence of all but trace amounts of hexoses or pentoses.

Slime preparations from salt solution purified with the CPC extraction were examined for uronic acids by the method of Galambos (10), for protein by the method of Lowry et al. (15), and for phosphate by the method of Chen and Toribara (3). Less than 1% of these materials was present on a weight basis. The sulfate and galactose content of slime preparations accounted for about 95% of the dry weight.

To test for β linkages, preparations were incubated in 0.1 M Tris buffer, *p*H 7.0, for 24 hr at 37 C or at room temperature, with β-galactosidase (1 mg/ml). After precipitation of the slime with three volumes of acetone and centrifugation, no polysaccharide or hexose could be detected in the supernatant by the phenol-sulfuric acid test.

The purified slime was also examined by infrared spectrophotometry. Figure 6 shows the spectrum obtained with the absorption at 820 cm^{-1} characteristic of C-O-S vibrations.

Kinetics of synthesis. When microplasmodia were transferred from a medium which permitted logairthmic growth to a salt solution, slime synthesis and spherule formation occurred readily. Figure 7 shows the relation between the glycogen content of the microplasmodia and of young spherules and the formation of slime. Spherules were first seen 23 hr after transfer to

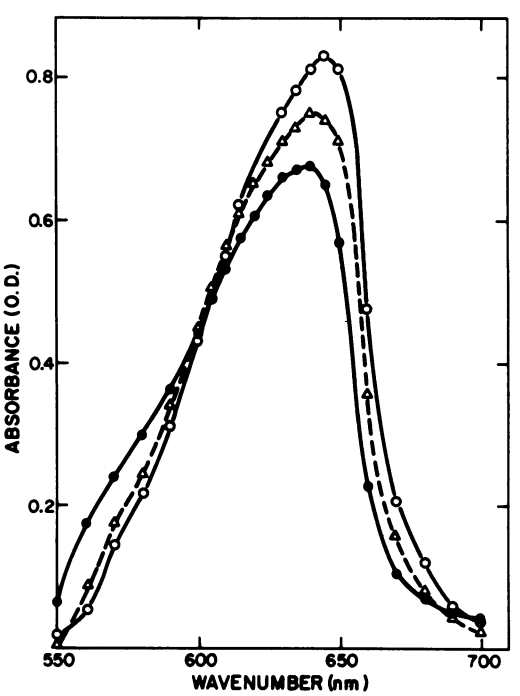

FIG. 5. *Profile of the A, B, and C fractions from Fig. 4 when reacted with a carbocyanine dye specific for acidic groups (Edstrom, 1969). Symbols: ○, A fraction; △, B fraction; ●, C fraction. The maximal reading of A is always at a slightly higher wavelength than B, and B slightly higher than C.*

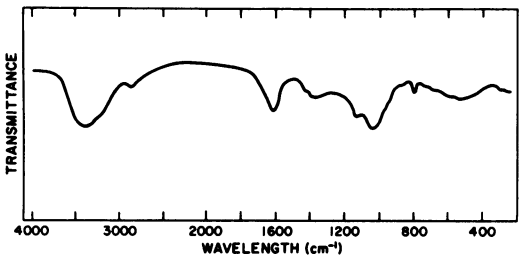

FIG. 6. *Infrared spectrum of P. polycephalum slime. Note the increased absorbance at 820 cm^{-1} characteristic of C-O-S vibration.*

