6-Phosphogluconate Dehydrogenase and the Assay of Uridine Diphosphate Glucose Pyrophosphorylase in the Cellular Slime Mould *Dictyostelium discoideum*

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1. 6-Phosphogluconate dehydrogenase activity is present in all morphogenetic stages during cell differentiation in the cellular slime mould. 2. The different ratios of 6-phosphogluconate dehydrogenase/UDP-glucose pyrophosphorylase observed during this process can render spectrophotometric assays of UDP-glucose pyrophosphorylase inaccurate. 3. The disputed occurrence of increases in specific activity of UDP-glucose pyrophosphorylase during cell differentiation in the cellular slime mould is discussed in the light of these observations.

Two distinct and conflicting models of cell differentiation in the cellular slime mould *Dictyostelium discoideum* have been proposed. Sussman & Sussman (1969) have suggested that during differentiation there is a series of ordered transcriptional and translational events as a consequence of which a new spectrum of enzyme activities is produced. By using morphologically deranged mutants it has been possible to correlate alterations in enzymic activity with morphogenetic stages and, by various means, it has been possible to study the cellular distribution of various enzymic activities within any particular stage (Newell *et al.*, 1969; Miller *et al.*, 1969). This work has led to the concept of a 'developmental programme' that controls the temporal and spatial synthesis (or destruction) of enzyme activities and hence morphogenesis (Newell & Sussman, 1970; Loomis, 1970). In contrast Wright (1968) has argued that changes in enzyme activity (if they occur) are irrelevant to the control of differentiation. She suggests that changes in the concentrations of key metabolites during differentiation are in themselves sufficient to cause the qualitative and quantitative changes in metabolic fluxes which are made manifest in the observed morphogenesis.

Both of these models have been used to account for the observed increase in amount of some carbohydrates that is such a notable feature of the morphogenesis (White & Sussman, 1961). Thus Ashworth & Sussman (1967) reported that the specific activity of UDP-glucose pyrophosphorylase increased tenfold during morphogenesis and then decreased. Since the increase was sensitive to the coincident inhibition of protein synthesis and the prior inhibition of RNA synthesis and was aberrant in morphologically deranged mutants it was concluded that the synthesis

was under transcriptional and translational control mechanisms, which themselves form part of the 'developmental programme'.

Wright & Anderson (1959) reported a greater than tenfold increase in UDP-glucose pyrophosphorylase activity during differentiation but, in later papers (e.g., Wright *et al.*, 1968), Wright and her co-workers accepted the lower value of a threefold increase reported by Pannbacker (1967b). Wright & Dahlberg (1968) had reported that UDP-glucose pyrophosphorylase was more labile in extracts prepared from early morphogenetic stages as compared with extracts prepared from later stages and it was therefore argued that the observed tenfold increase was, in part, an artifact of this differential stability. Newell & Sussman (1969) in an exhaustive study of the stability of UDP-glucose pyrophosphorylase showed that, provided the extracts were made in tricine buffers and the protein and pyrophosphate concentrations were controlled, the enzyme could be stabilized in extracts prepared from all stages and they reported an eight- to ten-fold increase in UDP-glucose pyrophosphorylase with their assay conditions.

Wright & Anderson (1959), Pannbacker (1967b), Wright & Dahlberg (1968) and Newell & Sussman (1969) all used a spectrophotometric assay for UDP-glucose pyrophosphorylase (reaction 1) based on the coupled reactions:

as suggested by Munch-Peterson (1955). This assay procedure involves adding excess of phosphoglucumutase (reaction 2) and glucose 6-phosphate dehydrogenase (reaction 3) and then determining the UDP-glucose- and pyrophosphate-dependent increase in NADPH. This spectrophotometric assay will only be valid if the extract contains little 6-phosphogluconate dehydrogenase activity (reaction 4):

or if the ratio of the activities of UDP-glucose pyrophosphorylase and 6-phosphogluconate dehydrogenase remains constant in extracts prepared from all developmental stages.

We report that neither of these assumptions is necessarily true and that this probably accounts, in part, for the different findings of Pannbacker (1967b) on the one hand and Ashworth & Sussman (1967) on the other, as the latter authors used a direct, radioisotope, assay of reaction (1).

