Uridine Diphosphate Glucose Pyrophosphorylase of Acanthamoeba castellanii

PURIFICATION, KINETIC, AND DEVELOPMENTAL STUDIES*

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SUMMARY

When cells of Acanthamoeba castellanii (Neff) are placed on a salts medium the amoebas encyst with the formation of a cellulose-containing cyst wall and an in vitro system synthesizing cellulose from UDP-glucose becomes detectable after about 12 hours of encystment. In the present study cellular levels of UDP-glucose were observed to increase 5-fold within 5 hours of the induction of encystment and to remain at elevated levels for 5 to 10 hours, apparently making UDP-glucose available to the cells at the time of cellulose synthesis. The increased level of UDP-glucose during encystment is not the reflection of an increase in uridine diphosphate glucose pyrophosphorylase activity detectable in vitro. The specific activity and the activity per cell were examined as a function of development in cell extracts. The specific activity was constant for the first 20 hours of encystment while the activity per cell decreased almost from the beginning of encystment.

The uridine diphosphate glucose pyrophosphorylase (EC 2.7.7.9) from trophozoites was purified to homogeneity as determined by polyacrylamide gel electrophoresis. The purified enzyme (final specific activity 15,833 units per mg) was highly specific for both UTP and UDP-glucose. The divalent cation requirement was most readily satisfied by magnesium and the pH optimum was 8 to 9 in the direction of UDP-glucose synthesis and 7.5 to 7.0 in the direction of pyrophosphorylase.

The enzyme was characterized kinetically as having an ordered mechanism of catalysis. Pyrophosphorylase was subject to product inhibition by UTP and pyrophosphate. The following $K_m$ values were obtained: UTP, $5.8 \times 10^{-2}$ M; glucose 1-phosphate, $1.4 \times 10^{-4}$ M; UDP-glucose, $5.0 \times 10^{-4}$ M; pyrophosphate, $2.4 \times 10^{-1}$ M.

The protozoan Acanthamoeba castellanii may be induced to encyst by transferring trophozoites from growth medium to minimal salts medium (1). Several events occur as a result of starvation. During the first 4 hours of encystment, cellular glycogen levels decrease to between one-half and one-third of that found in amoebas growing in enriched medium (2). The drop in the glycogen level is subsequently followed by the appearance of cellulose (3), which is undetectable in trophozoites. This polysaccharide is the major carbohydrate component of a complex cyst wall that is synthesized during encystment, accounting for one-third of the dry weight of the wall (4).

With a particulate enzyme system prepared from encysting A. castellanii it has been shown that $^{14}$C-labeled glucose from UDP-$^{14}$C-glucose is incorporated into cell wall β-(1→4)-glucans (3). Neither ADP-glucose, TDP-glucose, CDP-glucose, nor GDP-glucose were able to replace UDP-glucose as the glucosyl donor. These data suggest that the availability of UDP-glucose during encystment might play a key role in cell wall biosynthesis. For this reason a study of uridine diphosphate glucose pyrophosphorylase (UTP:α-glucose 1-phosphate uridyltransferase, EC 2.7.7.9) which catalyzes the synthesis of UDP-glucose from UTP and glucose 1-phosphate was undertaken.

The present communication describes the purification of the pyrophosphorylase to apparent homogeneity and some of its catalytic and kinetic properties. Both the level of UDP-glucose and pyrophosphorylase activity were examined as a function of encystment.

EXPERIMENTAL PROCEDURE

Materials

Phosphoglucomutase and glucose 6-phosphate dehydrogenase were obtained from Boehringer-Mannheim; NADP+, NAD+, UDP-glucose, crystalline bovine serum albumin, UTP, UDP-glucose dehydrogenase (type III), and Tricine buffer (N-tris(hydroxymethyl)methylglycine) from Sigma Chemical Co.; glucose 1-phosphate from Calbiochem; acrylamide gel reagents, N,N'-methylene bisacrylamide and N,N,N',N'-tetramethylethylenediamine from Eastman Organics; hydroxylapatite and Agarose 5m from Bio-Rad; DEAE-Sephadex A-50 from Pharmacia Fine Chemicals; and UDP-$^{14}$C-glucose from New England Nuclear (specific activity 227 mCi per mmol). All other chemicals were reagent grade.

