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POLYSACCHARIDES INVOLVED IN SLIME-MOLD DEVELOPMENT

I. WATER-SOLUBLE GLUCOSE POLYMER(S)

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SUMMARY

A water-soluble glucose polysaccharide fraction, synthesized and degraded during cellular slime-mold morphogenesis has been isolated and characterized. The kinetics of its appearance and disappearance are correlated with the overall rate of morphogenesis. Mutant strains incapable of accomplishing all or parts of the normal morphogenetic sequence exhibit altered patterns of synthesis and degradation.

INTRODUCTION

The cellular slime molds are free-living, phagotrophic amoebae which, after cessation of growth, aggregate in compact organized multicellular assemblies and construct fruiting bodies consisting of terminal spore masses and supportive tissues. A previous study[1], devoted to changes in some of the major cell constituents during the morphogenetic sequence, indicated that carbohydrate metabolism might be specifically linked to its various stages. Two interesting polysaccharide fractions were examined:

1. A trichloroacetic acid-insoluble, alkali-insoluble fraction containing glucose as the major constituent plus a trace of arabinose. This fraction increased from negligible levels in the vegetative amoebae to 3–4% of the dry weight of the completed fruiting bodies. Mutant strains incapable of constructing fruits did not synthesize the polysaccharide. It is probably a species of cellulose, known from histochemical[2] and X-ray diffraction[3,4] studies to exist as an outer sheath around the fruiting body stalk.

2. A trichloroacetic acid- and water-soluble, ethanol-insoluble fraction containing glucose and galactose as the major constituents. The level of this fraction increased to 1–2% of the dry weight during later stages of sorocarp construction. Its appearance was considerably altered or completely suppressed in mutant strains which could not accomplish all or parts of the normal morphogenetic sequence.

The second fraction has since been separated into two distinct species of polysaccharide. One is an acid mucopolysaccharide containing galactose, galactosamine, and galacturonic acid. It will be the subject of a separate report. The other, described in the present communication, is composed entirely of glucose. The kinetics of its appearance and disappearance during the developmental sequence are also presented.

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METHODS

Organism and cultivation

The organisms included: *Dictyostelium discoideum* wild type and several mutants and *D. mucoroides*. Stocks were grown on SM agar in association with *Aerobacter aerogenes*[5].

Collection of cell samples for analysis

Stationary phase amoebae were harvested from growth plates, washed 3 times by centrifugation (650 × *g* for 5 min), suspended in water and dispensed on washed agar at a density of $2 \cdot 10^8$ cells/plate. This represented about 25–35 % of the cell density on the growth plates. Samples were harvested at appropriate intervals between cell deposition and the termination of morphogenesis.

Analytical procedures

Cell homogenates were obtained by sonic oscillation. Total protein was determined by the Folin reaction[6], total reducing power by the method of PARK AND JOHNSON[7], hexose by the anthrone assay[8], and glucose by the glucostat micromethod[9]. Descending chromatography was performed at room temperature on Whatman No. 1 paper with either *n*-butanol–pyridine–water (3:2:1.5)[10] or *n*-butanol–acetic acid–water (4:1:5)[11]. The indicator sprays were aniline phthalate and ammoniacal $AgNO_3$.

RESULTS

Purification of the water-soluble glucose polysaccharide(s)

Cultures of *D. discoideum* (100 growth plates), incubated until immature fruiting bodies had appeared, were harvested and suspended in 0.05 M phosphate (pH 7.8). The cells were disrupted by sonication in a total volume of 100 ml, 2 volumes of 0.3 N $Ba(OH)_2$ and 2 volumes of equimolar $ZnSO_4$ were added to deproteinize the sonicate[13]. After centrifugation, the supernatant fluid was made slightly alkaline with NaOH and concentrated *in vacuo* at 50° to a volume of 10 ml. Ethanol was added to a final concentration of 75 % to precipitate the polysaccharide fraction. After incubation overnight at 8°, the precipitate was extracted with cold 1 N NaOH in 75 % ethanol, dissolved in water, and dialyzed against water. The glucose polysaccharide was then precipitated by addition of ethanol to a final concentration of 35–50 % (leaving the acid mucopolysaccharide in solution). The pellet was dried *in vacuo* and redissolved in water.

An alternative procedure involved removal of protein and nucleic acid by precipitation with 5 % trichloroacetic acid. The supernatant was extracted with ether to remove trichloroacetic acid, concentrated *in vacuo* at 50°, made 0.5 N with NaOH and incubated at 100° for 15 min. The polysaccharide was precipitated with 35 % ethanol and the hot alkali treatment was repeated 1–2 times. The final precipitate was dissolved in water and dialyzed against water.

By either method, the final yields were 20–25 mg (approx. 1 % of the starting material).

