Accumulation of UDP-Glucose Pyrophosphorylase by Axenically Grown Amoebae of *Dictyostelium discoideum*

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Amoebae of the slime mould *Dictyostelium discoideum* AX2 possess only low UDP-glucose pyrophosphorylase activity when grown on autoclaved *Klebsiella aerogenes* (approx. 30 units/mg of protein), but accumulate the enzyme to approx. 150–200 units/mg of protein during vegetative growth in axenic medium. The vegetative accumulation of UDP-glucose pyrophosphorylase by axenically grown cells is prevented if autoclaved *K. aerogenes* are included in the axenic medium, suggesting the absence of a specific inducer. Affinity chromatography using anti-(UDP-glucose pyrophosphorylase) antibody and sodium dodecyl sulphate/polyacrylamide-gel electrophoresis indicate that the enzyme accumulated during axenic growth and that normally accumulated during development are immunologically cross-reactive and that both are composed of two subunits with mol.wts. 55600 and 57500 present in approximately equal amounts in the active enzyme.

UDP-glucose pyrophosphorylase (EC 2.7.7.9) is a key enzyme in the development of the cellular slime mould *Dictyostelium discoideum*, since one of the products of the reaction catalysed is UDP-glucose, which serves as the precursor for the biosynthesis of glycogen, trehalose, cellulose and a mucopolysaccharide essential for fruiting-body formation. During growth and the early stages of development of amoebae previously grown on bacteria, UDP-glucose pyrophosphorylase activity is maintained at a low basal activity (approx. 30–40 units/mg of protein), but once the developing cells have entered into multicellular aggregates, enzyme specific activity rises to a peak of 400–450 units/mg of protein (Ashworth & Sussman, 1967; Newell & Sussman, 1970; Franke & Sussman, 1973; Edmundson & Ashworth, 1972). Accumulation of this enzyme is known to be essential for successful fruiting-body formation (Dimond *et al.*, 1976) and dependent on continual cell–cell interaction (Newell *et al.*, 1971). Experiments with actinomycin D alone (Newell *et al.*, 1971) or in combination with daunomycin (Firtel *et al.*, 1973) indicate that RNA transcription is required for the increase in enzyme specific activity, and Franke & Sussman (1973) have shown that the increase reflects a corresponding increase in enzyme protein and concluded that it is the direct result of substantial rise in the rate of UDP-glucose pyrophosphorylase synthesis compared with general protein synthesis. In contrast,

Gustafson & Wright (1973) and Gustafson *et al.* (1973), using similar techniques, have ascribed the rise in enzyme protein to a decrease in the rate of enzyme-protein degradation relative to that of other proteins.

Although developmental accumulation of UDP-glucose pyrophosphorylase requires specific cell–cell contacts, this enzyme is also accumulated during growth in axenic medium (Ashworth & Watts, 1970; Ashworth & Quance, 1972; Dimond *et al.*, 1976). During axenic growth of *D. discoideum* A3, UDP-glucose pyrophosphorylase is accumulated to a specific activity of approx. 100 units/mg of protein (assayed at 25°C), which is almost half that normally accumulated during development of bacterially grown amoebae. This raises the possibility that axenic medium contains an inducer that mimics the switch *in vivo*, causing developmental accumulation of UDP-glucose pyrophosphorylase without the requirement for formation of multicellular aggregates. However, Gustafson & Wright (1973) have provided evidence that the enzyme present in vegetative amoebae grown on bacteria is composed of subunits with a different molecular weight from those of developmentally accumulated enzyme, suggesting that two isoenzymes of UDP-glucose pyrophosphorylase may exist, one restricted to the growth phase and the other to development. To examine this phenomenon we report experiments designed to test whether the UDP-glucose pyrophosphorylase accumulated during axenic growth is the same enzyme protein as that normally accumulated during development and whether axenic medium does contain a specific enzyme inducer.

