Pentose Phosphate Metabolism during Differentiation in *Dictyostelium discoideum*

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Cell-free extracts of *Dictyostelium discoideum* contained the enzymes necessary for both oxidative and non-oxidative pentose phosphate metabolism. The specific activities of these enzymes changed little during differentiation. The properties of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase were studied with respect to K_m values for substrates and cofactor NADP^+ . The two dehydrogenases were relatively unstable in extracts prepared from early stages of development. The K_m value of phosphoglucose isomerase for glucose 6-phosphate was approximately 30-fold higher than that of glucose-6-phosphate dehydrogenase. Measurements of pentose phosphate pathway intermediates were made throughout development. All measured intermediates except fructose 1,6-bisphosphate appeared to accumulate between aggregation and culmination. Fructose 1,6-bisphosphate concentrations remained constant until culmination, then dropped 3-fold during sorocarp construction. Calculation of mass-action ratios for the pentose phosphate reactions suggested that glucose-6-phosphate dehydrogenase was the only reaction greatly displaced from equilibrium. These results are discussed in relation to factors controlling pentose phosphate metabolism during development in *D. discoideum*.

INTRODUCTION

The cellular slime mould *Dictyostelium discoideum* is an organism exhibiting one of the simplest kinds of morphogenesis, which involves two major cell types. In the presence of external nutrients the organism grows and multiplies as free-living single amoebae. Under starvation conditions growth ceases and the amoebae aggregate to form a multicellular colony which differentiates through several distinctive stages to form a sorocarp or fruiting body. During differentiation there is no net loss of carbohydrate. At aggregation most of the carbohydrate material is present as glycogen and RNA (Wright *et al.*, 1977). At the end of differentiation there is a net decrease in these macromolecules and a comparable increase in new polysaccharide end-products (trehalose, cellulose–glycogen wall complex and mucopolysaccharide) (Rosness & Wright, 1974). The utilization of carbon units from RNA for end-product synthesis would presumably involve metabolism via a hexose monophosphate shunt.

Hexose metabolism has been extensively studied during development in *D. discoideum* (Cleland & Coe, 1968, 1969; Wright, 1965). However, little information is available concerning alternative pathways for glucose metabolism in this organism. Previous studies have shown that the slime mould contains both an active glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase (Wright, 1960; Edmundson & Ashworth, 1972). These two enzymes comprise the oxidative limb of the pentose phosphate pathway and catalyse the irreversible conversion of carbon units from glucose 6-phosphate into pentose phosphate intermediates. It is known that oxidative pentose phosphate metabolism

serves as a major source of NADPH (McLean, 1958). Thus, the increase in NADPH during development in *D. discoideum* suggests enhanced activity of this pathway (Wright & Wasserman, 1964). Additional evidence for the existence of a pentose phosphate pathway in this organism comes from the glucose-labelling studies of Wright & Bloom (1961). During development, the $6\text{-}^{14}\text{CO}_2/1\text{-}^{14}\text{CO}_2$ ratio from $[6\text{-}^{14}\text{C}]\text{glucose}$ and $[1\text{-}^{14}\text{C}]\text{glucose}$ decreases and is minimal during sorocarp construction. Though there is preliminary evidence supporting oxidative pentose phosphate metabolism in *D. discoideum*, little information is available on the non-oxidative reactions of this pathway.

The present communication establishes the existence of a pentose phosphate pathway during development in *D. discoideum*. The activities of oxidative and non-oxidative enzymes of this pathway were measured and intermediates were quantified throughout development.

METHODS

Growth conditions. *Dictyostelium discoideum* strain NC-4 (ATCC 24697) was grown with *Escherichia coli* on nutrient agar sheets as described previously (Marshall *et al.*, 1970). Amoebae were harvested, washed free of bacteria, and spread on 2% (w/v) agar sheets (38 × 25 cm) containing 10 mM-phosphate buffer, pH 6.5, and 1 mM-EDTA. The agar sheets were incubated at 15 or 22 °C.

Preparation of extracts for metabolite determination. Cells (10 sheets per experiment) were removed from the agar at the appropriate stage of development and immediately frozen in liquid nitrogen. Cell samples were removed before freezing for dry weight determination. Extracts were prepared by thawing the cells in 70% (v/v) ethanol (200 ml) and heating at 80 °C for 3 min. After centrifuging at 27000 g for 15 min, the extract was concentrated to 80 ml in a rotary evaporator and lyophilized overnight. The lyophilized extracts were dissolved in 13 ml 0.05 M-acetic acid and centrifuged at 27000 g for 15 min. The supernatant was divided into 2 ml aliquots and 0.30 ml of saturated barium acetate was added to each. After centrifuging at 2000 g for 10 min, 4 vol. ethanol were added to each aliquot and the tubes were refrigerated overnight at 5 °C. The Ba^{2+} precipitates were collected by centrifuging at 2000 g for 15 min and dissolved in 10 ml 0.05 M-acetic acid. Ba^{2+} was removed from the preparation by adding 0.8 ml of saturated K_2SO_4 . After centrifuging at 2000 g for 15 min, the extract was lyophilized overnight. The final preparation was dissolved in 2.0 ml 0.05 M-acetic acid and assayed for metabolite concentrations.

