CHEMICAL COMPOSITION OF SLIME FROM THREE SPECIES OF MYXOMYCETES

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1. Introduction

The myxomycetes are commonly known as slime molds but little is known about the chemical makeup of the viscous, extracellular slime characteristically secreted by their naked, migrating plasmodia. We have previously shown [1] that slime production was much more abundant when pure cultures of plasmodia were grown in synthetic medium containing galactose, glucose or mannose. This was especially true if protein hydrolysate was also included in the medium.

In this study *Physarum flavicomun* variety 1, *P. polycephalum,* and *P. rigidum* were grown in pure culture in the synthetic liquid medium until slime production was maximal. The isolated, purified slimes were found to be glycoproteins of similar chemical composition. The carbohydrate portions were composed of the neutral sugar galactose and the proteins contained similar proportions of the common protein amino acids.

2. Materials and methods

Plasmodia of the three species were grown in pure culture in a synthetic medium [1] containing modified basal salts [2]. After the maximum growth yield was attained the microplasmodia were separated from the slime by low speed centrifugation. The slime was removed from the viscous supernatant by gradual winding on a glass rod after layering with 5 volumes of 95% ethanol [1]. The slime was dissolved in 1.5 volumes of distilled water at 75°C and then removed by winding on a glass rod from the cooled solution layered with 2 to 5 volumes of freshly prepared acidified acetone (1% (v/v) reagent grade conc. HCl in redistilled acetone). The slime could be dissolved as before and isolation with acidified acetone could be performed for a total of 3 times before extensive fragmentation occurred.

Protein content was determined and amino acid analyses performed as previously described [3]. Total carbohydrate content was determined by the anthrone method [4] using galactose as the standard. For hexosamine analyses, hydrolysis was performed with 4 N HCl for 5 to 7 hr at 100°C and the procedures of Boas [5] were followed. For determination of sialic acid, hydrolysis in 0.1 N H_2SO_4 for 1 hr at 80°C was used, together with the methods of Svennerholm [6, 7]. The determination of fucose was according to the method of Dische and Shettle [8] and uronic acids according to Dische [9]. For the recovery of neutral sugars, hydrolysis was performed with 2 N H_2SO_4 for 6 hr at 100°C and the ionic substances removed by the chromatographic method of Lamkin, et al. [10]. Glucose and galactose determinations were performed using the Glucostat and Galactostat Reagents (Worthington, Freehold, New Jersey).

Thin layer chromatography was performed with cellulose Chromagram sheets (Eastman Kodak, Rochester, New York) using the solvent and detection method of Lamkin, et al. [10]. For two dimensional chromatography the second solvent was butan-1-ol-acetone-0.1 M boric acid (4:5:1 v/v/v).

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Table 1
Amino acid composition of slime from *P. flavicomun, P. polycephalum,* and *P. rigidum.*

Amino acid	Moles/100 moles of amino acid	Nearest integer
Aspartic acid	10.2 ± 0.5[b]	10
Threonine	7.0 ± 0.3	7
Serine	6.9 ± 0.1	7
Glutamic acid	8.0 ± 0.5	8
Proline	5.1 ± 0.3	5
Glycine	8.4 ± 0.5	8
Alanine	7.8 ± 0.2	8
Half-cystine[a]	2.3 ± 0.1	2
Valine	6.1 ± 0.3	6
Methionine	1.7 ± 0.0	2
Isoleucine	5.2 ± 0.2	5
Leucine	7.6 ± 0.4	8
Tyrosine	3.2 ± 0.3	3
Phenylalanine	4.6 ± 0.1	5
Lysine	7.2 ± 1.3	7
Histidine	3.8 ± 0.8	4
Arginine	4.9 ± 1.0	5

[a]Determined as cysteic acid.
[b]Average of determinations ± average deviation from mean.

3. Results

Determinations for sialic acids, hexosamines, fucose and uronic acids were all negative. The carbohydrate portion of the slime was recovered in the neutral sugar fraction. The Glucostat test was performed on this fraction and was found to be negative. Thin layer chromatography of this fraction from all three species revealed a single sugar component which migrated similarly to galactose. Mixtures of each unknown with galactose revealed a single component after two dimensional chromatography. The Galactostat test was positive and accounted for the total sugar in the neutral sugar fraction from each of the three species.

The galactose to protein content of the slimes from the three species was highly variable from preparation to preparation but the average range of values was from about 62 to 72% galactose and 38 to 28% protein.

The amino composition of the protein portions of the slimes from the three different species was also similar and the averaged values are presented in table 1.

4. Discussion

Some vital functions performed by glycoproteins in animals include: to provide cells with a protective layer of mucus; to serve as lubricants; to function as carriers of vitamins, hormones and other substances; to regulate the water content and the diffusion of metabolites in the extracellular space [11]. By analogy, the glycoprotein nature of the extracellular slime should endow it with some unique properties which would favor survival of the migrating, naked, free-living plasmodium.

The chemical nature and similar composition of the slimes from the three different *Physarum* species indicates a genetically directed biosynthetic origin of the substance and refutes the generally accepted notion [12] that the slime is formed by the excretion of refuse material by the plasmodium.

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