Organism and Culture Conditions

Cultures of Acanthamoeba castellanii (Neff's Acanthamoeba sp.) were grown and induced to encyst as previously described (1, 3).
For enzyme purification, trophozoites were harvested at late log growth by centrifugation at 600 X g for 3 to 5 min, washed twice with deionized water, frozen in Dry Ice-acetone, and lyophilized. The lyophilized cells were then stored at -15°C. Fresh extracts were prepared when UDP-glucose pyrophosphorylase activity was examined as a function of encystment. For these determinations, encysting cells were harvested at 4°C, washed once with cold 0.1 m Tricine-NaOH, pH 7.8, and were resuspended in the same buffer. Crude extracts were prepared by passing the cells through the French Press at 10,000 p.s.i. until approximately 99% breakage was achieved. The extracts were assayed immediately for pyrophosphorylase activity as described.

**Assay Procedures**

**Assay A**—During purification, activity was routinely assayed in the direction of glucose 1-phosphate formation using a method analogous to that described by Munch-Petersen (5). Except where indicated, reactions contained, in a total of 1 ml, 1 μmol of UDP-glucose, 2 μmol of sodium pyrophosphate, 4 μmol of MgCl₂, 8 μg (0.05 unit) of phosphoglucose mutase, 1 μg (0.14 unit) of glucose 6-phosphate dehydrogenase, 1.0 μg of NAD⁺, 20 to 30 μg of the purified enzyme, and 100 μmol of Tricine-NaOH buffer, pH 7.6. Reactions were initiated by the addition of UDP-glucose pyrophosphorylase and the change in absorbance was monitored continuously at 340 nm with a Gilford recording spectrophotometer model 2400. The temperature of the assay mixtures was maintained at 37°C by circulating water through the jacket of the cuvette holder. The over-all reaction is:

\[
\text{UDP-glucose} + \text{PPi} + \text{NADP}^+ + \text{UTP} \rightarrow \text{UDP-glucose} + \text{Pi} + \text{NAD}^+ + \text{CO}_2
\]

One unit of activity is defined as the amount of enzyme necessary for the reduction of 1 μmol of NADP⁺ per min. The molar extinction coefficients of NADP⁺ and NADPH at 340 nm were taken to be 6.2 x 10⁴ M⁻¹ cm⁻¹.

The activity of the enzyme was stable under the conditions of the assay for at least 30 min at 37°C. NADPH added to crude cell extract appeared to be completely stable for at least 15 min at 37°C, indicating the absence of any significant amount of NADPH oxidase activity.

**Assay B**—The reaction was also measured, where indicated, in the direction of UDP-glucose synthesis. The reaction mixture contained, in a total volume of 1.0 ml, 2 μmol of glucose 1-phosphate, 1 μmol of UTP, 4 μmol of MgCl₂, 16 μmol of NAD⁺, 1 mg (0.05 units) of UDP-glucose dehydrogenase, 20 to 30 μg of the purified enzyme, and 100 μmol of Tricine-NaOH buffer, pH 8.0. Incubation temperature was 37°C. The over-all reaction is:

\[
\text{glucose}1\text{-phosphate} + \text{UTP} + 2 \text{NAD}^+ \rightarrow \text{UDP-glucuronate} + \text{Pi} + 2 \text{NAD}^+
\]

One unit of activity is defined as the amount of enzyme necessary for the reduction of 2.0 μmol of NAD⁺ per min.

The observed activities in assays lacking single components of either assay system are illustrated in Table I. All components were essential for the maximum rate of reaction with both the assays used.

**Electrophoresis**

Polyacrylamide disc gels were prepared with 5% separating gels and 3.5% spacer gels. Electrophoresis was performed at 5 mA per gel for 1 to 1.5 hours using Tris-glycine (30 g of Tris, 14.4 g of glycine per liter, pH 8.3) as reservoir buffer. Following electrophoresis, gels were stained with 0.2% Coomassie brilliant blue for 2 hours and then destained overnight or electrophoretically in methanol-acetic acid-H₂O (8:1:5).

**Protein Assays**

Protein was determined in the presence of interfering substances by Bennett's modification of the Lowry method (9). Bovine serum albumin was used as standard.