Attempts to measure concentrations of ribose 5-phosphate, ribulose 5-phosphate and xylulose 5-phosphate in cell extracts prepared using 6% (w/v) perchloric acid or 70% (v/v) ethanol were unsuccessful as these extracts contained high levels of phosphate which inhibited the standard pentose phosphate assay system. The measured intracellular phosphate concentration in *D. discoideum* is relatively high (approximately 5 mM) and increases 10-fold during differentiation (Gezelius & Wright, 1965). The Ba^{2+} precipitation procedure was utilized to separate hexose and pentose phosphates from intracellular phosphate. Control experiments using known amounts of authentic hexose and pentose phosphates yielded 100% recovery with 70% ethanol, the only exception being xylulose 5-phosphate for which 80% recovery was obtained. Ba^{2+} precipitation of known amounts of authentic pentose phosphates resulted in recoveries similar to hexose phosphates (glucose 6-phosphate, fructose 6-phosphate). Thus, in this study pentose phosphate concentrations were determined based on the recovery values for hexose phosphates.

Several control experiments were done using the Ba^{2+} precipitation procedure to evaluate the recovery of both hexose and pentose phosphates from *D. discoideum* extracts. Initial extraction with 70% ethanol resulted in higher recoveries for both glucose 6-phosphate (1.8-fold) and fructose 6-phosphate (2.8-fold) when compared with 6% perchloric acid extracts. Concentration of these extracts by Ba^{2+} precipitation demonstrated a relatively low recovery (15 to 20%) of the pentose phosphates (xylulose 5-phosphate, ribose 5-phosphate, ribulose 5-phosphate) with 6% perchloric acid as compared to 70% ethanol.

Expression of metabolite concentration. Hexose and pentose phosphate concentrations at each stage of differentiation are expressed as mM glucose equivalents, normalized to the initial dry weight/volume relationship at aggregation (Rosness & Wright, 1974). This method of calculation was chosen as both dry weight and volume decrease by 30 to 40% but total carbohydrate remains constant over the period of differentiation analysed (aggregation to sorocarp). At aggregation, 125 mg dry wt is equivalent to 1 ml packed cell volume (Walsh & Wright, 1978).

Metabolite assays. In general, metabolites were measured by the standard procedures given by Bergmeyer (1965). The two hexose phosphates, glucose 6-phosphate and fructose 6-phosphate, were assayed in the following system (total volume 1.0 ml): 200 μmol Tricine/KOH buffer (pH 7.6), 2 μmol MgCl_2 , 1 μmol NADP⁺ and 0.5 unit glucose-6-phosphate dehydrogenase. After completion of the above reaction, 2 units phosphoglucose isomerase were added for the determination of fructose 6-phosphate. The same assay

system was utilized for the quantification of 6-phosphogluconate with 0.1 unit 6-phosphogluconate dehydrogenase. Fructose 1,6-bisphosphate was assayed in the following system (total volume 1.0 ml): 300 μ mol glycylglycine/KOH buffer (pH 7.6), 3 μ mol NAD⁺, 4.5 μ mol sodium arsenate, 5 units glyceraldehyde-3-phosphate dehydrogenase and 0.5 units aldolase.

Pentose phosphates were assayed in a system similar to that described by Racker (1965). The standard reaction mixture contained (total volume 1.0 ml): 300 μ mol glycylglycine/KOH buffer (pH 7.6), 6 μ mol MgCl₂, 0.2 μ mol thiamin pyrophosphate, 4.5 μ mol sodium arsenate, 3 μ mol NAD⁺, 0.5 units aldolase, 5 units glyceraldehyde-3-phosphate dehydrogenase and 0.30 units transketolase. Aldolase was included in this system to remove fructose 1,6-bisphosphate before the addition of transketolase, which is not completely free from this enzyme. Xylulose 5-phosphate and ribulose 5-phosphate were measured in the above assay system by the addition of ribose 5-phosphate (1 μ mol) and ribulose-5-phosphate epimerase (0.25 units). Ribose 5-phosphate was assayed by the addition of xylulose 5-phosphate (1 μ mol). Total pentose phosphate was measured by addition of ribose 5-phosphate isomerase (10 units) and ribulose-5-phosphate epimerase (0.25 units) to the standard assay system.