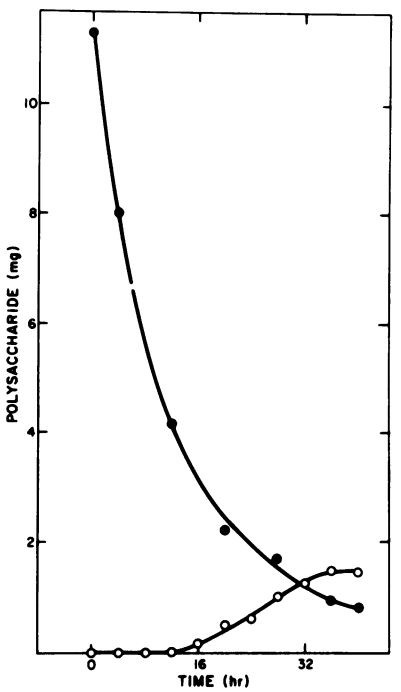

FIG. 7. *Relation of glycogen depletion (●) and slime synthesis (○) during the process of sperulation on salt solution. At ○ time, the logarithmically growing microplasmodia are transferred to salt solution.*

salt solution. About 95% of the microplasmodia were transformed to spherules by 35 hr.

When microplasmodia were left to shake in spent growth medium, slime synthesis and spherule formation also occurred. Figure 8 shows the relation of the major carbohydrates (glucose in the medium, glycogen, and slime) available to the microplasmodia during a period of exponential growth (about 12 to 60 hr) and during the lag phase that followed. Spherulation was about 95% complete at 105 hr in this experiment.

Spherulation induced by the polyol method of Chet and Rusch (4) failed to show any large increase in extracellular polysaccharide.

Sulfate with ^{35}S label, when added to salt solution at the beginning of starvation of the microplasmodia, was incorporated into the extracellular polysaccharide at the same rate that it was formed (Fig. 9). It appeared, therefore, that the ^{35}S was incorporated into the polysaccharide as it was being synthesized by the organism.

Figure 10 shows the effect of actinomycin D upon the formation of slime during the process of spherule formation. When the inhibitor was added at the time that the microplasmodia were

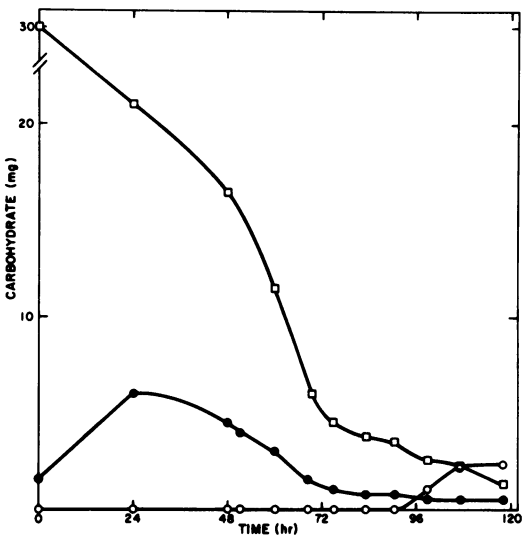

FIG. 8. *Relation of the amounts of glycogen (●), slime (○), and glucose (□) in the medium during the process of microplasmodia growth and spherulation (in spent growth medium). The formation of spherules was morphologically complete at about 105 hr. A 2.0-ml inoculum was used as described, and results are given per 3.0-ml sample.*

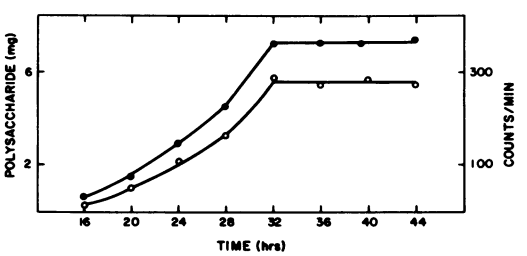

FIG. 9. *Relation of slime synthesis (●) and incorporation of ^{35}S-sulfate into the slime (○) during the process of spherule formation on salt solution.*

transferred to salt solution, only 50% as much slime accumulated in such cultures as in control cultures. Addition of actinomycin D at 7 or 14 hr after transfer of the microplasmodia to the salt solution had no effect upon spherule formation nor upon the total amount of extracellular polysaccharide synthesized. Actinomycin D added at 7 hr affected the kinetics of synthesis, whereas its addition at 14 hr had no effect on the kinetics of synthesis (Fig. 10). Synthesis of slime was complete at 34.5 hr in this study.