Materials and Methods

Chemicals

Actidione (cycloheximide), NAD^+ , NADP^+ , 6-phosphogluconate, UDP-glucose dehydrogenase (acetone powder), cysteine (as free base), UDP-glucose and tris(hydroxymethyl)aminomethane were obtained from Sigma Chemical Co., St. Louis, Mo., U.S.A. Glucose 6-phosphate dehydrogenase (140 units/mg), phosphoglucumutase (18 units/mg), 6-phosphogluconate dehydrogenase (13 units/mg), glutamate dehydrogenase (3 units/mg) and glucose 6-phosphate were purchased from Boehringer and Soehne G.m.b.H., Mannheim, Germany. *N*-Tris-(hydroxymethyl)glycine (tricine) was obtained from Calbiochem. Glucose 1-phosphate and tetra-sodium pyrophosphate, AnalaR grade, were purchased from B.D.H. Chemicals Ltd., Poole, Dorset, U.K.

Organism and culture conditions

D. discoideum, strain NC-4 (haploid) was grown in association with *Aerobacter aerogenes* on nutrient agar as described by Sussman (1966). Strain Ax-2 was grown axenically in a complex medium as described by Watts & Ashworth (1970).

For morphogenetic studies, the cells were harvested in cold water and washed free from bacteria, where necessary, by differential centrifugation for 5 min at 500g. The washing was repeated until a visual microscopic check showed the cells to be largely free of bacteria. Development was initiated by pipetting a suspension of washed cells on to Millipore filters (AABP047) supported on absorbent filter pads impregnated with a solution containing 50mm-

phosphate buffer, pH 6.4, salts (KCl , 1.5mg/ml; MgCl_2 , 0.5mg/ml) and streptomycin (0.5mg/ml) at a density of 1×10^8 cells/Millipore filter (Sussman & Lovgren, 1965). On incubation at 22°C, highly synchronous cell differentiation occurred and fruiting bodies were formed after 24 ± 1 h. With strain Ax-2, better synchrony was obtained by plating out cells at a density of 5×10^7 cells/Millipore filter.

Preparation of cell-free extracts

The cells were harvested in cold 0.05M-tricine buffer, pH 8.6. These suspensions were stored frozen at -15°C and then sonicated in an M.S.E. ultrasonic disintegrator for 1 min at a peak-to-peak amplitude of 8 μm . Spore suspensions at 10^8 spores/ml required up to 3×1 min sonications for complete rupture.

Cell debris was removed by centrifugation at 10000g for 15 min and the supernatants containing between 0.5 and 5.0 mg of protein were used as crude extracts and assayed immediately. All the above operations were done between 0° and 4°C . Spore and stalk cells were separated by washing mature fruiting bodies on double-thickness cheesecloth; the spores filter through and the stalks remain. The stalks were resuspended and washed until free from spores.

Enzyme assays

All assays were performed at 25°C , and protein was determined by the method of Lowry *et al.* (1951).

UDP-glucose pyrophosphorylase. This enzyme catalyses the reaction (1).

Assay system 1. The enzyme is assayed in the direction of glucose 1-phosphate formation from UDP-glucose by the method of Munch-Peterson (1955), which involves reactions (1), (2) and (3) and the net result of which is the formation of 1 mol of NADPH for each mol of UDP-glucose utilized.

Each cuvette contained in 1.0 ml: MgCl_2 , 5 μmol ; cysteine, 2.5 μmol ; NADP^+ , 0.5 μmol ; UDP-glucose, 1.0 μmol ; phosphoglucumutase, 0.14 unit; glucose 6-phosphate dehydrogenase, 0.60 unit; cell extract and 50mm-tricine buffer, pH 8.6, to a volume of 0.98 ml. The reaction was initiated by the addition of 0.02 ml of 0.15M-pyrophosphate solution and the reduction of NADP^+ was measured at 340 nm in a Unicam SP.800 recording spectrophotometer. After a short initial lag, the increase in E_{340} was linear with time for at least 5 min; a rate of 0.60 extinction unit/min was not exceeded, to ensure that UDP-glucose pyrophosphorylase was the rate-limiting step. A unit of UDP-glucose pyrophosphorylase activity is defined as that amount which converts 1 nmol of NADP^+ into NADPH/min at 25°C .