Abbreviation used: IgG, immunoglobulin G.

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Experimental

Materials

Cemusol NPT-12 (a detergent widely used for lysis of *D. discoideum* amoebae) was a gift from Rhone-Poulenc Chimie Fine, 21 Rue Jean Goujon, 75360 Paris, France. DEAE-cellulose, CM-cellulose and Sepharose 4B were obtained from Sigma (London) Chemical Co., Poole, Dorset, U.K.; acrylamide and other polyacrylamide-gel materials were purchased from Bio-Rad Laboratories, Richmond, CA, U.S.A. All other chemicals used were of the highest purity commercially available and obtained from Sigma.

Methods

Growth and development of *D. discoideum*. *D. discoideum* AX2 amoebae were grown in HL5 axenic medium supplemented with 86mM-glucose as described by Watts & Ashworth (1970) to a final cell density of 1.4×10^6 – 6.0×10^6 /ml, harvested by centrifugation (500g, 5 min), washed twice with water, and plated on washed Millipore filters at 2.5×10^7 – 3.0×10^7 cells per filter as described by Hames & Ashworth (1974). Development was then allowed to occur in a humid atmosphere at 22°C with continuous exposure to overhead light, resulting in fruiting-body formation approx. 25h after deposition of the myxamoebae. Alternatively, for bacterially grown *D. discoideum* AX2, *K. aerogenes* was grown overnight at 37°C in SM broth (Sussman, 1966) and harvested and washed in 0.04M- $\text{Na}_2\text{HPO}_4/\text{KH}_2\text{PO}_4$ buffer, pH 6.5, by centrifugation at 20000g for 30 min. The bacterial pellet was resuspended in this buffer to approx. $7 A_{650}$ units/ml and the suspension autoclaved [104 kPa (15 lbf/in^2) 20 min]. After cooling to 22°C, *D. discoideum* amoebae were inoculated into the autoclaved *K. aerogenes* suspension, supplemented with 86mM-glucose, at 5×10^4 cells/ml. Amoebae were harvested for enzyme assay when they had reached a final cell density of 1.60×10^6 – 3.60×10^6 cells/ml. For some assays *D. discoideum* AX2 myxamoebae were grown on autoclaved *K. aerogenes* suspended in HL5 medium supplemented with 86mM-glucose under conditions similar to those described above for bacterially grown amoebae.

Assay of UDP-glucose pyrophosphorylase

At various times during development, amoebae were harvested from four Millipore filters into 5 ml of ice-cold 0.1M-Tricine [*N*-[2-hydroxy-1,1-bis-(hydroxymethyl)ethyl]glycine]/NaOH, pH 7.6, centrifuged (500g, 5 min), and resuspended in 0.5 ml of this buffer. Complete cell lysis was achieved by freeze-thaw in the presence of 0.15% (v/v) Cemusol NPT-12. Cell extracts from vegetative amoebae were prepared by a similar protocol. UDP-glucose pyrophosphorylase activity was measured immediately at

37°C by using the assay described by Franke & Sussman (1971). A unit of UDP-glucose pyrophosphorylase is that capable of converting 1 nmol of UDP-glucose into glucose 1-phosphate/min at 37°C.

Assay of 6-phosphogluconate dehydrogenase

Cell extracts, prepared as described above, were assayed for 6-phosphogluconate dehydrogenase activity by the method of Edmundson & Ashworth (1972). A unit of activity is defined as that amount which converts 1 nmol of NADP^+ into NADPH/min at 37°C.