Preparation of cell-free extracts. Cells at the appropriate stage of development were washed off the agar with 50 mM-Tris/HCl buffer (pH 7.6) (approx. 10 ml buffer per non-nutrient sheet). The cell suspensions were stored frozen at -20°C. The frozen cells were allowed to thaw at room temperature and disrupted by passage through a Yeda pressure cell at 110 MPa. After centrifuging the extract at 27000 g for 20 min, the crude cell-free supernatant was dialysed overnight at 6°C against 100 volumes of 50 ml Tris/HCl buffer (pH 7.6).

Protein determination. Protein was determined by Lowry's method, using bovine serum albumin as the standard.

Enzyme assays. Glucose-6-phosphate dehydrogenase [D-glucose-6-phosphate:NADP⁺ 1-oxidoreductase; EC 1.1.1.49], phosphoglucose isomerase [D-glucose-6-phosphate ketol-isomerase; EC 5.3.1.9] and 6-phosphogluconate dehydrogenase [6-phospho-D-gluconate:NADP⁺ 2-oxidoreductase (decarboxylating), EC 1.1.1.44] were assayed in the following system (total volume 1.0 ml): 200 μ mol Tricine/KOH buffer (pH 7.6), 2 μ mol MgCl₂, 1 μ mol NADP⁺, 2 μ mol substrate (glucose 6-phosphate, fructose 6-phosphate or 6-phosphogluconate) and enzyme extract. Assays for phosphoglucose isomerase included 2 units glucose-6-phosphate dehydrogenase in the assay mixture.

The pentose phosphate enzymes were assayed by a procedure similar to that of Cooper *et al.* (1958). The standard assay system contained (final volume 1.0 ml): 300 μ mol glycylglycine/KOH buffer (pH 7.6), 6 μ mol MgCl₂, 0.2 μ mol thiamin pyrophosphate, 4.5 μ mol sodium arsenate, 3 μ mol NAD⁺, 5 μ mol ribose 5-phosphate, 5 units glyceraldehyde-3-phosphate dehydrogenase and enzyme extract. For assays of ribose-5-phosphate isomerase [D-ribose-5-phosphate ketol-isomerase; EC 5.3.1.6], ribulose-5-phosphate epimerase (0.25 units) and transketolase (0.30 units) were included in the standard reaction mixture. Assays for ribulose-5-phosphate epimerase [D-ribulose-5-phosphate 3-epimerase; EC 5.1.3.1] contained ribose-5-phosphate isomerase (10 units) and transketolase (0.30 units). Transketolase [sedoheptulose-7-phosphate:D-glyceraldehyde-3-phosphate glycolaldehydetransferase; EC 2.2.1.1] activity was determined by the addition of ribose-5-phosphate isomerase (10 units) and ribulose-5-phosphate epimerase (0.25 units) to the standard assay system. Alternatively, transketolase was assayed in the absence of isomerase and epimerase with xylulose 5-phosphate (0.5 μ mol) as the acceptor. Transaldolase [sedoheptulose-7-phosphate:D-glyceraldehyde-3-phosphate dihydroxyacetone transferase; EC 2.2.1.2] activity was measured in the following assay system (final volume 1.0 ml): 300 μ mol glycylglycine/KOH buffer (pH 7.6), 4.5 μ mol sodium arsenate, 3 μ mol NAD⁺, 0.4 μ mol erythrose 4-phosphate, 10 μ mol fructose 6-phosphate, 5 units glyceraldehyde-3-phosphate dehydrogenase and enzyme extract.

All enzyme assays were followed by monitoring the increase in absorbance at 340 nm. Several concentrations of each prepared extract were assayed to ensure that the reaction rate was not limited by effects on one of the coupling enzymes. The temperature of the assay mixture was maintained at approximately 23°C. A molar absorption coefficient of 6.22×10^3 l mol⁻¹ cm⁻¹ was used to quantify NADH or NADPH formation at 340 nm. One unit of activity is defined as the amount of enzyme catalysing the synthesis of 1 μ mol NADH or NADPH min⁻¹ at 23°C. Specific activity is expressed as units (mg protein)⁻¹.