Assay system 2. This is similar in principle to assay 1, but includes the additional reaction (4) catalysed by 6-phosphogluconate dehydrogenase.

Thus the net result of reactions (1–4) is the formation of 2mol of NADPH for each mol of UDP-glucose utilized. Each assay cuvette contained in 1.0ml: MgCl_2 , $5\mu\text{mol}$; cysteine, $2.5\mu\text{mol}$; NADP^+ , $2.0\mu\text{mol}$; UDP-glucose, $1.0\mu\text{mol}$; phosphoglucosmutase, 0.07 unit; glucose 6-phosphate dehydrogenase, 0.30 unit; 6-phosphogluconate dehydrogenase, 0.60 unit; cell extract and 50mm-tricine buffer, pH8.6, to a volume of 0.98ml. The reaction was initiated by the addition of 0.02ml of 0.15M-pyrophosphate solution as before. The kinetics and validity of complex auxiliary enzyme assay systems have been examined by McClure (1969); this assay is valid provided that the rate of the observed UDP-glucose pyrophosphorylase activity is not greater than an increase in E_{340} of 0.10 extinction unit/min. The 6-phosphogluconate dehydrogenase supplied by Boehringer as a suspension in 3M- $(\text{NH}_4)_2\text{SO}_4$ required dialysis before use in this assay system, otherwise the $(\text{NH}_4)_2\text{SO}_4$ concentration in the cuvette became too great and adversely affected the phosphoglucosmutase. For UDP-glucose pyrophosphorylase, a unit of activity is defined as that amount converting 2nmol of NADP^+ into NADPH/min at 25°C.

Assay system 3. The enzyme is assayed in the direction of UDP-glucose formation by using the NAD^+ -linked UDP-glucose dehydrogenase, by the method of Strominger *et al.* (1957).

Thus the formation of 1mol of UDP-glucose by reaction (1) results in the formation of 2mol of NADH by reaction (5). Each assay cuvette contained in 1.0ml: MgCl_2 , $5\mu\text{mol}$; cysteine, $2.5\mu\text{mol}$; UTP, $1.0\mu\text{mol}$; NAD^+ , $1.6\mu\text{mol}$; UDP-glucose dehydrogenase, 250 units (as defined by Sigma Chemical Co. Ltd.); cell extract and 50mm-tricine buffer, pH8.6, to a volume of 0.99ml. After equilibration, the reaction was initiated by the addition of 0.01ml of 0.1M-glucose 1-phosphate solution and measured at 340nm as before. A unit of activity is defined as that amount which converts 2nmol of NAD^+ into NADH/min at 25°C. The commercially available UDP-glucose dehydrogenase preparation was unsatisfactory, because it contained lactate dehydrogenase and NADH oxidase activity; therefore a batch was partially purified by the following procedure. The acetone-dried powder was extracted with cold water (20ml/g) and the extract was centrifuged at 10000g for 15min. The dark green supernatant was brought to 40% saturation at 0°C with $(\text{NH}_4)_2\text{SO}_4$. After equilibration and centrifugation the precipitate was discarded and the supernatant

was adjusted to 55% saturation and centrifuged. This precipitate was redissolved in 2ml of 0.01M-sodium acetate buffer, pH4.9; it was kept in a water bath at 50°C for 1–2min and was then rapidly cooled to 0°C, when the precipitate was centrifuged down. The supernatant was adjusted to pH8.6 with ammonia solution and used as described above.

6-Phosphogluconate dehydrogenase. The enzyme catalyses reaction (4) and was assayed by the method of Horecker & Smyrniotis (1951). Each cuvette contained in 1.0ml: 6-phosphogluconate, $1\mu\text{mol}$; NADP^+ , $0.5\mu\text{mol}$; cell extract and 50mm-tricine buffer, pH8.6, to a final volume of 1.0ml. The reaction was initiated by the addition of the 6-phosphogluconate and the reduction of the NADP^+ was measured at 25°C and 340nm as before. The reaction was linear with time for at least 5min after the addition of the 6-phosphogluconate. One unit of activity is defined as that amount which converts 1nmol of NADP^+ into NADPH/min at 25°C.