Preparation of anti-(UDP-glucose pyrophosphorylase) antibody

D. discoideum mutant KY-3 was grown in association with *K. aerogenes* on 2% (w/v) agar plates as described by Franke & Sussman (1971). This mutant is morphogenetically deficient and stops development before fruiting-body formation, but accumulates UDP-glucose pyrophosphorylase to the same activity as in the wild type, although the activity fails to disappear (Ashworth & Sussman, 1967). The UDP-glucose pyrophosphorylase accumulated during development of mutant KY-3 appears to be catalytically and immunochemically indistinguishable from the wild type (Franke & Sussman, 1971). After the amoebae had reached their terminal morphogenetic stage, they were harvested in ice-cold 0.05M- PP_i/P_i buffer, pH 7.5 (0.05M-tetrasodium pyrophosphate adjusted to pH 7.5 with NaH_2PO_4), lysed by freeze-thaw, and centrifuged at 20000g for 20 min to remove residual bacteria plus cell debris. UDP-glucose pyrophosphorylase was purified from the supernatant by a modification of the method of Gustafson *et al.* (1973). Antisera were raised against UDP-glucose pyrophosphorylase by intramuscular injection into rabbits of 100 μg of electrophoretically pure enzyme in Freund's complete adjuvant, followed by a similar injection 5 weeks later in Freund's incomplete adjuvant. Bleeding was undertaken 1 and 2 weeks later and IgG prepared by DEAE-cellulose and CM-cellulose chromatography as described by Schimke *et al.* (1973).

Preparation of immunoadsorbent

Two types of immunoadsorbent were prepared: rabbit anti-(UDP-glucose pyrophosphorylase) antibody linked to Sepharose 4B and pre-immune rabbit γ -globulin linked to Sepharose 4B. The pre-immune serum was taken through the same purification procedure as for preparation of anti-(UDP-glucose pyrophosphorylase) IgG described above. In each case Sepharose 4B was activated with CNBr and linked to the corresponding γ -globulin by the method of Livingston (1973), the final immunoadsorbent

being stored in 0.015 M-NaCl/0.05 % NaN_3 /0.01 M-sodium phosphate, pH 7.2.

Immunoadsorption of vegetative and developmental UDP-glucose pyrophosphorylase

Vegetative *D. discoideum* AX2 amoebae at 1×10^7 – 2×10^7 cells/ml in HL5 medium supplemented with 86 mM-glucose or developing cells at the 18 h stage of development were harvested and resuspended in 0.15 % (v/v) Cemusol NPT-12 in 0.1 M- PP_i/P_i buffer, pH 7.5, at 5×10^8 cells/ml. After lysis by freeze-thaw, cell debris was removed by centrifugation at 20000g for 20 min followed by centrifugation at 250000g for 2.5 h. The supernatant was removed, avoiding the floating pellicle, and diluted with 2 vol. of 0.1 M- PP_i/P_i buffer, pH 7.5. After addition of $(\text{NH}_4)_2\text{SO}_4$ to 40 % saturation and incubation in ice for 30 min, the resulting precipitate was removed by centrifugation (15000g/15 min). The supernatant was re-adjusted to 70 % $(\text{NH}_4)_2\text{SO}_4$ saturation, the protein pellet recovered by centrifugation, dissolved in 0.01 M- PP_i/P_i buffer, pH 7.5, and dialysed against this buffer overnight at 4°C. Any precipitate that formed on dialysis was removed by centrifugation at 15000g for 10 min. The dialysed supernatant was made 0.3 M in NaCl and 2 % (v/v) in Triton X-100 and passed slowly through a 5 ml column of pre-immune rabbit γ -globulin–Sepharese pre-equilibrated in 0.3 M-NaCl/2 % Triton X-100/0.01 M- PP_i/P_i , pH 7.5. All column eluates were monitored for UDP-glucose pyrophosphorylase activity and for protein content by measuring the A_{300} . The enzyme eluted from the column was passed immediately through a 3.5 ml column of anti-(UDP-glucose pyrophosphorylase) antibody–Sepharese. The bound enzyme was washed successively with 0.3 M-NaCl/2 % (v/v) Triton X-100/0.01 M- PP_i/P_i , pH 7.5, then 1 M-NaCl/2 % (v/v) Triton X-100/0.05 M- PP_i/P_i , pH 7.5, then 1 M-NaCl/0.05 M- PP_i/P_i , pH 7.5, and finally eluted with 0.05 M- PP_i , adjusted to pH 10.5 with NH_3 . Immediately after enzyme elution the pH of the eluate was adjusted to neutrality by addition of NaH_2PO_4 and the column stored in 0.1 M-NaCl/0.05 % NaN_3 /0.01 M- PP_i/P_i buffer, pH 7.5.