Cycloheximide at a concentration of 10 μg/ml, a level sufficient to inhibit protein synthesis, was administered at the time that microplasmodia were transferred to salt solution. After 32 hr, microplasmodia were almost completely lysed. Despite this, there was no inhibition of slime synthesis. The total amount of slime and the time course of its synthesis did not differ significantly from that of control cultures.

Function of the polysaccharide. Spherule walls are first seen shortly after the period of rapid slime synthesis has begun. It was suggested, therefore, that the slime might be, in part, a precursor to the spherule walls. To test this hypothesis, microplasmodia in salt solution were incubated with CPC-purified slime labeled with ^{14}C. When spherule formation was complete, less than 5% of the radioactivity was associated with the completed spherules; the remainder was recovered in the slime. This suggests that the slime is not a precursor of the wall.

Some fungi have been shown to use extracellular polysaccharide as a reserve carbon source (7, 21). To test this possibility, microplasmodia were incubated for 30 days in salt solution.

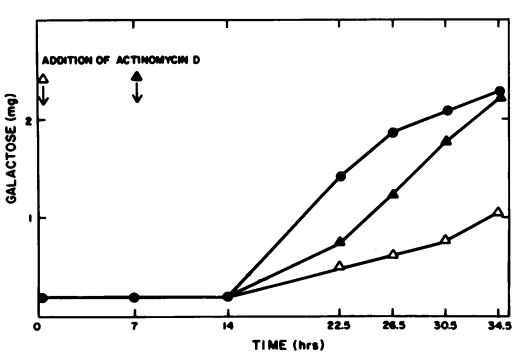

FIG. 10. *Effect of actinomycin D on formation of slime during spherule formation on salt solution. Control culture (●), actinomycin D added at 7 hr (▲), actinomycin D added at 0 hr (△). Actinomycin D, when added at 14 hr, gave results identical to that of the control. At 34.5 hr, slime values reached a plateau and did not increase further.*

Maximal slime synthesis occurred in less than 48 hr, and there was no decrease in the amount present at the end of 30 days. It is possible, however, that the extracellular polysaccharide is metabolized by *P. polycephalum* under conditions other than starvation. Therefore, spherules and microplasmodia were incubated in growth medium (with or without glucose) containing added CPC-purified ^{14}C slime. No growth occurred unless the medium also contained glucose. As the cultures neared the end of logarithmic growth in the medium which contained glucose, more than 95% of the ^{14}C-labeled material was recovered as slime. It appears, therefore, that the slime cannot be metabolized under these conditions.

DISCUSSION

In the process of spherule formation in spent growth medium or salt solution, *P. polycephalum* shows a 10- to 20-fold increase in extracellular polysaccharide over a 14- to 16-hr period. During logarithmic growth in shaken flasks, the organism produces slime at a very slow rate. Indeed, the amount of slime produced during such conditions of growth is so limited that it forms only a thin layer over the top of the microplasmodial pellet after centrifugation. This layer is so thin that it cannot be quantitatively separated from the microplasmodial pellet by the techniques employed for spherule samples.

Our evidence indicates that the slime in the starvation medium and that which surrounds the outer walls of the spherules are identical. Under stationary conditions, the slime tends to adhere to the outer wall of the spherules during the formation and forms a thick coating, and little or no polysaccharide is found in the medium. When the spherules are in shaken flasks, however, much of the slime is separated from the spherules and is found in the medium. Thus, it would appear that the slime is the same regardless of its location.

The slime is a sulfated galactose polymer containing trace amounts of rhamnose. The ratio of galactose to rhamnose appears to be greater than 50 to 1 (as judged by chromatography). The slime migrates as a single homogeneous band near the top of a gel after electrophoresis. It is Alcian Blue-positive, indicating the presence of acidic groups and periodic acid Schiff reagent-positive, indicating the presence of 1,2-glycol moieties.