Chemicals. Glucose 6-phosphate, 6-phosphogluconate, ribose 5-phosphate, fructose 6-phosphate, xylulose 5-phosphate, ribulose 5-phosphate, erythrose 4-phosphate and NADP⁺ were obtained from Sigma; NAD⁺ was from Boehringer. Glyceraldehyde-3-phosphate dehydrogenase (77 units mg⁻¹), phosphoriboisomerase (245 units mg⁻¹), glucose-6-phosphate dehydrogenase (340 units mg⁻¹), phosphoglucose isomerase (750 units mg⁻¹), xylulose-5-phosphate epimerase (55 units mg⁻¹), transketolase (18 units mg⁻¹), 6-phosphogluconate dehydrogenase (54 units mg⁻¹), transaldolase (23 units mg⁻¹) and aldolase (10 units mg⁻¹) were obtained from Sigma.

Table 1. *Activities of enzymes of the pentose phosphate pathway in D. discoideum*

Extracts were prepared from cells at the appropriate stages of development and assayed for enzyme activities as described in Methods. Assays of the two dehydrogenases at aggregation were done immediately without prior overnight dialysis (see Table 2). The results are expressed as mean values $\pm$ S.E.M., with the number of determinations indicated in parentheses. Each determination was made on a separate population of cells.

Enzyme	Activity [units (mg protein) ⁻¹]			
	Aggregation	Pseudoplasmodium	Culmination	Sorocarp
Glucose-6-phosphate dehydrogenase	0.150 $\pm$ 0.016 (3)	0.145 $\pm$ 0.018 (4)	0.149 $\pm$ 0.012 (6)	0.150 $\pm$ 0.012 (5)
6-Phosphogluconate dehydrogenase	0.037 $\pm$ 0.006 (3)	0.032 $\pm$ 0.004 (5)	0.034 $\pm$ 0.005 (5)	0.034 $\pm$ 0.007 (4)
Phosphoglucose isomerase	0.229 $\pm$ 0.022 (3)	0.317 $\pm$ 0.033 (3)	0.373 $\pm$ 0.020 (3)	0.334 $\pm$ 0.033 (3)
Ribose-5-phosphate isomerase	0.226 $\pm$ 0.035 (3)	0.254 $\pm$ 0.033 (4)	0.262 $\pm$ 0.015 (6)	0.303 $\pm$ 0.009 (4)
Ribulose-5-phosphate epimerase	0.019 $\pm$ 0.004 (3)	0.022 $\pm$ 0.004 (4)	0.024 $\pm$ 0.002 (6)	0.025 $\pm$ 0.002 (5)
Transketolase	0.021 $\pm$ 0.002 (3)	0.028 $\pm$ 0.004 (4)	0.024 $\pm$ 0.004 (4)	0.031 $\pm$ 0.003 (3)
Transaldolase	0.072 $\pm$ 0.023 (3)	0.079 $\pm$ 0.010 (4)	0.077 $\pm$ 0.022 (3)	0.082 $\pm$ 0.023 (3)

Table 2. *Stability of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase in D. discoideum extracts prepared from cells at early and late stages of development*

Extracts were prepared from cells at the appropriate stage of development and assayed for enzyme activities as described in Methods. Where indicated, glucose 6-phosphate (10 mM) or 6-phosphogluconate (10 mM) was added prior to cell disruption. Initial assays were carried out immediately after cell disruption and centrifuging at 27000 g for 15 min. Cell extracts were reassayed after overnight dialysis at 6°C. The results are expressed as mean values $\pm$ S.E.M. for three determinations. Each determination was made on a separate population of cells.

Enzyme	Stage	Addition	Activity [units (mg protein) ⁻¹]	
			Initial	After dialysis
Glucose-6-phosphate dehydrogenase	Aggregation	—	0.150 $\pm$ 0.016	0.029 $\pm$ 0.009
	Aggregation	Glucose 6-phosphate	0.144 $\pm$ 0.013	0.048 $\pm$ 0.008
	Culmination	—	0.156 $\pm$ 0.017	0.146 $\pm$ 0.016
6-Phosphogluconate dehydrogenase	Aggregation	—	0.037 $\pm$ 0.006	0.006 $\pm$ 0.001
	Aggregation	6-Phosphogluconate	0.035 $\pm$ 0.004	0.009 $\pm$ 0.002
	Culmination	—	0.036 $\pm$ 0.008	0.034 $\pm$ 0.010

