UDP-GLUCOSE PYROPHOSPHORYLASE SYNTHESIS IN MYXAMOEBAE OF *DICTYOSTELIUM DISCOIDEUM*

G.L. Gustafson and B.E. Wright

Department of Developmental Biology
Boston Biomedical Research Institute
Boston, Massachusetts 02114

Received November 28,1972

SUMMARY A mixture of [^{14}C] amino acids and [^{35}S] methionine are rapidly incorporated into the UDP-glucose pyrophosphorylase of myxamoebae of the cellular slime mold over a period of differentiation in which a significant change in the amount of enzyme does not occur. There is no evidence for a change in the rate of enzyme synthesis prior to or during the initiation of enzyme accumulation. Therefore, there is no evidence for the involvement of specific periods of transcription or translation in controlling the synthesis of this enzyme prior to or during differentiation.

INTRODUCTION

UDP-glucose pyrophosphorylase (E.C.2.7.7.9.) is present at all stages of the life cycle in *Dictyostelium discoideum*. During growth, when UDP-glucose is required for glycogen synthesis, the enzyme must be synthesized at a rate such that it doubles at every cell division, or every four hours. During differentiation, the rate of enzyme protein accumulation (1) can roughly be accounted for by this rate of synthesis (2). This communication deals with the transition period between growth and multicellular differentiation. During this time the rate of isotope incorporation into enzyme relative to acid insoluble protein also indicates a rate of enzyme synthesis comparable to that during growth. As enzyme is not accumulating during this transition period, rapid enzyme turnover is implicated. Protein turnover has been studied previously in *D. discoideum*, using [^{35}S] methionine (3). The latter investigation as well as others (4) have demonstrated that cellular permeability to a variety of metabolites can change by an order of magnitude during differentiation. Thus, the rate of isotope incorporation into a specific enzyme protein must be compared to that of some other intracellular

component, in this case, acid-insoluble protein.

MATERIALS

Crystalline phosphoglucomutase (rabbit muscle) and crystalline glucose-6-P dehydrogenase (yeast) were purchased from Calbiochem; $NADP^+$, UDP-glucose and crystalline bovine serum albumin from Sigma Chemical Co.; acrylamide gel reagents from Canalco; [^{35}S] methionine (specific activity 180 c/mMole) from New England Nuclear Corporation; [^{14}C] amino acid mixture from International Chemical and Nuclear Corporation, and ultrapure Tris and ultrapure ammonium sulfate from Schwarz/Mann. All other chemicals were reagent grade.

METHODS

Strain NC-4 was grown according to the method of Liddel and Wright (5). Protein was determined using a modification of the Folin assay (6).

Preparation of Enzyme and Antisera: The assay for enzyme activity was similar to that of Munch-Peterson (7). One unit of enzyme is defined as that amount catalyzing the synthesis of 1 µmole NADPH (or 1 µmole of glucose-1-P) per min at 25 C. Enzyme was purified 500 fold from culminating cells to a specific activity of 200 units/mg (8). At 37 C, purified enzyme had a specific activity of 400 units/mg. Details of the purification procedure will be published elsewhere. Antibody against UDP-glucose pyrophosphorylase was prepared by injection of 2 mg purified enzyme, emulsified with Freund's complete adjuvant, into toe pads of rabbits (9). After four weeks, rabbits were bled at two week intervals. The serum was subjected to ammonium sulfate fractionation; the protein precipitating between 0 and 50% salt saturation was collected after centrifugation, dissolved in 0.85% NaCl, dialyzed 12 hours at 4 C, and stored at -20 C.

Isotope Incorporation and Enzyme Isolation: Vegetative amoebae, harvested from nutrient-agar, were washed free of bacteria and aliquots equivalent to approximately 0.7 ml packed cell volume were spread on 12.5

cm diameter Whatman No. 50 filter papers and exposed to isotope (10). Following incubation the cells were washed from the filter papers with 30 ml cold water, and were then pelleted by centrifugation. Cell pellets were suspended in 3 ml 50 mM tetrasodium pyrophosphate pH 7.4, frozen in a liquid nitrogen bath, and thawed at room temperature. Streptomycin sulfate (0.4 ml of a 50% solution) was added to thawed extracts with stirring. Following centrifugation for 15 min at 27,000 x g, the supernatant liquids were collected and adjusted to pH 7.3 by addition of 2 ml 50 mM PP_i-P_i pH 8.5. The neutralized, cell-free extracts were then incubated 20 min at 37 C followed by centrifugation for 30 min at 105,000 x g. The supernatant liquids from this treatment were incubated with an excess of antisera for 40 min at room temperature. This was sufficient to precipitate 80% of the enzyme protein. Three to four units of enzyme, or 15-20 μg of enzyme protein was reacted with antisera in each isolation. Precipitated enzyme was subjected to electrophoresis (to be described elsewhere). UDP-glucose pyrophosphorylase protein was identified from its position of migration in the gels relative to standard proteins and purified enzyme. For quantitation of stained protein, acrylamide gels were scanned with a Joyce-Loeble microdensitometer equipped with a 595 nm filter in the light path. The amount of protein in gels was shown to be directly proportional to the amount of stain absorbed as determined from the area under peaks in microdensitometer tracings. Following quantitation of protein, the gels were cut into 2.5 mm sections. Each section was placed in tightly capped scintillation vials with 0.5 ml 30% hydrogen peroxide and incubated at 70 C for 1 1/2 hours in order to dissolve the gel. The vials were cooled, 10 ml Aquasol (New England Nuclear Corp) was added, and the vials were counted in a Beckman scintillation spectrometer. Each sample was counted 10 min.