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1 The abbreviation used is: Tricine, N-tris(hydroxymethyl)methylglycine.
phosphate buffer, pH 7.6, containing 28 mM 2-mercaptoethanol. Most of the cells were broken during lyophilization and the rest were broken by passing the extract through the French Press one time at 10,000 p.s.i. or by homogenizing with 10 strokes of a "tight-fitting" Dounce homogenizer. The extract was then diluted with the same phosphate buffer and centrifuged at 49,000 × g for 30 min. The pellet was discarded and additional 2-mercaptoethanol was added to the supernatant (1 ml per liter of supernatant).

Streptomycin Sulfate Precipitation—A 15% streptomycin solution (0.1 ml per liter of supernatant) was added slowly with gentle stirring. The pH was kept between 7.5 and 8.0. A small precipitate was removed by centrifugation at 20,000 × g for 15 min. The supernatant was saved.

Ammonium Sulfate Precipitation—Ammonium sulfate was added slowly to the supernatant with gentle stirring to a final concentration of 40%. The precipitate was removed by centrifugation and the supernatant was brought to 60% saturation with additional ammonium sulfate. The pellet obtained after centrifugation was resuspended in 0.05 M potassium phosphate buffer, pH 7.2, containing 14 mM 2-mercaptoethanol and dialyzed against two changes of the same buffer over 4 hours. The dialysate was centrifuged at 49,500 × g for 15 min and any pellet that appeared was discarded.

Agarose Chromatography—The enzyme solution was further fractionated by exclusion chromatography on a column of Agarose 5m (2.5 × 160 cm) equilibrated with 0.05 M potassium phosphate buffer, pH 7.2, and containing 14 mM 2-mercaptoethanol. The flow rate was maintained at 1.8 ml per min. The results of the fractionation are shown in Fig. 1. The peak active fractions, which eluted after the void volume (120 ml), were pooled and the enzyme was further purified on hydroxyapatite.

Hydroxyapatite Chromatography—The pooled fractions from the Agarose column were applied directly to a hydroxyapatite column (2.5 × 10 cm) equilibrated with 0.05 M phosphate buffer, pH 7.2, containing 14 mM 2-mercaptoethanol. The column was washed with 0.1 M phosphate buffer, pH 7.6, containing 14 mM 2-mercaptoethanol (Fig. 2). After applying the sample, the column was washed with 0.1 M phosphate buffer, pH 7.6, containing 14 mM 2-mercaptoethanol before starting a linear phosphate gradient (0.1 to 0.5 M phosphate buffer, pH 7.6, containing 14 mM 2-mercaptoethanol; total 160 ml). The peak enzyme activity fractions which eluted with 0.22 to 0.25 M phosphate were pooled and concentrated under N₂ in a Diaflow ultrafiltration cell with a PM-30 filter at a pressure of 50 p.s.i. UTP was added at a final concentration of 1 mM to protect the enzyme. The concentrate was dialyzed for 4 hours against two changes of 0.1 M Tris-HCl, pH 7.6, containing 14 mM 2-mercaptoethanol and 0.1 M NaCl.

DEAE-Sephadex A-50 Chromatography—The dialyzed enzyme was washed with 0.1 M phosphate buffer, pH 7.6, containing 14 mM 2-mercaptoethanol, before a linear 0.1 to 0.5 M phosphate buffer, pH 7.6, gradient was started; 3-ml fractions were collected. The bracket indicates the combined fractions. Assay A was used.
FIG. 3. DEAE-Sephadex A-50 chromatography of UDP-glucose pyrophosphorylase. The concentrated, dialyzed preparation from the hydroxylapatite column (see the text) was applied to a DEAE-Sephadex A-50 column (1.5 × 15 cm) equilibrated with 0.1 M Tris-HCl, pH 7.6, containing 14 mM 2-mercaptoethanol and 0.1 M NaCl. After washing, the column was developed with a linear gradient of NaCl (0.1 to 0.5 M). Two-milliliter fractions were collected. The bracket indicates the combined fractions. Assay A was used.

