CELL WALL POLYSACCHARIDE SYNTHESIS IN VITRO CATALYZED BY AN ENZYME FROM SLIME MOLD MYXAMOEBAE LACKING A CELL WALL*

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Introduction. - Differentiation of the cellular slime mold, D. discoideum, terminates with the rapid accumulation of cell wall polysaccharides during construction of the fruiting body or sorocarp. The alkali-insoluble cell wall material has been shown to be composed of a complex of glycogen and cellulose (Ward and Wright, 1965). It is present at an insignificant level at the beginning of differentiation in amoeboid cells. An enzyme catalyzing the incorporation of glucose-^{14}C from uridine diphosphoglucose-^{14}C (UDPG-^{14}C) into these polymers (largely the glycogen moiety) is bound to the cell wall and has not thus far been solubilized nor made dependent upon a primer. The enzyme is relatively stable when isolated at the culminating stages of development, and becomes activated in vitro in the presence of EDTA. Its activity is stimulated 2-5 fold by glucose-6-phosphate (G-6-P). When prepared at the earlier stages of differentiation, however, the enzyme is very difficult to detect and, when detected, it is strikingly unstable. It has, therefore, not been possible to determine relative specific enzyme activities as a function of the stage of morphogenesis (Wright, 1966). It has now been demonstrated that a cytoplasmic enzyme capable of catalyzing the incorporation of radioactive glucose from UDPG-^{14}C into alkali-insoluble cell wall polysaccharides can be found in amoeboid cells at the earliest stages of differentiation. This enzyme is located in the

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100,000 x g pellet fraction; its activity is completely dependent upon the presence of cell wall primer and G-6-P, and is stimulated by EDTA. These circumstances suggest the possibility that an enzyme partially responsible for the accumulation of cell wall material towards the end of the differentiation process has always existed in the cell, but shifts from one site to another, or uses a new (insoluble) primer, or both.

Materials and Methods. - The preparation of UDPG-^{14}C has been described previously (Ward and Wright, 1965). Adenosine diphosphoglucose (ADPG-^{14}C) was obtained through the courtesy of Dr. H. Nikaido; α-amylase (highest purity from hog pancreas) was obtained from Sigma Chemical Company; α-glucan phosphorylase from Worthington Biochemical Corporation; a cellulase preparation from Streptomyces sp. QM B814 was a gift from Dr. E.T. Reese; preparations of phosphorylase b and amylo-1,6-glucosidase were kindly supplied by Dr. E. Bueding; a sample of amylose was a gift from Dr. P. Bernfeld.

Conditions for growing and harvesting cells of D. discoideum have been described (Ward and Wright, 1965). After rupture of the cells, cell husk material is removed by centrifugation at 2,000 x g for 10 min, and the supernatant fluid centrifuged for one hr at 100,000 x g. The pellet is then washed once in the tris-EDTA buffer and again sedimented at 100,000 x g for one hr. Such preparations are stable for about 2 weeks if kept at -20°. Enzyme activity is assayed in a total volume of 0.2 ml in the presence of tris, 20 μmoles, pH 8.5; EDTA, 2 μmoles, UDPG-^{14}C (2 x 10^6 cpm/μmole), 0.01 μmole; G-6-P, 1.0 μmole; alkali-insoluble primer and 50-100 μg enzyme protein. Following a 60 min incubation at 37° radioactive product insoluble in hot 1 per cent NaOH is purified and counted.

Results and Discussion. - The first table demonstrates the complete dependence of this system on alkali-insoluble primer material. Treatment of washed sorocarps with 1 per cent alkali gives active primer; further treatments with cellulase or EDTA has increased the activity of the primer. Consistently

inactive are: various non-alkali-treated sorocarp preparations, cellulose, insoluble cellodextrins, chitin and fractions thereof. Amylose is insoluble in 1 per cent alkali and is always active as a primer; freshly prepared samples are the most active. Although a complete dependency on G-6-P is always observed with aged enzyme, fresh enzyme preparations frequently show a partial dependence, suggesting alterations *in vitro* such as a change in the ratio of G-6-P independent to dependent forms (Larner, *et al.*, 1964). Preparing the enzyme in the presence of EDTA is beneficial, as is the addition of EDTA during an assay using enzyme prepared with or without EDTA. The amount of EDTA producing maximal activity varies somewhat with protein concentration and the age of the enzyme preparation. The range of effective concentrations, however, is similar to that required for activation of the cell wall-synthesizing enzyme bound to the cell wall of sorocarps. Also in common with the latter system is the fact that ADPG-^{14}C has less than 1/10 the activity of UDPG-^{14}C.

TABLE 1: Primer and G-6-P Dependence

mg Primer	μmoles glucose incorp. (x 10^3)	G-6-P (M x 10^3)	μmoles glucose incorp. (x 10^3)
0	0.03	0	0.05
0.08	0.53	1	2.62
0.20	0.96	5	6.80
0.40	1.49	10	5.81
0.40 (boiled control)	0.01		

In order to compare the radioactive product of this reaction, completely dependent on cell wall material as primer, to the product synthesized in the terminal stages of development, the following experiment was done. Large scale incubations were carried out under the usual conditions. The radioactive product (3,600 cpm) was divided into two parts and incubated with phosphorylase *b* and with phosphorylase *b* plus amylo-1,6-glucosidase, respectively. Incubation for 3 hr solubilized 47 per cent of the counts in both cases, indicating the lack of synthesis of α-1,6-linkages. Further

successive treatments of the insoluble product with phosphorylase and amylase resulted in the solubilization of 79 per cent of the counts. Thus the insoluble product synthesized by the pellet enzyme is similar to that formed by the enzyme bound to cell wall preparations of young sorocarps; i.e., most of the radioactive glucose is incorporated into α-1,4-linkages of the glycogen fraction (Ward and Wright, 1965).

Table 2 summarizes three experiments in which the specific activity of the enzyme in the 100,000 x g pellet fraction was determined at various stages of development. No appreciable differences in enzyme stability have been observed for preparations from various stages of morphogenesis. It is clear, therefore, that the enzyme specific activity decreases as differentiation progresses. In contrast, stable cell wall-bound enzyme peaks in activity in preparations from young sorocarps (Wright, 1966).

Table 2: Specific Enzyme Activity as a Function of Stage of Differentiation

Stage	Per Cent Counts Incorporated/100 µg Protein		
	Exp I	Exp II	Exp III
Aggregation	2.80	1.80	0.51
Pseudoplasmodium	0.63		0.20
Preculmination		0.67	
Culmination			0.00
Sorocarp, 2 hr	0.16		

Preliminary evidence suggests that the latter enzyme is in part derived from the pellet fraction enzyme as differentiation progresses. An interesting model system for the latter mechanism has been described, in which enzymes are entrapped into the lattice of an insoluble, synthetic polymer as it forms (Bernfeld and Wan, 1963). It has in fact been possible to demonstrate in vitro the attachment of the pellet enzyme to cell wall primer. The

latter can then be washed to remove free pellet enzyme and, in a second incubation, be used as a source of both enzyme and primer to synthesize insoluble cell wall material from UDPG-^{14}C.

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