Results

6-Phosphogluconate dehydrogenase

Characterization of the reaction. The reaction of 6-phosphogluconate with NADP^+ catalysed by crude extracts from all morphogenetic stages was unaffected by the presence or absence of EDTA or metal ions (Mg^{2+} , Mn^{2+}) and there was no reaction between 6-phosphogluconate and NAD^+ . The rate of reduction of NADP^+ was linear with the amount of protein added to the reaction mixture over the range 0–1.0mg with extracts prepared from all stages of development. Kinetic experiments with such extracts gave Michaelis constants of:

$$K_m \text{ 6-phosphogluconate} = 4.0 \times 10^{-5} \text{ M} \quad (5 \text{ mM-NADP}^+);$$

$$K_m \text{ NADP}^+ = 1.8 \times 10^{-5} \text{ M} \quad (1 \text{ mM-6-phosphogluconate}).$$

The reaction:

is an equilibrium reaction and so to demonstrate the stoichiometry it was coupled with the reaction:

by using excess of glutamate dehydrogenase, α -oxoglutarate and NH_4^+ . Under these conditions it was possible to demonstrate an equivalence between 6-phosphogluconate disappearance and CO_2 appearance with cell extracts. Chromatographic analysis of the reaction mixtures showed that as the reaction proceeded 6-phosphogluconate disappeared and one

phosphorylated sugar, indistinguishable from ribulose 5-phosphate, appeared. With incubation mixtures in which the NADP^+ /6-phosphogluconate ratio was 5:1 or more, it was also possible to demonstrate an equivalence between NADPH appearance and 6-phosphogluconate disappearance. Under all assay conditions ribulose 5-phosphate appeared to be both stable and the only phosphorylated reaction product.

The enzyme had a pH optimum of 8.6–8.8 in both tris and tricine buffers, but it was more active in tricine buffers. Addition of glycylglycine to tris buffers also 'activated' the 6-phosphogluconate dehydrogenase and thus this effect of tricine was probably due to the fact that it is a zwitterion. Similar effects of zwitterionic buffers have been reported before (Ashworth & Sussman, 1967; Good *et al.*, 1966). The enzyme activity in extracts prepared from all morphogenetic stages was stable to freezing and

thawing and an extract incubated at 0°C for 24h retained at least 70% of its original activity.

Activity during development and in different cell types. Myxamoebae of strains NC-4 and Ax-2 have similar specific activities irrespective of whether they are grown on bacteria (crude extracts of which have a specific activity of 200 nmol/min per mg of protein) or, in the case of Ax-2, axenically. During the differentiation of these myxamoebae (Fig. 1) there is a linear decrease in specific activity of crude extracts from the myxamoebal value of 35–50 nmol/min per mg of protein to the value characteristic of the fruiting body (20–25 nmol/min per mg of protein). Mature fruiting bodies were obtained by incubating myxamoebae on Millipore filters for 36 h at 22°C, and were fractionated into spore and stalk cells by filtering suspensions of fruiting bodies through several layers of muslin. The stalks remain trapped in the muslin.

Fig. 1. Specific activity of 6-phosphogluconate dehydrogenase in crude cell-free extracts during cell differentiation of *D. discoideum*

Crude extracts of the spores so prepared had a specific activity of 24nmol/min per mg of protein and the crude extracts of the stalks had a specific activity of less than one-tenth this value (2nmol/min per mg of protein).