Composition

Analysis of the product by the anthrone reaction showed that about 95 % of its dry weight could be accounted for as glucose (Table I). Its folin reactivity was less

than 0.5 % of the dry weight (standardized against bovine serum albumin). 100 % of the anthrone reactivity of the unhydrolyzed material appeared as free glucose (by glucostat assay) after 2.5 h hydrolysis with 1 N HCl at 90°. A hydrolyzed sample, chromatographed on paper with butanol–pyridine–water or butanol–acetic acid–water yielded only one spot when sprayed with either ammoniacal $AgNO_3$ or aniline phthalate. In each case, the R_F value was identical to that of a simultaneously run glucose standard.

TABLE I

THE GLUCOSE CONTENTS OF TWO SEPARATELY PURIFIED SAMPLES OF THE NON-DIALYZABLE GLUCOSE POLYSACCHARIDE FRACTION

The anthrone assays were performed on unhydrolyzed material; the glucostat determinations, on material hydrolyzed with 1 N HCl at 90° for 2.5 h.

Expt.	*Dry mass (mg)*	*Glucose content by anthrone*		*Glucose content by glucostat*	
		(mg)	*(%)*	*(mg)*	*(%)*
1	2.225	2.100	93.5	2.070	92
2	2.450	2.270	92.6	2.310	94.5

TABLE II

SUSCEPTIBILITY OF THE NON-DIALYZABLE GLUCOSE POLYSACCHARIDE TO AMYLASES

Substrate in Expt. 1 was purified by the first method described previously. Substrate in Expt. 2 was purified by repeated treatment with 0.5 N NaOH at 100°. Assays: saturating amounts of enzyme were employed as compared with equivalent amounts of potato starch. The reactions were followed by 3,5-dinitrosalicylate assay[16] against a maltose standard. Reaction conditions: α-amylase, in 0.02 M phosphate (pH 6.9) with trace of NaCl; β-amylase, in 0.02 M acetate (pH 4.8); mixture, in 0.02 M phosphate (pH 6.0) with trace of NaCl. Temperature 30°.

Expt.	*Enzyme(s)*	*Per cent of total reducing power released*
1	β-Amylase	0
	α-Amylase	38
	Both	54
2	α-Amylase, β-Amylase, or both	44

The non-dialyzable product purified by the first method was insensitive to cellulase or dextranase as measured by either increase in total reducing power or liberation of free glucose. However, as Table II shows, it was extensively hydrolyzed by α-amylase or a mixture of α- and β-amylase but not by β-amylase alone. Preparations purified by treatment with hot alkali were sensitive to β-amylase. Paper chromatography of the enzymic digests in butanol–acetic acid–water revealed the presence of maltose as the major reaction product. The pattern of enzymic sensitivity plus the fact that the purified material yielded a red color in spot tests with I_2 in KI solution suggest that the non-dialyzable product is rich in α-1,4-glucosidic linkages but is highly branched. The reason for the failure of β-amylase to attack the polysaccharide when purified

without hot alkali treatment is not apparent at present. The presence of an enzyme inhibitor in this product was ruled out by the fact that when mixed with the alkali-treated polysaccharide it did not affect the enzymic digestion of the latter.

Dialyzable bound glucose

In addition to the aforementioned polysaccharide, the trichloroacetic acid-soluble fraction was also found to contain dialyzable material which, upon acid hydrolysis, released free glucose (confirmed by glucostat assay and paper chromatography). Susceptibility to hydrolysis by polysaccharidases was not examined.

Kinetics of appearance and disappearance during morphogenesis

Cells were grown on SM agar to the stationary phase, were harvested, washed free of bacteria by repeated centrifugations, and dispensed on washed agar. Under these conditions populations have been shown to undergo the normal course of morphogenesis with a very high degree of synchrony[1]. Thus samples taken at intervals between cell deposition and fruiting body construction are homogeneous and can provide valid indicators of changes in biochemical activities associated with the morphogenetic sequence.

The cell samples were sonicated and the trichloroacetic acid-soluble polysaccharide fractions isolated. Bound glucose was determined as the difference between hydrolyzed and unhydrolyzed aliquots by glucostat assay. The dialyzable polysaccharide was determined as the difference between the bound glucose contents of aliquots taken before and after dialysis. Free glucose was determined before hydrolysis. Fig. 1 summarizes the results of one representative experiment using *D. discoideum*. Starting from a low initial amount prior to aggregation of the cells, the total quantity of water-soluble glucose polysaccharide increased during a 20-h period more than three-