Electrophoretic characterization of immunoadsorbed UDP-glucose pyrophosphorylase

For this, 4 % (w/v) polyacrylamide gels (1.3 cm diameter, 11 cm long) were pre-electrophoresed at 10 mA/gel for 1 h in Tris/glycine/ PP_i buffer, pH 8.8 (9 g of Tris, 36 g of glycine 13.5 g of tetrasodium pyrophosphate per litre). The enzyme eluted from the immunoadsorbent was precipitated by addition of $(\text{NH}_4)_2\text{SO}_4$ to 70 % saturation and incubation in ice for 30 min. After recovery of the enzyme precipitate by centrifugation at 50000g for 1 h at 4°C, it was dissolved in 100 μl of 10-fold diluted Tris/glycine/ PP_i buffer, pH 8.8, containing 20 % (v/v) glycerol and

Bromophenol Blue as electrophoretic marker. The sample was loaded on the pre-electrophoresed 4 % polyacrylamide gels, electrophoresed overnight at 10 mA/gel, and sliced into 3 mm slices. Each slice was macerated in 2 ml of 0.1 M- PP_i/P_i buffer, pH 7.5, left overnight at 4°C, and then gel pieces were removed by centrifugation (15000g, 15 min). The supernatant was assayed for UDP-glucose pyrophosphorylase activity, and a 50 μl sample was mixed with 50 μl of 2 % sodium dodecyl sulphate/0.2 % mercaptoethanol/20 % glycerol/1 M-Tris/HCl, pH 6.8, heated at 100°C for 5 min and then electrophoresed for 16 h at 150 V on a 6–18 % polyacrylamide gradient slab gel by using the sodium dodecyl sulphate buffer system of Laemmli (1970). Slab gels were stained with 0.1 % Coomassie Brilliant Blue R-250 in methanol/water/acetic acid (5:5:2, by vol.) and destained in 5 % methanol/10 % (w/v) acetic acid.

Results

When grown in axenic culture, amoebae of *D. discoideum* AX2 accumulate cellular UDP-glucose pyrophosphorylase activity such that the specific activity of this enzyme is approx. 4 times that when the amoebae are grown on autoclaved *K. aerogenes* (Table 1). One of the inherent problems of the spectrophotometric assay for UDP-glucose pyrophosphorylase activity is the possibility of error due to variation in 6-phosphogluconate dehydrogenase activity between the cell samples being compared (Edmundson & Ashworth, 1972). The specific activity of the latter enzyme remains constant, irrespective of the growth medium used here, and this results in an increased ratio of 6-phosphogluconate dehydrogenase to UDP-glucose pyrophosphorylase activities in bacterially grown cells as compared with axenically grown myxamoebae (Table 1). If anything, this would lead to an overestimate of UDP-glucose pyrophosphorylase activity in bacterially grown amoebae and mask an even greater disparity of UDP-glucose pyrophosphorylase activities in the two cell types than we observe; in fact, control experiments involving the addition of excess 6-phosphogluconate dehydrogenase to the reaction mixture before assay did not alter the ratio of UDP-glucose pyrophosphorylase activity between the two cell extracts (B. D. Hames, unpublished work).