TABLE II
Purification of UDP-glucose pyrophosphorylase

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Total activity</th>
<th>Protein</th>
<th>Specific activity</th>
<th>Purification</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Crude extract</td>
<td>244</td>
<td>616</td>
<td>396</td>
<td>1.0</td>
<td>100</td>
</tr>
<tr>
<td>2. 49,500 × g supernatant</td>
<td>219</td>
<td>290</td>
<td>732</td>
<td>1.8</td>
<td>90</td>
</tr>
<tr>
<td>3. Streptomycin supernatant</td>
<td>222</td>
<td>280</td>
<td>793</td>
<td>2.0</td>
<td>91</td>
</tr>
<tr>
<td>4. 40-60% (NH₄)₂SO₄ Pellet</td>
<td>223</td>
<td>113</td>
<td>1,973</td>
<td>5.0</td>
<td>91</td>
</tr>
<tr>
<td>5. Active fraction eluted from Agarose 5m</td>
<td>138</td>
<td>56</td>
<td>2,404</td>
<td>6.2</td>
<td>57</td>
</tr>
<tr>
<td>6. Active fraction eluted from hydroxylapatite</td>
<td>146</td>
<td>27</td>
<td>5,407</td>
<td>13.7</td>
<td>60</td>
</tr>
<tr>
<td>7. Active fraction eluted from DEAE-Sephadex A-50</td>
<td>57</td>
<td>3.6</td>
<td>15,833</td>
<td>40.0</td>
<td>23</td>
</tr>
</tbody>
</table>

from hydroxylapatite was applied to a column (1.5 × 15 cm) of DEAE-Sephadex A 50 which had been equilibrated with 0.1 M Tris-HCl, pH 7.6, containing 14 mM 2-mercaptoethanol and 0.1 M NaCl. After washing, the column was developed with a linear NaCl gradient (0.1 to 0.5 M). Two peaks of 280 nm absorbing material were eluted from the column (Fig. 3). Pyrophosphorylase activity was found to be associated with the first peak of protein. The peak fractions were pooled, concentrated, made 1 mM with respect to UTP, and dialyzed as described for hydroxylapatite chromatography. The purified enzyme was stored at 4°, and used in the experiments reported below. At this stage, UDP-glucose pyrophosphorylase was purified about 40-fold with a recovery of about 23% (Table II). The protein obtained after DEAE-Sephadex chromatography yielded a single band when stained with Coomassie blue after electrophoresis (Fig. 4). In addition, the active fractions from the DEAE-Sephadex column were combined, concentrated, and dialyzed against 0.1 M phosphate buffer, pH 7.6, containing 14 mM 2-mercaptoethanol. The dialyzed enzyme was rechromatographed on a column of Sepharose 4B and eluted with the phosphate-mercaptoethanol buffer. This resulted in a single symmetrical peak of enzyme activity which coincided exactly with the single protein peak observed.

General Catalytic Properties

Effect of Time and Protein Concentration—UDP-glucose pyrophosphorylase activity was linear with time over a 16- to 18-min incubation period when assayed either in the direction of glucose 1-phosphate formation (Fig. 5) or UDP-glucose synthesis (Fig. 6), except for a lag phase of about 2 min. This is attributed to
Fig. 5. Effect of time and protein concentration on pyrophosphorolysis of UDP-glucose. □, 4.1 μg of protein; ●, 12.3 μg of protein; △, 16.4 μg of protein; ○, 32.8 μg of protein. Assay A was used.

Fig. 6. Effect of time and protein concentration on synthesis of UDP-glucose. □, 12.1 μg of protein; △, 24.2 μg of protein; ○, 48.5 μg of protein. Assay B was used.

the coupling system, since a similar lag occurred when glucose 1-phosphate and UDP-glucose were added to the respective coupling systems. The linear portion of the curves were used to measure reaction rates.

In the direction of glucose 1-phosphate formation the assay was linear over an approximate 8-fold range of protein concentrations (4.1 to 32.8 μg), while in the other direction, the assay was linear over a 3-fold range (12.1 to 48.4 μg). The rate in the direction of glucose 1-phosphate formation is 2 to 3 times that in the direction of UDP-glucose formation.

pH Optima—Fig. 7 shows that, in the direction of UDP-glucose synthesis, activity is optimal between 8 and 9. In the direction of glucose 1-phosphate formation, a relatively sharp peak is seen at 7.5 to 7.6. The coupling enzymes present in the assay mixtures were shown to be in excess at all pH values tested.

Specificity—Table III shows that none of the other common nucleoside triphosphates could replace UTP, and none of the corresponding nucleotide sugars could replace UDP-glucose.