UDP-glucose pyrophosphorylase

Characterization of the reaction. The pyrophosphorolysis of UDP-glucose, as measured by method 1, was dependent upon the simultaneous presence of Mg^{2+} , PP_i , UDP-glucose and enzyme. UDP-glucose synthesis, as measured by method 3, required the presence of Mg^{2+} , UTP, glucose 1-phosphate and enzyme (Table 1). Cysteine had little effect, but was included in the assay mixtures as it stimulated phosphoglucomutase in methods 1 and 2 and UDP-glucose dehydrogenase in method 3. The requirement for Mg^{2+} could be partially satisfied by Mn^{2+} , but not by Ni^{2+} . The optimum concentration of Mg^{2+} was between 5 and 10mM and high concentrations of Mg^{2+} inhibited pyrophosphorolysis, but had no observable effect in the reverse direction. High concentrations of Mg^{2+} and pyrophosphate caused precipitation and under the conditions in the assay cuvette, 20mM- Mg^{2+} exceeded the solubility product of Mg_2PP_i . Optimum results were obtained with a Mg^{2+}/PP_i ratio of 2:1, which could indicate that the true substrate is Mg_2PP_i , at least in the direction of pyrophosphorolysis.

Kinetic experiments suggested that the rate of pyrophosphorolysis was almost twice that of synthesis of UDP-glucose (Table 1). The K_m values for the various substrates, determined in the presence of 5mM- Mg^{2+} and saturating concentrations of the other substrates, were found to be: K_m UDP-glucose = $9.0 \times 10^{-5}M$, K_m PP_i = $4.0 \times 10^{-4}M$ (assay method 1); K_m UTP = $8.2 \times 10^{-4}M$, K_m glucose 1-phosphate = $1.1 \times 10^{-4}M$ (assay method 3).

Assay method 3, with low concentrations of glucose 1-phosphate substrate (50 μ M) and UTP substrate

(100 μ M), was used to study the effect upon enzyme activity of a series of compounds, each at 1mM. The following compounds had no significant effect (i.e. <10%) on enzyme activity: (a) nucleotides: UMP, UDP, UTP, AMP, ADP, ATP, GMP, GDP; but with GTP a 10% inhibition was found; (b) compounds of the glycolytic sequence: pyruvate, phosphoenolpyruvate, glycerol 3-phosphate, fructose 1,6-diphosphate, glucose 6-phosphate, fructose 6-phosphate, ribose 5-phosphate; (c) intermediates of the tricarboxylic acid cycle: citrate, isocitrate, α -oxoglutarate, glutamate, succinate, malate, oxaloacetate; (d) glucose and fructose, and P_i .

It was not possible to record the effects of these compounds on the reaction in the reverse direction (pyrophosphorolysis) as most of them affected the auxiliary enzymes in assay method 1.

The enzyme had a pH optimum of 8.4–8.9 in the direction of synthesis of UDP-glucose and 8.1–8.6 in the direction of phosphorolysis with both tris-HCl and tricine-NaOH buffers. Tricine enhanced the activity of both reactions, particularly at lower pH values.

At 0–4°C crude extracts from all stages of development were stable in tricine buffers for at least 30min. In extracts prepared from myxamoebae or early developmental stages in tris buffers the enzyme activity was unstable, as reported by Wright & Dahlberg (1968). We confirmed that the degree of instability depended on the protein concentration of the extract and could be suppressed by the addition of PP_i (Newell & Sussman, 1969). Prolonged incubation of crude extracts prepared in tricine buffers at pH8.5 and 25°C led to a loss of activity, which was greater with extracts prepared from myxamoebae (after 2.5h myxamoebal extracts lost 45% of their initial activity whereas fruiting body extracts lost only 15%). Addition of bovine serum albumin (2mg/ml) decreased the rate of loss of activity from the myxamoebal extract but not that from extracts prepared from fruiting bodies.

Table 1. *Characterization of reactions catalysed by UDP-glucose pyrophosphorylase*

Requirements for pyrophosphorolysis		Requirements for synthesis	
Modification to assay system	Enzyme activity* (units/ml)	Modification to assay system	Enzyme activity† (units/ml)
None	242	None	139
Minus UDP-glucose	4	Minus UTP	3
Minus pyrophosphate	1	Minus glucose 1-phosphate	4
Minus Mg^{2+}	1	Minus Mg^{2+}	1
Minus enzyme	1	Minus enzyme	1
Minus enzyme, plus boiled extract	1	Minus Mg^{2+} , plus 5mM- Mn^{2+}	110
Minus Mg^{2+} , plus 5mM- Mn^{2+}	187	Minus cysteine	135

* Conditions as described in assay method 1.