The large difference in UDP-glucose pyrophosphorylase activity between bacterially and axenically grown *D. discoideum* AX2 is not due to the presence of cellular enzyme activators or inhibitors, since mixtures of the two extracts always display UDP-glucose pyrophosphorylase activity that is the sum of the activities of the individual preparations (B. A. Hodson, unpublished work). However, when amoebae are grown in axenic medium that also contains autoclaved *K. aerogenes*, the amoebal UDP-glucose

Table 1. *Variation in UDP-glucose pyrophosphorylase activity with growth conditions*
Results are means of three separate experiments $\pm$ s.d. Abbreviation: n.d., not determined.

	Growth conditions		
	HL5 medium	<i>K. aerogenes</i>	HL5 medium + <i>K. aerogenes</i>
UDP-glucose pyrophosphorylase specific activity (units/mg)	146 $\pm$ 20.0	34.3 $\pm$ 9.7	29.7 $\pm$ 7.3
6-Phosphogluconate dehydrogenase specific activity (units/mg)	60.3 $\pm$ 7.3	51.6 $\pm$ 5.5	n.d.

pyrophosphorylase activity is decreased to values characteristic of bacterially grown cells (Table 1). Thus the increased activity of UDP-glucose pyrophosphorylase in axenically grown amoebae does not appear to be due to the presence of an inducer in the axenic medium. On occasions, axenically grown amoebae have been observed to accumulate up to 200 units of UDP-glucose pyrophosphorylase/mg of protein, but interestingly even these amounts do not prevent the developmental accumulation of this enzyme occurring at the normal stage; assuming that the slow decrease in vegetatively accumulated enzyme activity continues throughout development, the amount of UDP-glucose pyrophosphorylase normally accumulated during development of bacterially grown cells is still accumulated by axenically grown cells (Fig. 1). This raises the question as to whether the enzyme accumulated during axenic growth results from a premature accumulation of the same gene product as is accumulated during development or whether the two activities represent separate structural-gene isoenzymic variants. In an attempt to answer this question, we chose to compare vegetative and developmentally accumulated UDP-glucose pyrophosphorylase immunologically and electrophoretically.

Antibodies were raised in rabbits against UDP-glucose pyrophosphorylase purified from developing amoebae of *D. discoideum* mutant KY-3, which accumulates UDP-glucose pyrophosphorylase at the same stage of development as the wild-type organism, but unlike the wild-type the enzyme activity fails to decay, providing a convenient experimental enzyme source. Enzyme purified from developing cells of *D. discoideum* KY-3 and the wild-type organism are immunochemically and catalytically identical (Franke & Sussman, 1971). The anti-(UDP-glucose pyrophosphorylase) antibody was coupled to Sepharose 4B by using CNBr activation, and a similar immuno-adsorbent was also prepared by using pre-immune rabbit γ -globulin. *D. discoideum* AX2 amoebae at the 18 h stage of development were lysed and the protein precipitating between 40 and 70% $(\text{NH}_4)_2\text{SO}_4$ saturation was dissolved in 0.3M-NaCl/2% (v/v) Triton X-100 and passed down a column of pre-

Fig. 1. *Developmental kinetics of UDP-glucose pyrophosphorylase*

The cells were harvested at various stages of development and assayed for UDP-glucose pyrophosphorylase immediately after cell lysis. ●, *D. discoideum* AX2 amoebae grown to 1.60×10^6 cells/ml in HL5 medium supplemented with 86mM-glucose. The broken curve shows UDP-glucose pyrophosphorylase accumulation by *D. discoideum* NC4 grown on bacteria (Newell *et al.*, 1971). *D. discoideum* AX2 grown on *K. aerogenes* gives an almost identical developmental profile for this enzyme (Edmundson & Ashworth, 1972).

immune γ -globulin-Sepharose. This step should remove any protein which binds non-specifically to rabbit γ -globulin, although non-specific adsorption is also minimized by the presence of a high salt concentration and Triton X-100. Both the bulk of the sample protein and all of the UDP-glucose pyrophosphorylase activity passed unretarded through the column (Fig. 2a) and was immediately loaded on a second column containing anti-(UDP-glucose pyrophosphorylase) antibody-Sepharose. All of the UDP-glucose pyrophosphorylase activity was retained by