Cation Requirement—The purified UDP-glucose pyrophosphorylase showed an absolute requirement for a divalent cation for catalysis of both synthesis and pyrophosphorolysis of UDP-glucose (Table I). Fig. 8 illustrates that magnesium ion was more effective than either cobalt or calcium ions in supporting the catalysis of the reversible reaction. Manganese precipitated out of the assay mixture so that its effect on the reaction rates could not be tested. In the direction of pyrophosphorolysis maximal activity was obtained when magnesium was present at a concentration of at least 2 mM i.e. at a concentration equal to that of PPi. In the direction of UDP-glucose synthesis a magnesium concentration of 1 mM elicited maximum reaction rates, a concentration equal to that of UTP.

Gustafson and Gander (8) have demonstrated for UDP-glucose pyrophosphorylase from Sorghum vulgare a direct relationship between the requirement for magnesium ion and the concentration of PPi in the direction of pyrophosphorolysis, and that the major function of magnesium ion is in forming MgPPi."
DIVALENT CATION (mM)

**Fig. 8.** Effects of divalent cations on initial rates of pyrophosphorolysis and UDP-glucose synthesis. Assay in the direction of glucose 1-phosphate formation (-----); assay in the direction of UDP-glucose formation (- - -). ○ or □, Mg^{2+}; ▲ or △, Co^{2+}; ■ or ▼, Ca^{2+}. Velocity units (V) are micromoles of product per min per ml.

**Fig. 9.** Initial velocity of UDP-glucose synthesis as a function of the concentrations of glucose 1-phosphate (GTP) and UTP. Assay B was used. In the primary plot the reciprocal of the initial velocities of UDP-glucose synthesis in micromoles per min per ml was plotted as a function of the reciprocal of UTP concentration at each concentration of glucose 1-phosphate as indicated. Inset, the slopes and intercepts of the primary plots are replotted as functions of the reciprocal of glucose 1-phosphate concentration.

Since the same direct relationship exists between cation concentration and concentration of either PP_{i} or UTP in this study, it suggests that here, as well, the major function of magnesium is in forming either a MgPP_{i}^{-2} or MgUTP^{-2} complex. It was assumed that all of the PP_{i} formed a complex with magnesium ions.

**Initial Velocity and Product Inhibition Studies**—Initial velocities were measured as a function of substrate concentrations in the absence of added products. Fig. 9 illustrates a reciprocal plot of initial velocities of UDP-glucose synthesis as a function of the concentrations of glucose 1-phosphate and UTP when magnesium was in excess of UTP. A reciprocal plot of the initial velocities of pyrophosphorolysis as a function of UDP-glucose and PP_{i} is shown in Fig. 10. As before, magnesium was added to all reaction mixes at a concentration in excess of PP_{i}. Replots of the slopes and intercepts against the reciprocal of the glucose 1-phosphate and PP_{i} concentrations (Figs. 9 and 10) were linear. The Michaelis constant (Table IV) for each substrate was calculated from Lineweaver-Burk plots for each substrate in the presence of an excess of the second substrate.

**TABLE IV**

<table>
<thead>
<tr>
<th>Michaelis constants</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>K_{UTP}</td>
<td>5.8 × 10^{-4}</td>
</tr>
<tr>
<td>K_{glucose 1-phosphate}</td>
<td>1.4 × 10^{-4}</td>
</tr>
<tr>
<td>K_{UDP-glucose}</td>
<td>5.0 × 10^{-2}</td>
</tr>
<tr>
<td>K_{PP_{i}}</td>
<td>2.4 × 10^{-2}</td>
</tr>
</tbody>
</table>

Initial velocity experiments were performed with MgCl_{2} added at a concentration of 1.0 mM in excess of the UTP or the PP_{i} concentration tested. Fits of 1/v versus 1/s made in the presence of an excess of the second substrate.
FIG. 11. Product inhibition of pyrophosphorolysis by UTP with UDP-glucose (UDPG) as the variable substrate. Mg²⁺ was added at a concentration 1.0 mM in excess of the concentration of either UTP (as indicated) or PPᵢ (2 mM), whichever was greater. In the primary plot the reciprocal of initial velocity of glucose 1-phosphate formation (micromoles per min per ml) is plotted as a function of the reciprocal of UDP-glucose concentration. The slopes of the primary plot are replotted in inset as a function of UTP concentration. Assay A was used.