† Conditions as described in assay method 3.

Table 2. *UDP-glucose pyrophosphorylase in different cell types*

Results are quoted as means $\pm$ S.E.M. for at least four determinations done on crude extracts of the appropriate samples. Specific activities are reported as follows: for assay 1, nmol of NADP⁺ reduced/min per mg of protein; for assays 2 and 3, $\frac{1}{2}$ nmol of NAD⁺ reduced/min per mg of protein.

Type	Specific activity		
	Assay 1 (phosphorolysis)	Assay 2	Assay 3 (synthesis)
<i>A. aerogenes</i>	46 $\pm$ 11	—	35 $\pm$ 9
Myxamoebae Ax-2*	49 $\pm$ 4	36 $\pm$ 3	23 $\pm$ 2
Myxamoebae NC-4	40 $\pm$ 5	30 $\pm$ 3	19 $\pm$ 3
Mature fruiting bodies (30h)	136 $\pm$ 11	128 $\pm$ 10	74 $\pm$ 8
Spores	151 $\pm$ 12	140 $\pm$ 11	89 $\pm$ 10
Stalks	10 $\pm$ 2	9 $\pm$ 2	6 $\pm$ 2

* Myxamoebae Ax-2 grown in axenic media (Watts & Ashworth, 1970) and harvested when in the logarithmic phase of growth.

Table 3. *Stoichiometry of the reaction catalysed by UDP-glucose pyrophosphorylase with assay method 1*

Incubation mixtures were as described for assay method 1 at 25°C, but contained only 50 nmol of UDP-glucose.

Cell extract	Product NADPH formed (nmol)	Molar ratio: product formed/substrate added
Myxamoebae NC-4	66	1.32
At 8 h stage*	63	1.25
At 22 h stage*	56	1.12
At 28 h (fruiting body*)	53	1.06

* See Fig. 1.

Activity in different cell types. Table 2 shows the characteristic specific activity of the various cell types. The contribution of the *A. aerogenes* to the value for the myxamoebae of strain NC-4 was probably less than 1% and the fact that the value for the myxamoebae grown axenically was typically higher than when the same myxamoebae were grown on bacteria agrees with the idea that the contribution made by the contaminating bacteria can be neglected. Assay methods 1 and 3 gave markedly different results.

Stoichiometry in crude extracts. The stoichiometry of the reaction:

was studied in the direction of synthesis of UDP-glucose with assay method 3, by measuring the change in E_{340} due to NAD⁺ reduction as a function of glucose 1-phosphate (or UTP) addition in the presence of excess of UTP (or glucose 1-phosphate). The expected stoichiometry of 2 mol of NADH formed per mol of UDP-glucose formed could not be demonstrated, although chromatographic analysis of the incubation mixtures showed that none of the limiting substrate and no UDP-glucose was present

when NADH production had ceased. This phenomenon has been observed before (Strominger *et al.*, 1957) and we have no explanation for this discrepancy. This lack of exact stoichiometry explains the differences between assay method 3 and methods 1 and 2 (Table 2).

We were also unable to demonstrate the expected stoichiometry of 1 mol of NADPH formed/mol of UDP-glucose added in assay method 1 (Table 3) and the extent of over-reduction of the added NADP⁺ varied with the morphogenetic stage. An obvious reason for this discrepancy is the presence in the crude extracts of 6-phosphogluconate dehydrogenase activity (Fig. 1). When the two activities are comparable (as they are in the myxamoebae and in early morphogenetic stages) the contribution of the 6-phosphogluconate dehydrogenase activity to the apparent UDP-glucose pyrophosphorylase activity might be significant. Assay method 2 was developed to overcome this problem and contains an excess of 6-phosphogluconate dehydrogenase activity in addition to the coupling enzymes of assay method 1. Table 2 shows the values obtained for activities in various cell types with assay methods 1 and 2. The difference is significant with the myxamoebal extracts

Fig. 2. Specific activity of UDP-glucose pyrophosphorylase in crude cell-free extracts during cell differentiation of *D. discoideum*