RESULTS AND DISCUSSION

As indicated in Table I, enzyme protein can achieve a higher specific radioactivity than acid-insoluble protein in the absence of significant

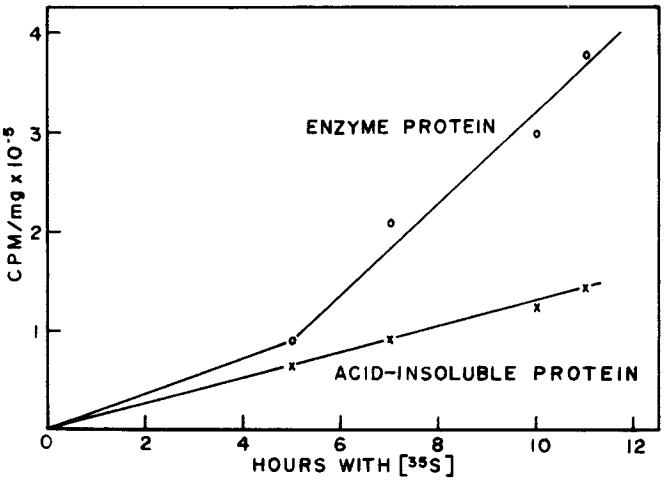

Figure 1: The specific radioactivity of enzyme protein and acid insoluble protein. Cells were exposed to [^{35}S] methionine at 0 time and aliquots harvested at successive time periods beginning at 5 hrs. Specific radioactivity is expressed as CPM/mg protein x 10^{-5}.

TABLE I

SPECIFIC RADIOACTIVITY OF UDP-GLUCOSE PYROPHOSPHORYLASE RELATIVE TO ACID INSOLUBLE PROTEIN

Exp.	Source of Label	Hour Added[2]	Hours Exposed	% change Total enz. units	Spec. radio. enz. / Spec. radio. a.i.p.
I	[^{14}C]amino acids	1.5	4	none	1.24
II	[^{14}C]amino acids[1]	1.25	3	none	0.85
		1.25	5	none	0.93
		1.25	7	none	1.23
III	[^{35}S]methionine	1.5	3	none	1.28
IV	[^{35}S]methionine[1]	1.0	5	none	1.37
		1.0	7	+5%	2.25
		1.0	10	+15%	2.38
		1.0	11	+18%	2.60

[1]Stage studies

[2]From the initiation of starvation

changes in the amount of enzyme. Data from Experiment IV are plotted in Figure 1. Following an initial lag period, the rate of increase in specific radioactivity of enzyme was greater than the corresponding rate for acid-insoluble protein. That enzyme synthesis is not reflected by a significant

increase in the amount of enzyme protein/cell aliquot suggests that enzyme protein is being degraded at a rate equal to synthesis. Protein turnover throughout differentiation is, roughly, 7%/hr (3). Considering all experiments (only some of which are reported here) carried out prior to the period of rapid enzyme accumulation, enzyme protein is labelled between 2-4 times the rate of acid-insoluble protein, following an initial lag period. A rate of enzyme synthesis of about 20%/hr can account for the extent of enzyme accumulation later in differentiation, in the absence of enzyme degradation. Thus, the rate of enzyme synthesis appears to be fairly constant during and after the transition between growth and differentiation, indicating a lack of regulation at the levels of transcription and translation (11).

ACKNOWLEDGEMENT

This investigation was supported by grant #GB15055 from the National Science Foundation.

G.L. Gustafson is a postdoctoral fellow of the National Institute of General and Medical Sciences (F02GM31775). Present address: Department of Agricultural Chemistry, Washington State University, Pullman, Washington 99163.

REFERENCES

1. Franke, J., and Sussman, M., J. Biol. Chem. 246, 6381 (1971).
2. Gustafson, G.L., and Wright, B.E., In: Critical Reviews in Microbiology, Laskin, A.I. and Lechevalier, H., eds. Vol. 1(4), p. 453, CRC Press, Cleveland, Ohio (1972).
3. Wright, B.E., and Anderson, M.L., Biochim. Biophys. Acta 43, 67 (1960).
4. Wright, B.E., In: Biochemistry and Physiology of Protozoa, Hutner, H.S., ed. Vol. 3, p. 341, Academic Press, London, 1964.
5. Liddel, G.U., and Wright, B.E., Dev. Biol. 3, 265 (1961).
6. Lowry, O.H., Rosenbrough, N.J., Farr, A.L., and Randall, R.J., J. Biol. Chem. 193, 265 (1951).
7. Munch-Peterson, A., Acta Chem. Scand. 9, 1523 (1955).
8. Gustafson, G.L., and Wright, B.E., Fed. Proc. 30, 1069 (1971).
9. Leskowitz, S., and Waksman, B.J., J. Immunol. 84, 58 (1960).
10. Newell, P.C., and Sussman, M., J. Biol. Chem. 244, 2990 (1969).
11. Wright, B.E., Critical Variables in Differentiation, Prentice-Hall, Inc., Englewood Cliffs, N.J. (1972).