FIG. 12. Product inhibition of UDP-glucose synthesis by PPᵢ with UTP as the variable substrate and glucose 1-phosphate added at a fixed, nonsaturating concentration (1.0 mM). Mg²⁺ was added 1.0 mM in excess of the concentration of either UTP or PPᵢ (as indicated), whichever was greater. In the primary plot the reciprocal of initial velocity of UDP-glucose synthesis is plotted as a function of the reciprocal of concentration of UDP-glucose. Inset, the slopes and intercepts are replotted as a function of PPᵢ concentration. Assay B was used.

FIG. 13. Product inhibition of UDP-glucose synthesis by PPᵢ with glucose 1-phosphate (G1P) as variable substrate. Mg²⁺ was added in 1.0 mM excess of the concentration of either UTP (1 mM) or PPᵢ (as indicated), whichever was greater. In the primary plot the reciprocal of the initial velocity of UDP-glucose synthesis is plotted as a function of the reciprocal of concentration of glucose 1-phosphate. Inset, the slopes and intercepts are replotted as a function of PPᵢ concentration. Assay B was used.

FIG. 14. Product inhibition of UDP-glucose synthesis by PPᵢ with UTP as variable substrate, and glucose 1-phosphate added at a fixed, saturating concentration (10 mM). Mg²⁺ was added 1.0 mM in excess of the concentration of either UTP or PPᵢ (as indicated), whichever was greater. In the primary plot the reciprocal of initial velocity of UDP-glucose synthesis in micromoles per min per ml is plotted as a function of the reciprocal of concentration of UTP. Inset, the slopes of the primary plot are replotted as a function of the concentration of PPᵢ. Assay B was used.
as a function of UDP-glucose concentration (Fig. 11), inhibition was found to be competitive. A replot of slopes as a function of UTP concentration was linear, suggesting that UTP combines with one form of pyrophosphorylase. In contrast, PPi, acted as a noncompetitive inhibitor of UDP-glucose when either UTP (Fig. 12) or glucose 1-phosphate (Fig. 13) was the variable substrate. Under either condition the replots of the slopes and intercepts as a function of PPi concentration were linear indicating that PPi, too, combines with one form of the enzyme. The data in Fig. 12 were collected adding glucose 1-phosphate at a fixed, nonsaturating concentration (0.001 M). However, if glucose 1-phosphate is present at saturation levels (0.01 M) and UTP is the variable substrate, the data obtained suggest uncompetitive inhibition between PPi and UTP (Fig. 14).

**UDP-Glucose Pyrophosphorylase Activity As Function of Encystment**—The specific activity and activity per cell of the enzyme during development were obtained from assays of crude extracts. Fig. 15 illustrates that the relative enzyme specific activity is constant for the first 20 hours after induction of encystment. Thereafter, the specific activity decreases, until it has dropped 4-fold by 48 hours. The enzyme activity per cell, however, declines continually during the initial 20 hours of encystment to stabilize at a level 4-fold lower than the zero time value. After 20 hours, enzyme activity per cell remains constant.

**Levels of UDP-Glucose during Encystment**—Table V shows the levels of UDP-glucose found in cells as they were induced to encyst. After 5 hours in encystment medium, the amount of cellular UDP-glucose increases approximately 5-fold. The level remains higher than the zero time value, but begins to decline slowly for the next 5 hours. By 23 hours cellular UDP-glucose approaches the initial level and by 48 hours has dropped below the initial level.

**DISCUSSION**

A number of UDP-glucose pyrophosphorylases have been prepared from a variety of sources, including plants (8, 12, 13), mammals (14–17), Escherichia coli (18, 19), and Dictyostelium discoideum (20). In comparing the protozoan enzyme with these, A. castellanii UDP-glucose pyrophosphorylase appears to be most similar to the slime mold enzyme with respect to purification properties, specificity toward substrates and activating metal ions, and pH activity optima.