○, Assay method 1. Values are expressed in nmol of NADPH formed/min per mg of protein. ●, Assay method 2. Values are expressed as $\frac{1}{2}$ nmol of NADPH formed/min per mg of protein.

use in the assay. Also, the final step is essentially an equilibrium reaction, and McClure (1969) recommends that, to be valid, only the initial 3–5% of the reaction should be used. With these reservations the assay method was satisfactory and was linear with time (after an initial lag) and the amount of protein added, provided no more than 10–15 units of UDP-glucose pyrophosphorylase activity was added to the cuvette. The stoichiometry of assay method 2 was determined in the same fashion as with assay method 1. Values of 1.8–2.0 mol of NADPH formed/mol of limiting substrate added were observed and there was no consistent variation of the stoichiometry with morphogenetic stage.

Activity during development. In Fig. 2 the changes in UDP-glucose pyrophosphorylase specific activity during the cell differentiation measured with assay methods 1 and 2 are shown. The contribution of the endogenous 6-phosphogluconate dehydrogenase activity to the apparent activity as measured by assay method 1 is clearly shown in the early stages of development. With assay method 1 it can be seen that there is a ninefold increase in specific activity and with assay method 2 there is a 13-fold increase.

Discussion

Pannbacker (1967b) reported that there is a threefold increase in UDP-glucose pyrophosphorylase activity during cell differentiation, whereas Ashworth & Sussman (1967) reported a tenfold increase. This difference appears to be caused by two technical differences in the procedures used by these authors. First, as reported by Wright & Dahlberg (1968), this enzyme is labile in the tris buffers used by Pannbacker (1967b) but stable (Newell & Sussman, 1969) in the tricine buffers used by Ashworth & Sussman (1967) and by Roth *et al.* (1968). Secondly, spectrophotometric assays of this enzyme in crude extracts may be in error because of the presence, in these extracts, of 6-phosphogluconate dehydrogenase. The error involved can be minimized by suitable assay conditions (Newell & Sussman, 1970) but, since it is possible to alter the relative amounts of various enzymes by altering the growth conditions (Quance & Ashworth, 1972), this source of error can never be neglected.

There seems to be no doubt that the original conclusion of Ashworth & Sussman (1967), that there is a tenfold increase in the activity of UDP-glucose pyrophosphorylase during development of *D. discoideum*, was valid. Thus the observed dependence of this increase reported by these workers and by Roth *et al.* (1968) on concomitant protein synthesis and prior RNA synthesis also remains valid. It thus seems that the synthesis of this enzyme can be said to form part of the 'development programme' in the sense

(30–40%) and much less in extracts from fruiting bodies (10%). However, as a routine assay, method 2 is subject to some serious limitations, as multi-step coupled-enzyme assay systems display complex kinetics (McClure, 1969). Systems having more than two linking steps are rarely practicable, as they require increasingly large amounts of the auxiliary enzymes to ensure an accurate reflection of the activity of the primary enzyme. In this particular system, the final enzyme, 6-phosphogluconate dehydrogenase, is commercially available as a suspension in 3M-ammonium sulphate solution with a maximum activity of 13 units/ml. As this activity is comparatively low, large amounts are required, and, as the presence of much ammonium sulphate adversely affects the activity of the phosphoglucomutase, the enzyme must therefore be dialysed before

in which this term has been used by Sussman & Sussman (1969). There remains, however, the question of whether these transcriptional and translational controls remain the only controls that regulate the UDP-glucose flux and hence carbohydrate synthesis. As Pannbacker (1967*a*) noted, it would seem possible to account for the observed increase in the flux through the UDP-glucose pool with a smaller increase in UDP-glucose pyrophosphorylase activity than the 10–13-fold increase observed by us. This raises the general problem of the relationship, during development, of alterations in enzyme activities and consequent alterations in metabolic fluxes. Although the amount of an enzyme present may alter in order to affect a metabolic flux and thus accomplish a morphogenetic event, is this also a sufficient explanation of the biochemical cause of the event? This general problem is discussed in the papers that follow (Ashworth & Quance, 1972; Quance & Ashworth, 1972; Weeks & Ashworth, 1972; Hames *et al.*, 1972).

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