Direct sulfate analysis indicates a ratio of sulfate to galactose of about 1 to 4, whereas dye-binding studies suggest a ratio of 1 to 3. Since Edstrom (9) gives no dye-binding data on a

material with a 1 to 4 ratio and since no such control material was available, the results of the direct analysis are probably more reliable.

The failure of β-galactosidase to attack agarose, an alternating polymer of α-1-3–, β-1-4–linked galactose units (McCormick, *unpublished data*), suggests that the enzyme cannot act against internal β linkages. Thus, the failure of β-galactosidase to attack slime suggests that it is not made up only of β-linked galactose units. Furthermore, the slime is antigenically related to type XIV *Pneumococcus* polysaccharide (*personal communication*, M. Heidelberger) which is understandable since both contain galactose. At an early stage in the investigation, it was thought that the slime might be similar to carrageenan, which is a α1-3–, β1-4–linked sulfated galactose polymer synthesized by a number of marine algae (1). However, Dowex chromatography of authentic carrageenan shows that its elution profile has little in common with the polysaccharide of *P. polycephalum*.

After the completion of this work, Simon and Henney (20) reported that the slime of *P. polycephalum* and two related species consisted of glycoproteins containing galactose. Their data showed a "highly variable" galactose-to-protein ratio which probably resulted from the contamination of the glycoprotein with the polysaccharide we described in the present paper. Our results show that these two substances can be readily separated by gel electrophoresis.

Although gel electrophoresis of the polysaccharide suggests a homogeneous polymer, fractionation on a Dowex column reveals that the polymer consists of three fractions whose ratios of sulfate to galactose differs only slightly, if at all. The fractions migrate at slightly different rates with some overlap between the bands A and B, and B and C (Fig. 4). Electrophoresis of the CPC-purified polysaccharide on "Sepraphore III" revealed one broad band covering approximately the same area as bands A, B, and C. Thus, this procedure does not have as high a resolution, under our conditions, as does Dowex column chromatography.

Although the function of the large amounts of slime formed during spherulation is not precisely known, its sticky, gel-like characteristics may play a role as a cementing agent to hold spherules to surfaces; to act as a hydrophilic agent in retaining water which would aid germination; to protect the organisms from foreign materials including bacteria by entrapment or by serving as an ion-exchange material; and to serve as a source of carbon when needed. The latter suggestion is erroneous, however, as shown in this report and in view of the fact that Daniel and Baldwin (5) previously showed that galactose could not be used as a source of carbon.

Slime formation appears to be a secondary and not an essential part of spherulation, since spherule formation may be induced by the addition of mannitol with only a minimal amount of slime production (4). Spherules initiated in this manner exist as individual units rather than as a packet surrounded by a thick layer of slime. It is also of interest that the synthesis of slime can continue even though spherulation is inhibited by cycloheximide. It would appear, therefore, that enzyme synthesis is unnecessary for the synthesis of slime. The partial blockage of slime formation by actinomycin D is not consistent with the above interpretation, but the effect of this inhibitor may be secondary to its influence on ribonucleic acid synthesis, owing to the extremely high levels (300 μg/ml) used.

ACKNOWLEDGMENTS

This work was supported by Public Health Service grants CA-07175 and CA-5002 from the National Cancer Institute, and by a grant from the Alexander and Margaret Steward Trust Fund.

We thank Gene Sherman for assistance with the infrared spectroscopy and LaVila Winnie for her technical assistance.