The data illustrated in Figs. 11 to 14 demonstrate that A. castellanii pyrophosphorylase is subject to product inhibition by UTP and PPi. The product inhibition of UTP is competitive with UDP-glucose, but PPi acts as a noncompetitive inhibitor of UDP-glucose with either UTP or glucose 1-phosphate as variable substrate. Similar mutually competitive interactions between UTP- and UDP-glucose for the pyrophosphorylase have been reported by Teuboi et al. (17) for erythrocyte, dog cardiac muscle, and mung bean, and by Gustafson and Gander (8) for Sorghum vulgare. The noncompetitive inhibition between PPi and glucose 1-phosphate, and the shift from noncompetitive to uncompetitive inhibition between PPi and UTP when glucose 1-phosphate is increased from a nonsaturating to a saturating concentration (Figs. 12 and 14) has also been shown to occur in Sorghum (8). The velocity and product inhibition data indicate an ordered binding of substrates and release of products, as described by Cleland (9, 10), in which the nucleotide is the first substrate to bind and the last product to leave.

![Fig. 15. Activity of UDP-glucose pyrophosphorylase as a function of encystment. Enzyme activity was measured using crude extracts (as the source of the enzyme) prepared from cells after incubation in encystment medium for the time indicated as described under "Experimental Procedures." Upper panel, relative specific activity; lower panel, relative activity per cell.](http://www.jbc.org/)

**Table V**

<table>
<thead>
<tr>
<th>Time of encystment</th>
<th>Cysts</th>
<th>UDP-glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment 1</td>
<td>Experiment 2</td>
<td>Experiment 1</td>
</tr>
<tr>
<td>0 hr</td>
<td>% cell population</td>
<td>nmol/10⁶ cells</td>
</tr>
<tr>
<td>0</td>
<td>15</td>
<td>4.4</td>
</tr>
<tr>
<td>5</td>
<td>20</td>
<td>30</td>
</tr>
<tr>
<td>10</td>
<td>60</td>
<td>53</td>
</tr>
<tr>
<td>23</td>
<td>85</td>
<td>87</td>
</tr>
<tr>
<td>48</td>
<td>91</td>
<td>91</td>
</tr>
</tbody>
</table>

Levels of UDP-glucose during encystment were obtained from assays of crude extracts (as the source of the enzyme) prepared from cells after incubation in encystment medium for the time indicated as described under "Experimental Procedures." The percentage of cells in the population was determined by light microscopy at the time that cell samples were taken. The figures reported are the sum of immature and mature cyst forms (11).
from Fig. 15 indicate that activity of the pyrophosphorylase per cell decreases during the first 20 hours after the induction of encystment. Cellular protein has, as well, been shown to decline during this same time span (11). Griffiths and Hughes (21) have demonstrated that during the first 24 hours of encystment of this amoeba large quantities of material, including proteins, are ejected from the cytoplasm so that the mass of the cyst is greatly reduced when compared to that of the trophozoite. Some of the pyrophosphorylase may be part of this elimination, or the enzyme may simply be inactivated or destroyed as a result of starvation. Data from Fig. 15 also indicate that the specific activity of pyrophosphorylase remains constant during the same time that activity per cell is dropping.

Whatever the explanation, these results suggest that the increased levels of UDP-glucose observed in the cells after 5 hours of encystment is not a reflection of an increase in UDP-glucose pyrophosphorylase specific activity measured in vitro. These studies do not provide data about possible localized concentration increases within the cell, or in vivo regulation by metabolites. During the first 5 hours of encystment there is a considerable decrease in cellular glycogen levels and it is also possible that the increased concentration of UDP-glucose reflects an increase in the availability of glucose 1-phosphate (and/or UTP) for pyrophosphorylase activity (see Reference 22).

In contrast to the results obtained with A. castellanii are those reported for the cellular slime mold (20, 22, 23). During the developmental program in Dictyostelium discoideum several polysaccharides synthesized from UDP-glucose accumulate. In these cells about a 10-fold increase in UDP-glucose pyrophosphorylase specific activity occurs during particular stages of fruiting body construction. Raizada and Krishna-Murti (24) have reported that the specific activity of UDP-glucose increases during the encystment of Hartmannella culbertsoni (a related soil amoeba) on agar plates. However, this result is difficult to interpret because the extracts used were prepared from encysting populations from which mature cysts were excluded prior to homogenization. It is possible that the results might relate more to amoeboid forms which are incapable of encystment than to the usual encystment sequence.

REFERENCES
1. NEFF, R. J., HAY, S., BENTON, W., AND WILBORN, M. (1964) *Methods Cell Physiol.* 1, 55
Uridine Diphosphate Glucose Pyrophosphorylase of *Acanthamoeba castellanii* : PURIFICATION, KINETIC, AND DEVELOPMENTAL STUDIES
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