RESULTS

Crude cell-free extracts prepared from *Dictyostelium discoideum* were assayed under one set of conditions for individual enzymes of the pentose phosphate pathway. The specific activities of the non-oxidative pentose phosphate enzymes, ribose-5-phosphate isomerase, ribulose-5-phosphate epimerase, transketolase and transaldolase, changed little during differentiation (Table 1). This was also true of the two oxidative enzymes, glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase, provided activities were measured immediately after cell disruption. Initial studies of these two oxidative enzymes had indicated an increase in activity (approximately 5- to 7-fold), between aggregation and culmination. However, Wright and coworkers have found that several enzymes prepared from cells at early stages of development are unstable (Wright, 1960; Wright & Dahlberg, 1968). Results shown in Table 2 suggest that both dehydrogenases are unstable in crude extracts prepared from cells at aggregation. Assays of these enzymes immediately after preparation of the extracts yielded specific activities similar to those in culmination extracts, but after overnight dialysis these activities fell 5- to 7-fold. Preparation of the extract in the presence of substrate (10 mM-glucose 6-phosphate or 6-phosphogluconate) afforded some protection against a loss in activity. The non-oxidative pentose phosphate enzymes and phosphogluconate isomerase appeared to be relatively stable in extracts prepared from cells at early stages of development (Table 1). Overnight dialysis of these enzymes caused less than a 10% loss in activity.

In general, the activities of the enzymes of the pentose phosphate pathway were somewhat lower than those of the glycolytic enzymes in *D. discoideum* (Cleland & Coe, 1968). However, the activities of glucose-6-phosphate dehydrogenase and ribose-5-phosphate isomerase were about 4- to 6-fold higher than those of the other pentose phosphate enzymes. The activity of the glycolytic enzyme phosphogluconate isomerase was similar to the values reported previously by Cleland & Coe (1968). Of the pentose phosphate enzymes, ribulose-5-phosphate epimerase had the lowest specific activity, ribose-5-phosphate isomerase having a 10-fold higher activity.

In view of the relatively low activity of the pentose phosphate enzymes, crude extracts were tested for the presence of inhibitors for the enzymes assayed. Commercially prepared pentose phosphate enzymes (Sigma) were assayed before and after mixing with crude *D. discoideum* extracts. No evidence of significant inhibition was indicated for either oxidative or non-oxidative pentose phosphate enzymes when mixed with either early (aggregation) or late (culmination) extract.

Estimations of the individual role of the oxidative and non-oxidative reactions in the

Table 3. *Intracellular concentrations of pentose phosphate metabolites in D. discoideum during development*

Extracts were prepared from cells at the appropriate stage of development and assayed for metabolite concentration as described in Methods. Metabolite concentrations are expressed as mM glucose equivalents normalized to the initial dry weight/volume relationship at aggregation (see Methods). Results are given as mean values $\pm$ s.e.m., with the number of determinations indicated in parentheses. Each determination was made on a separate population of cells.

Metabolite	Concentration (mM glucose equivalents)			
	Aggregation	Pseudoplasmodium	Culmination	Sorocarp
Glucose 6-phosphate	0.088 ± 0.029 (3)	0.133 ± 0.015 (5)	0.216 ± 0.033 (4)	0.117 ± 0.017 (5)
Fructose 6-phosphate	0.030 ± 0.009 (3)	0.042 ± 0.004 (5)	0.071 ± 0.009 (4)	0.037 ± 0.004 (5)
6-Phosphogluconate	0.006 ± 0.003 (3)	0.013 ± 0.002 (4)	0.018 ± 0.002 (4)	0.010 ± 0.004 (4)
Fructose 1,6-bisphosphate	0.065 ± 0.005 (3)	0.048 ± 0.007 (4)	0.050 ± 0.002 (4)	0.024 ± 0.006 (4)
Ribose 5-phosphate	0.012 ± 0.003 (3)	0.018 ± 0.003 (5)	0.026 ± 0.003 (4)	0.017 ± 0.003 (5)
Ribulose 5-phosphate	0.011 ± 0.004 (3)	0.016 ± 0.002 (5)	0.024 ± 0.002 (4)	0.013 ± 0.002 (4)
Xylulose 5-phosphate	0.006 ± 0.002 (3)	0.012 ± 0.004 (5)	0.014 ± 0.003 (4)	0.008 ± 0.002 (5)

pentose phosphate shunt have been based on the quantitative measurements of the activities of the corresponding enzymes in extracts (Gumaa & McLean, 1969). When assayed under near-physiological conditions (pH 7.6), the ratio of 6-phosphogluconate dehydrogenase to glucose-6-phosphate dehydrogenase activity during development was about 0.25. This ratio changed markedly as the pH of the assay was increased. At pH 8.6, 6-phosphogluconate dehydrogenase activity was doubled whereas glucose-6-phosphate dehydrogenase activity was halved. Typical hyperbolic kinetics were indicated when glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase prepared from culminating cells were assayed at varying concentrations of either substrate. The apparent Michaelis constants (K_m) of glucose-6-phosphate dehydrogenase for glucose 6-phosphate and NADP^+ were approximately 4.2×10^{-5} M and 5.4×10^{-5} M, respectively. The measured K_m values of 6-phosphogluconate dehydrogenase for 6-phosphogluconate and NADP^+ were 2.9×10^{-5} M and 3.2×10^{-5} M at pH 7.6.