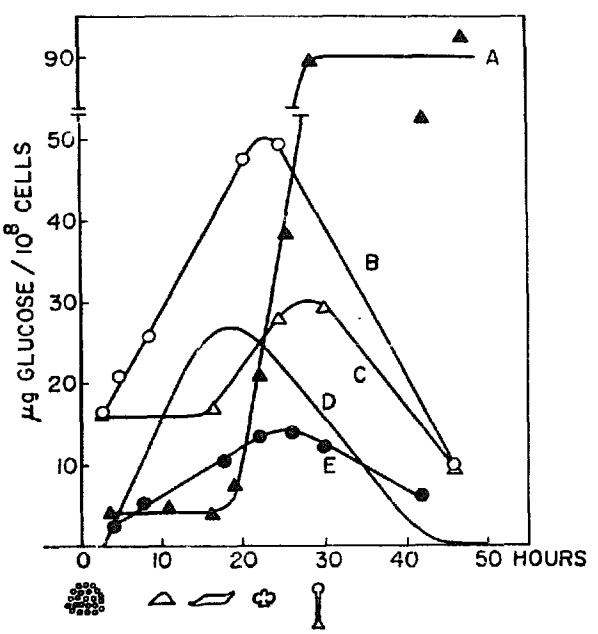

Fig. 1. Developmental kinetics of the soluble glucose polysaccharide fraction in *D. discoideum*. A, Alkali-insoluble polysaccharide; B, total soluble glucose polysaccharide; C, non-dialyzable component of B; D, dialyzable component of B (obtained as the difference between B and C); E, free glucose.

fold per cell (almost 6-fold per milligram dry wt. of cell material, since the cells lose about 50 % of their dry weight during this time[1]). The peak was reached during actual construction of the fruiting bodies and was followed by a sharp decline. This decline was coincident with a very rapid increase in the alkali-insoluble polysaccharide fraction. The dialyzable and non-dialyzable portions of the trichloroacetic acid-soluble fraction are seen to have risen from different initial values to peaks at different times during the developmental sequence. Free glucose performed in coincidence with the total soluble polysaccharide fraction.

A corresponding examination of another species, *D. mucoroides*, showed that it too contains both dialyzable and non-dialyzable trichloroacetic acid-soluble glucose polymers. The developmental kinetics of these fractions were similar to those found in *D. discoideum* with the exception that the maxima were attained sooner and the disappearances were more abrupt. However, this is in keeping with the greater overall rate of morphogenesis encountered in *D. mucoroides*.

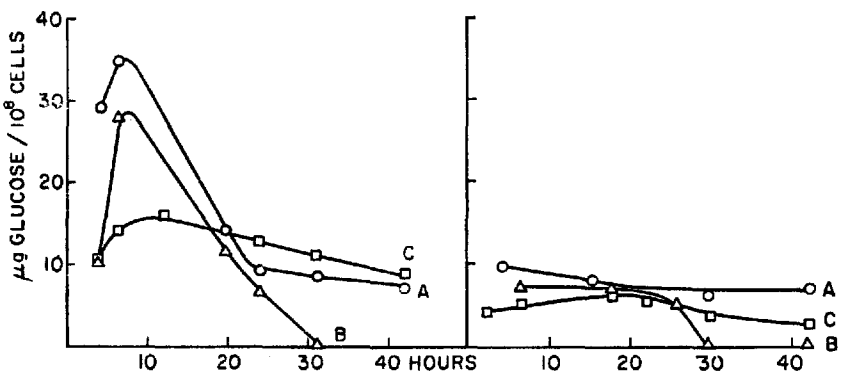

Fig. 2. Developmental kinetics in two mutant strains: left, Fr-17; right, Fr-16. A, Non-dialyzable glucose polysaccharide; B, dialyzable glucose polysaccharide; C, free glucose.

The developmental kinetics of these fractions were also studied in several mutants of *D. discoideum* which display morphogenetic deficiencies. Fig. 2 shows the pattern in Fr-16 which is able to form cell aggregates but cannot construct fruiting bodies. In 7 other deficient mutants the synthesis of both trichloroacetic acid-soluble fractions was either partially or completely inhibited. The pattern displayed by still another deficient mutant, Fr-17, was especially interesting. In Fr-17 the rate of morphogenesis is considerably increased and the sequence of morphogenetic events is altered. At the cell densities employed a recognizable fruiting body is not formed. Instead, the terminal structure is an amorphous, flat mound. Yet many of the end-products of normal morphogenesis appear although in geometric disarray, *i.e.* spores, large highly vacuolate cells which may be related to stalk cells, pigment, the acid mucopolysaccharide, the alkali-insoluble polysaccharide, antigens known[14] to be associated with the stalk and spore mass of the wild type fruit, fibrils which may be cellulosic, etc. As Fig. 2 indicates, Fr-17 also accumulates free glucose and the dialyzable and non-dialyzable polysaccharide fractions, comparable in quantity to the wild type, but the peaks are reached at earlier times after deposition of the cells. These peaks occur at a time when the first spores can be discerned. Thus the kinetics of appearance and disappearance of these components seem to be under the same metabolic controls which regulate the total morphogenetic rate.

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