LITERATURE CITED

1. Anderson, N. S., T. C. S. Dolan, and D. A. Rees. 1965. Evidence for a common structural pattern in the polysaccharide sulphates of the *Rhodophyceae*. Nature (London) **205**:1060–1062.
2. Caldwell, R. C., and W. Pigman. 1965. Disc electrophoresis of human saliva in polyacrylamide gel. Arch. Biochem. Biophys. **110**:91–96.
3. Chen. P. S., and T. Y. Toribara. 1956. Microdetermination of phosphorus. Anal. Chem. **28**:1756–1758.
4. Chet, I., and H. P. Rusch. 1969. Induction of spherule formation in *Physarum polycephalum* by polyols. J. Bacteriol. **100**: 673–678.
5. Daniel, J. W., and H. H. Baldwin. 1964. Methods of culture for plasmodial myxomycetes, p. 9–41. *In* D. M. Prescott (ed.), Methods in cell physiology, vol. 1. Academic Press Inc., New York.
6. Davis, B. J. 1964. Disc electrophoresis II. Methods and application to human serum proteins. Ann. N.Y. Acad. Sci. **121**:404–427.
7. Davis, E. N., R. A. Rhodes, and H. R. Shulke. 1965. Fermentative production of exocellular glucans by fleshy fungi. Appl. Microbiol. **13**:267–271.
8. Dubois, M., K. A. Giles, J. K. Hamilton, P. A. Rebers, and F. Smith. 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. **28**:350–356.
9. Edstrom, R. D. 1969. A colorimetric method for the determination of mucopolysaccharides and other acidic polymers. Anal. Biochem. **29**:421–432.
10. Galambos, J. T. 1967. The reaction of carbazole with carbohydrates. I. Effect of borate and sulfamate on carbazole of sugars. Anal. Biochem. **19**:119–132.
11. Goodman, E. M., H. W. Sauer, L. Sauer, and H. P. Rusch. 1969. Polyphosphate and other phosphorus compounds during growth and differentiation of *Physarum polycephalum*. Can. J. Microbiol. **15**:1325–1331.
12. Guttes, E., and S. Guttes. 1963. Starvation and cell wall forma-

tion in the myxomycete *Physarum polycephalum*. Ann. Bot. 27:49–53.
13. Krisman, C. R. 1962. A method for the colorimetric estimation of glycogen with iodine. Anal. Biochem. 4:17–23.
14. Lloyd, P. F., B. Evans, and R. J. Fielder. 1969. Determination of sulphate and of barium in carbohydrate sulphates by flame photometry. Carbohyd. Res. 11:129–136.
15. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193:265–275.
16. Pearce, R. H., J. M. Mathieson, and B. J. Grimmer. 1968. Fractionation of anionic glycosaminoglycans by ion-exchange chromatography. Anal. Biochem. 24:141–156.
17. Pedrini, V., and A. Pedrini-Mille. 1968. Keratan sulfate-protein complexes from human costal cartilage, p. 139–151. *In* G. Quintarelli (ed.), The chemical physiology of the Mucopolysaccharides. Little, Brown and Co., New York.
18. Schiller, S., G. A. Slover, and A. Dorfman. 1961. A method for the separation of acid mucopolysaccharides: its application to the isolation of heparin from the skin of rats. J. Biol. Chem. 236:983–987.
19. Scott, J. E. 1960. Aliphatic ammonium salts in the assay of acidic polysaccharides, p. 145–196. *In* D. Glick (ed.), Methods of biochemical analysis, vol. 8. Interscience Publishers, Inc., New York.
20. Simon, H. L., and H. R. Henney, Jr. 1970. Chemical composition of slime from three species of myxomycetes. FEBS Letters 7:80–82.
21. Szaniszlo, P. J., C. Wirsen, Jr., and R. Mitchell. 1968. Production of a capsular polysaccharide by a marine filamentous fungus. J. Bacteriol. 96:1474–1483.
22. Van Handel, E. 1965. Estimation of glycogen in small amounts of tissue. Anal. Biochem. 11:256–265.
23. Zacharius, R. M., T. E. Zell, J. H. Morrison, and J. J. Woodlock. 1969. Glycoprotein staining following electrophoresis on acrylamide gels. Anal. Biochem. 30:148–152.