The glycolytic enzyme phosphoglucose isomerase can serve as a source of glucose 6-phosphate units for the oxidative reactions of the pentose shunt. However, in the reverse direction this enzyme can also provide fructose 6-phosphate units for the non-oxidative reactions. The activity of the slime mould isomerase changed little during development. The specific activity of the isomerase in prepared extracts was about 2-fold higher than that of glucose-6-phosphate dehydrogenase (Table 1). The apparent K_m values of the culmination enzyme for glucose 6-phosphate and fructose 6-phosphate were 14×10^{-4} M and 2×10^{-4} M, respectively.

The intermediates of the pentose phosphate pathway were measured to correlate enzyme activities with endogenous substrate concentrations. Metabolite concentrations generally provide a more accurate evaluation of cellular conditions than enzyme profiles (Hess & Brand, 1965). The two hexose phosphates, glucose 6-phosphate and fructose 6-phosphate, increased 3-fold and 2-fold, respectively, between early and late stages of development (Table 3). Wright *et al.* (1964) found similar changes in glucose 6-phosphate concentrations over this same period. Cleland's (1969) values for fructose 6-phosphate are lower than those measured in this study. Table 3 indicates that fructose 6-phosphate concentrations are about 3-fold lower than those of glucose 6-phosphate. Although the concentration of 6-phosphogluconate increased approximately 2-fold between aggregation and culmination, these concentrations were 10- to 12-fold lower than those of glucose 6-phosphate. The measured values for 6-phosphogluconate at culmination were similar to the concentrations observed in animal cells (Gumaa & McLean, 1968). In contrast to the above metabolites, fructose 1,6-bisphosphate concentrations remained relatively constant until culmination. The ratio of glucose 6-phosphate to fructose 1,6-bisphosphate dropped at an increasing rate over the course of development. After culmination, all of the measured hexose phosphate concentrations decreased at a similar rate.

Quantitative determinations of the pentose phosphate intermediates (ribose 5-phosphate, ribulose 5-phosphate, xylulose 5-phosphate) were compared with the intracellular concentrations of glucose 6-phosphate throughout differentiation (Table 3). Although the concentrations of these metabolites were low, they were similar to those measured previously in animal cells (Kauffman *et al.*, 1969). The observed values for ribose 5-phosphate, ribulose 5-phosphate and xylulose 5-phosphate were in the proportion of 1.1:1.0:0.5 rather than in the *in vitro* measured equilibrium ratio of 2.8:1.2:0.9 (Tabachnick *et al.*, 1958). Between aggregation and culmination, the total pentose phosphate pool increased about 2-fold. Throughout differentiation, the ratio of glucose 6-phosphate to total pentose phosphate remained constant at about 4:1. Attempts to measure intracellular concentrations of sedoheptulose 7-phosphate and erythrose 4-phosphate were unsuccessful. Values for these metabolites were either low or they were not recoverable by the procedures used in this study. Kauffman *et al.* (1969) have found erythrose 4-phosphate concentrations in mouse brain to be less than $2.0 \mu\text{mol kg}^{-1}$.

Table 4. Comparison between mass-action ratios and apparent equilibrium constants for reactions of the pentose phosphate pathway during development in *D. discoideum*

The mass-action ratios were calculated from the concentration of metabolites given in Table 3. Substrate concentrations are expressed as mM glucose equivalents based on packed cell volume at aggregation (see Methods). The pentose phosphate pathway enzymes are considered in the direction towards oxidative decarboxylation of glucose 6-phosphate. The mass-action ratio was calculated from the general expression $[C][D]/[A][B]$, using the NADPH/NADP⁺ values of Wright & Wasserman (1964) and the [CO₂] value of 1.16 mM (Krebs & Veech, 1969).

Enzyme	Calculated mass-action ratio				Apparent equilibrium constant*
	Aggregation	Pseudo-plasmodium	Culmination	Sorocarp	
Glucose-6-phosphate dehydrogenase	0.093	0.134	0.100	0.163	2.74×10^4 (1)
6-Phosphogluconate dehydrogenase	2.91	1.95	2.47	2.88	2.3 to 5.0 (2)
Phosphoglucose isomerase	0.341	0.316	0.329	0.316	0.32 to 0.47 (3)
Ribose-5-phosphate isomerase	0.916	0.889	0.923	0.764	0.35 to 0.77 (4)
Ribulose-5-phosphate epimerase	0.546	0.750	0.583	0.615	1.25 to 1.40 (5)

* The apparent equilibrium constants are from: 1, Gumaa & McLean (1969); 2, Horecker & Smyrniotis (1952); 3, Hess (1963); 4, Dickens & Williamson (1956); 5, Hurwitz & Horecker (1956).