Fig. 2. Affinity chromatography of UDP-glucose pyrophosphorylase by using IgG-Sephacrose

(a) Cell extracts were prepared from axenically grown cells at the 18h stage of development and protein precipitating between 40% and 70% $(\text{NH}_4)_2\text{SO}_4$ saturation was loaded on a column of pre-immune rabbit IgG-Sephacrose 4B. Column eluates were assayed for UDP-glucose pyrophosphorylase activity ($\circ$) and A_{300} (for protein) ($\bullet$). (b) Fractions eluted from the pre-immune rabbit IgG-Sephacrose which contained UDP-glucose pyrophosphorylase activity were loaded on a column of anti-(UDP-glucose pyrophosphorylase) IgG-Sephacrose. The column was loaded in and washed with 0.3M-NaCl/2% Triton X-100/0.01M- PP_i/P_i , pH 7.5, then 1M-NaCl/2% Triton X-100/0.05M- PP_i/P_i , pH 7.5 (buffer 1), then 1M-NaCl/0.05M- PP_i/P_i , pH 7.5 (buffer 2), and enzyme eluted with 0.05M- PP_i adjusted to pH 10.5 with NH_3 (buffer 3).

the immunoadsorbent, whereas most of the sample protein was eluted unretarded once more (Fig. 2b). Some protein was bound non-specifically, despite the prior passage through pre-immune rabbit γ -globulin-Sephacrose, and this was eluted by raising the salt concentration to 1M. A final wash with 1M-NaCl/0.05M- PP_i/P_i , pH 7.5, served to remove Triton X-100, which otherwise interferes with the recovery of UDP-glucose pyrophosphorylase after column chromatography, and then the enzyme was eluted by raising the pH of the column buffer. A further wash with 0.05M- PP_i/P_i /0.1M-glycine/HCl, pH 2.8, failed to elute any more enzyme. Provided that the antibody-Sephacrose is restored to pH 7.5 immediately after enzyme elution it remains active and can be used repeatedly.

Developmentally accumulated UDP-glucose pyrophosphorylase eluted from the immunoadsorbent was electrophoresed on 4%-polyacrylamide rod gels in Tris/glycine/pyrophosphate buffer, pH 8.8, the gels sliced and each slice assayed for enzyme activity (Fig. 3a). Gel fractions were then eluted and samples analysed on 6-18% polyacrylamide gradient slab gels by using the sodium dodecyl sulphate buffer system of Laemmli (1970). Only two polypeptides were observed in all gel fractions analysed, and the concentration of these, as estimated from the staining intensity of the polypeptide bands, correlated exactly with the activity of UDP-glucose pyrophosphorylase (Fig. 3b). The molecular weights of the polypeptides were 55600 and 57500 as determined by mobility comparison with molecular-weight markers. When UDP-glucose pyrophosphorylase present in vegetative amoebae of axenically grown *D. discoideum* AX2 was purified by the same protocol, again these two polypeptides were present and coincided exactly with UDP-glucose pyrophosphorylase activity (Figs. 3c and 3d). A third polypeptide of lower molecular weight was also observed, but, since its distribution in the polyacrylamide gel failed to correlate with enzyme activity, it is probably a protein contaminant. The different distances migrated by vegetatively and developmentally accumulated UDP-glucose pyrophosphorylase in the 4% acrylamide gel before sodium dodecyl sulphate/polyacrylamide-gel electrophoresis are the result of different periods of electrophoresis and do not represent inherent differences in enzyme mobility. Co-electrophoresis of the polypeptides detected in gel fractions containing vegetative and developmentally accumulated UDP-glucose pyrophosphorylase showed that they have identical mobilities (Fig. 4a), and A_{570} scans of the stained polypeptides suggest that in both cases they are present in equal amounts in the active enzyme (Fig. 4b).