Endogenous mass-action ratios for several of the pentose phosphate reactions were calculated from the concentration of intermediates given in Table 3. Values for the calculated mass-action ratios during differentiation were compared with the apparent equilibrium constants for each reaction (Table 4). Values for the ratio NADPH/NADP⁺, used in calculating the mass-action ratios, were taken from Wright & Wasserman (1964): these were 1.37 for aggregation and slug, 1.10 at preculmination, 1.20 at culmination and 1.91 at sorocarp. The results shown in Table 4 show no clear-cut changes in the mass-action ratios during differentiation. Of the reactions studied, only that catalysed by glucose-6-phosphate dehydrogenase was greatly displaced from equilibrium. Apparently, this enzyme represents a rate-limiting step in the conversion of hexose phosphates to pentose phosphates. The reactions catalysed by 6-phosphogluconate dehydrogenase, phosphoglucose isomerase and by the non-oxidative pentose phosphate enzymes (ribose-5-phosphate isomerase and ribulose-5-phosphate epimerase) were all close to equilibrium. The mass-action ratio determined for phosphoglucose isomerase was similar to values previously calculated in glycolysing yeast cells (Hess, 1963). Because intracellular concentrations of sedoheptulose 7-phosphate and erythrose 4-phosphate could not be measured, mass-action ratios for transketolase and transaldolase were not calculated. However, these reactions were close to equilibrium in yeast and animal systems (Racker, 1961; Gumaa & McLean, 1969).

DISCUSSION

The results of this study indicate that *D. discoideum* contains all the enzymes necessary for both oxidative and non-oxidative pentose phosphate metabolism during development. Figure 1 illustrates the interconversions between metabolites of the oxidative and non-oxidative reactions of the pentose phosphate pathway. Other enzymes participating in the conversion of pentose phosphate into glucose 6-phosphate, such as phosphoglucose isomerase and fructose-bisphosphate aldolase are also known to occur in this organism (Cleland, 1969). Cleland & Coe (1968) surveyed the glycolytic enzymes in *D. discoideum* and found specific activity changes of less than 3-fold between early and late stages. They

Fig. 1. Oxidative and non-oxidative reactions of the pentose phosphate pathway. The enzymes involved in these reactions are: (1) glucose-6-phosphate dehydrogenase; (2) 6-phosphogluconate dehydrogenase; (3) phosphoglucose isomerase; (4) ribose-5-phosphate isomerase; (5) ribulose-5-phosphate epimerase; (6) transketolase; (7) transaldolase. The concentration of the metabolites in boxes were measured in this study.

estimated that glycolytic activity occurs at a constant rate during development. The overall contribution of the hexose monophosphate pathway to development in *D. discoideum* has yet to be evaluated; however, results in this study allow several observations to be made.

The specific activities of the pentose phosphate enzymes remain relatively constant throughout development. The glucose-labelling studies of Wright & Bloom (1961), utilizing [6- ^{14}C]glucose and [1- ^{14}C]glucose, suggest active pentose phosphate metabolism occurs during early (aggregation) and late (sorocarp) stages. Since glucose dehydrogenase and the Entner–Doudoroff enzymes are absent from this organism, the release of $^{14}\text{CO}_2$ from [1- ^{14}C]glucose is attributed mainly to the enzymes of the pentose phosphate pathway. The C-1/C-6 ratio between pseudoplasmodium and culmination stages was closer to unity, indicating relatively greater activity by the glycolytic enzymes. Wright & Bloom (1961) also found that the relative rates of CO_2 formation from [1- ^{14}C]glucose and [6- ^{14}C]glucose decrease 10-fold as differentiation proceeds. During development in *D. discoideum*, cells utilize protein degradation as the major source of energy (Wright, 1963). These non-labelled intermediates would enter the Krebs cycle and dilute the labelled intermediates arising from [^{14}C]glucose. Thus, the interpretation of labelling studies observed in this organism must take into account the less efficient utilization of carbohydrate as an energy source.

A logical control point for operation of oxidative pentose phosphate metabolism is the enzyme glucose-6-phosphate dehydrogenase. The reaction catalysed by this enzyme is essentially irreversible and is situated at the branch point between the two pathways of carbohydrate oxidation. Determination of the mass-action ratios for the *D. discoideum* glucose-6-phosphate dehydrogenase indicated that this reaction was displaced far from equilibrium. This is in contrast to the mass-action ratio determinations for the 6-phosphogluconate dehydrogenase reaction, which appears to be close to equilibrium (Table 4).