Routinely, only about 40% of the UDP-glucose pyrophosphorylase activity originally bound to the immunoadsorbent is recovered whether vegetatively or developmentally accumulated enzyme is chromato-

Fig. 3. Electrophoretic analysis of UDP-glucose pyrophosphorylase

UDP-glucose pyrophosphorylase accumulated by axenically grown amoebae during vegetative growth or at the 18h stage of development was purified by column affinity chromatography and then loaded on to a 4% (w/v) polyacrylamide rod gel in Tris/glycine/pyrophosphate buffer, pH 8.8. After electrophoresis, the gel was sliced and slices eluted. After assay of gel eluates for UDP-glucose pyrophosphorylase activity (a, c) a portion of each was analysed on a 6–18% gradient polyacrylamide slab gel in the sodium dodecyl sulphate buffer system of Laemmli (1970). The photographs (b, d) show the only stained polypeptide bands detectable after electrophoresis; track numbers refer to gel slice numbers from the 4% polyacrylamide gel. The first track contained molecular-weight standards; bovine serum albumin (68000), ovalbumin (45000), lactate dehydrogenase (36000) and chymotrypsinogen (25000); (a), (b) refer to UDP-glucose pyrophosphorylase purified from cells at the 18h stage of development. (c), (d) refer to UDP-glucose pyrophosphorylase purified from vegetative amoebae at a cell density of 1.0×10^7 cells/ml in axenic medium.

graphed. Rather than being the result of residual binding of enzyme to the immunoadsorbent, this appears to be due to enzyme inactivation during elution.

Discussion

Axenically grown *D. discoideum* AX2 amoebae are known to accumulate a number of enzymes, including

N-acetylglucosaminidase and α -mannosidase, which are only present in low amounts when the cells are grown in association with bacteria (Ashworth & Quance, 1972). Unlike the enzymes above, which are non-essential for fruiting-body formation (Dimond *et al.*, 1973; Free & Loomis, 1975), mutants that are defective in UDP-glucose pyrophosphorylase synthesis fail to culminate (Dimond *et al.*, 1976), emphasizing the developmental importance of this

Fig. 4. Comparison of UDP-glucose pyrophosphorylase from vegetative and developing cells

(a) Electrophoretic analysis was on a 6–18% gradient polyacrylamide slab gel in sodium dodecyl sulphate buffer (Laemmli, 1970) of UDP-glucose pyrophosphorylase purified from: (i) vegetative amoebae; (ii) cells at the 18 h stage of development. Also shown (b) is an A_{570} scan of the stained polypeptide bands of UDP-glucose pyrophosphorylase from vegetative amoebae, obtained by using a Unicam SP.1800 spectrophotometer fitted with a gel-scanning attachment. Abbreviations used: L, large subunit, mol. wt. 57500; S, small subunit, mol. wt. 55600; UDP-glucose pyrophosphorylase from 18 h-developmental-stage amoebae gave an identical A_{570} profile.

enzyme. During development of *D. discoideum* NC4 cells grown on the bacteria, the enzyme specific activity increases 10-fold to reach a peak of approx. 400–450 units/mg of protein before culmination. Accumulation only occurs once developmental cell-cell contacts have been established and ceases if these contacts are broken (Newell *et al.*, 1971). Furthermore, repeated disaggregation and reaggregation results in further accumulation of enzyme, despite the fact that normal developmental amounts of UDP-glucose pyrophosphorylase have already been accumulated. Surprisingly, despite the requirement for cell-cell contact for developmental accumulation of UDP-glucose pyrophosphorylase, amoebae grown vegetatively in axenic medium also accumulate this enzyme, although to amounts lower than those observed during development. Ashworth & Watts (1970) originally observed this phenomenon in *D. discoideum* AX2, and Ashworth & Quance (1972) demonstrated that accumulation occurs throughout the exponential phase of growth, reaching a final plateau value when the cell density exceeds $1 \times$

10^6 cells/ml of axenic medium supplemented with 86 mM-glucose. Dimond *et al.* (1976) demonstrated that *D. discoideum* A3 also accumulates UDP-glucose pyrophosphorylase during axenic growth to approx. 100 units/mg of protein assayed at 25°C, which compares well with our value of 146 units/mg of protein for *D. discoideum* AX2 assayed at 37°C. The reason for this accumulation is still unclear, although our data suggest that it is probably not due to the presence of a specific inducer in the axenic medium.

One of the more important questions that this raises is whether the enzyme accumulated during growth represents a premature expression of the gene normally expressed during development or a separate gene product. Gustafson *et al.* (1973) analysed UDP-glucose pyrophosphorylase of *D. discoideum* NC4 grown on bacteria and found that the enzyme accumulated during development comprised two types of subunits with differing mobilities on sodium dodecyl sulphate/polyacrylamide gels, although the resolution obtained was apparently insufficient to allow molecular-weight characterization or quantification. However, UDP-glucose pyrophosphorylase isolated from the same cells in vegetative growth comprised only one subunit, with a higher mobility than either of the subunits detected during development. We have been unable to detect this smaller polypeptide. Rather our data suggest that UDP-glucose pyrophosphorylase present at both stages of the life cycle is composed of two distinct polypeptide subunits with mol. wts. 55600 and 57500 present in equal amounts. Furthermore, by virtue of the immunoadsorption purification step, it is clear that the enzyme accumulated by axenically grown cells of *D. discoideum* AX2 cross-reacts with antibody raised against the developmentally accumulated enzyme. Franke & Sussman (1971) also noted serological identity of UDP-glucose pyrophosphorylase from vegetative and developing amoebae of *D. discoideum* NC4, although they were only able to detect one subunit, of mol. wt. 55000, by using non-gradient sodium dodecyl sulphate/polyacrylamide-gel electrophoresis.

Despite the apparent identity of vegetative and developmental forms of UDP-glucose pyrophosphorylase subunits, there is evidence in the literature for at least two forms of *D. discoideum* UDP-glucose pyrophosphorylase differing in sedimentation coefficient and stability (Newell & Sussman, 1969), but since the major form is reported to account for 90% of the total observed activity yet the two types of subunit that we observe are present in equal amounts, it is unlikely that there are two different multimer forms of UDP-glucose pyrophosphorylase each composed of identical subunits. This is also indicated by mutants defective in UDP-glucose pyrophosphorylase synthesis where all the activity is affected at all stages of the life cycle (Dimond *et al.*, 1976). The UDP-

glucose pyrophosphorylase of *D. discoideum* has been shown to be octomeric (Franke & Sussman, 1971), as has the enzyme from human, calf and rabbit liver. Both the human and calf enzymes exhibit molecular aggregation to produce dimer forms of the native octomeric enzyme, and with the calf liver UDP-glucose pyrophosphorylase trimers and tetramers are also observed (Levine *et al.*, 1969; Turnquist *et al.*, 1974). Thus the observed polymeric forms of *D. discoideum* UDP-glucose pyrophosphorylase are probably different-sized aggregates of the basic octomeric enzyme rather than different structural isoenzymes.

The two polypeptide subunits that we observe may either be distinct structural-gene products or represent translational modification of a single gene product. Although the available genetic evidence has hitherto been used to argue in favour of the latter (Dimond *et al.*, 1976), it does not exclude a situation where both of two distinct gene products are required for native enzyme activity or where only one of the two subunits possesses an active site, the other being involved in regulation of enzyme activity, as with aspartate transcarbamoylase (Gerhart & Schachman, 1965), although there is currently no evidence for allosteric control of UDP-glucose pyrophosphorylase activity (Franke & Sussman, 1971). These possibilities are all readily amenable to experimental investigation.

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