Glucose 6-phosphate limitation is probably not a factor in regulating the activity of glucose-6-phosphate dehydrogenase, as the intracellular activities of this metabolite were

well above the K_m of this enzyme. Phosphoglucose isomerase, which competes with glucose-6-phosphate dehydrogenase for glucose 6-phosphate, had an apparent K_m some 5-fold higher than the measured concentrations of glucose 6-phosphate at culmination. As a result, a substantial part of the glucose 6-phosphate pool would be metabolized by the oxidative pentose phosphate pathway as compared to the non-oxidative reactions and glycolysis.

One factor limiting the oxidative pentose phosphate reactions is the rate of reoxidation of NADPH (McLean, 1960; Osmond & Aprees, 1969). The presence of artificial electron acceptors (phenazine methosulphate) or substrates which require NADPH for their metabolism has been shown to stimulate the rate of formation of $^{14}\text{CO}_2$ from $[1-^{14}\text{C}]$ glucose in animal cells (Cahill *et al.*, 1958; Hers, 1957). Addition of glyceraldehyde (100 mM) to *D. discoideum* cells incubated with $[1-^{14}\text{C}]$ glucose stimulated $^{14}\text{CO}_2$ formation 2-fold (B. E. Wright, unpublished results). Thus, it would appear that pentose phosphate metabolism in the slime mould is limited by the availability of oxidized forms of the coenzyme. According to Wright & Wasserman (1964), the intracellular concentrations of NADPH increase 1.4-fold between early and late stages of development. However, NADP^+ concentrations remain relatively constant at about $25 \mu\text{mol (ml packed cell volume)}^{-1}$. This is close to the measured K_m for NADP^+ of 6-phosphogluconate dehydrogenase. Glucose-6-phosphate dehydrogenase has a 2-fold higher apparent K_m value for NADP^+ and is therefore more likely to be the rate-limiting reaction.

6-Phosphogluconate dehydrogenase may also play a part in the control of oxidative pentose phosphate reactions (Kauffman *et al.*, 1969). Concentrations of 6-phosphogluconate measured in the early stages of development were 2- to 3-fold lower than the K_m of 6-phosphogluconate dehydrogenase. Carter & Parr (1967) have shown that fructose 1,6-bisphosphate is a competitive inhibitor of 6-phosphogluconate dehydrogenase. When fructose 1,6-bisphosphate is present in equimolar amounts with substrate, 25% inhibition of the 6-phosphogluconate dehydrogenase reaction was indicated. The high ratio of fructose 1,6-bisphosphate to 6-phosphogluconate (5 to 7) in early stages of development, suggests that this might be an important control factor.

Evaluations of the activity of the non-oxidative pentose phosphate reactions in *D. discoideum* is complicated by the inability to measure intracellular concentrations of sedoheptulose 7-phosphate and erythrose 4-phosphate. If one assumes that the transketolase and transaldolase reactions are at equilibrium, calculations based on equilibrium constants (Datta & Racker, 1961; Venkataraman & Racker, 1961) and glyceraldehyde 3-phosphate concentrations determined by Cleland (1969) yield sedoheptulose 7-phosphate and erythrose 4-phosphate values of $0.028 \mu\text{mol (ml packed cell volume)}^{-1}$ and $0.004 \mu\text{mol (ml packed cell volume)}^{-1}$, respectively, at culmination. These are close to the steady state values observed previously in animal cells (Gumaa & McLean, 1968). Transketolase is the limiting enzyme of the pentose phosphate pathway when measurements are made under optimum conditions *in vitro* (Novello & McLean, 1968). In *D. discoideum*, ribulose-5-phosphate epimerase activity was found to be slightly lower than transketolase activity; however, optimum conditions may not have been utilized for the preparation and assay of these enzymes.

For total cell carbohydrate to remain constant during differentiation, a source of carbon units other than glycogen must be present (Wright *et al.*, 1977). Unpublished results from that laboratory indicate that intracellular concentrations of glucose 6-phosphate increase when cells from late stages are incubated with ribose. It is generally recognized that the non-oxidative reactions of the pentose phosphate pathway can serve as a salvage pathway for ribose (Eldan & Blum, 1975). During differentiation about 40% of the cellular RNA is degraded (White & Sussman, 1961). The results of this study indicate that *D. discoideum* does contain the enzymes necessary to utilize pentose carbon made available from RNA degradation. Enzyme and isotope studies similar to those of Gumaa & McLean (1969)

will be needed for further evaluation of the relative importance of oxidative and non-oxidative pentose phosphate reactions to carbohydrate metabolism in *D. discoideum*.

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