Biosynthesis of Bacterial Glycogen: Purification and Properties of Salmonella typhimurium LT-2 Adenosine Diphosphate Glucose Pyrophosphorylase

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The adenosine diphosphate glucose pyrophosphorylase from a Salmonella typhimurium LT-2 mutant, JP102, derepressed in the glycogen biosynthetic enzymes was purified to homogeneity. The enzyme was found to be identical with the parent wild-type enzyme with respect to regulatory properties, immunological reactivity, and kinetic constants for the allosteric effectors and for the substrate, adenosine triphosphate. The JP102 enzyme was composed of four identical subunits, each with a molecular weight of about 48,000. This was supported by the findings that (i) gel electrophoresis under denaturing conditions showed only one component; (ii) digestion with carboxypeptidase B released stoichiometric amounts of arginine; and (iii) amino-terminal sequencing showed a single sequence for the first 27 residues. The properties of the purified S. typhimurium enzyme were compared with the properties of the previously purified Escherichia coli B enzyme.

The biosynthesis of the α-1,4-glucosidic linkages of bacterial glycogen is believed to occur via the following reactions (for reviews on the subject, see references 21 and 31):

ATP + α-glucose 1-phosphate \[\rightarrow\]
ADP-glucose + PP,

(1)

ADP-glucose + α-glucan \[\rightarrow\]
α,1,4-glucosyl-glucan + ADP

(2)

ADP-glucose pyrophosphorylase (EC 2.7.7.27; glucose 1-phosphate adenylyltransferase) catalyzes reaction 1, and in most cases has been shown to be activated by glycolytic intermediates and inhibited by either AMP, Pₐ, or ADP (21, 31). The particular glycolytic intermediate that may activate is dependent on the source of the ADPglucose pyrophosphorylase. For example, many enteric bacteria contain an ADP-glucose pyrophosphorylase that is activated by fructose 1,6-diphosphate, NADPH, and pyridoxal phosphate (31, 34, 35). Evidence obtained in the study of mutants (5, 13, 32, 33, 35) and in correlating in vivo concentrations of fructose 1,6-diphosphate with rates of glycogen accumulation (7, 8) strongly suggest that fructose 1,6-diphosphate is a physiologically important activator of ADPglucose pyrophosphorylase and therefore of glycogen synthesis in Escherichia coli and Salmonella typhimurium.

Recent studies have identified the activator binding site of the E. coli ADPglucose pyrophosphorylase (28, 29). It was therefore of interest to compare the structural and chemical properties of the E. coli B enzyme with other enteric ADPglucose pyrophosphorylases. This report is concerned with the purification and characterization of the S. typhimurium LT-2 ADPglucose pyrophosphorylase and comparison of its properties with the ADPglucose pyrophosphorylase of E. coli.

MATERIALS AND METHODS

Reagents. Carboxypeptidase A (DFP treated; 21 mg/ml, 55 U/mg), yeast inorganic pyrophosphatase (600 U/mg), and carboxypeptidase B (DFP treated; 5 mg/ml, 90 U/mg) were obtained from Sigma Chemical Co. Iodoacetic acid was purchased from Sigma and recrystallized from CCl₄. Iodo-[^14]C)acetic acid (12.6 mCi/mmol) and α-d-[U-^3]C]glucose 1-phosphate (150 mCi/mmol) were obtained from Amersham Corp., and 32P, was from New England Nuclear Corp. All other reagents were obtained from commercial sources at the highest possible purity.

Bacteria. The following microorganisms were used: S. typhimurium LT-2 (obtained from S. Kustu, University of California, Davis) and S. typhimurium JP102, a glycogen-excess mutant containing three- to fourfold higher levels of ADPglucose pyrophosphorylase activity than the parent wild type, S. typhimurium LT-2. The isolation and characterization of this derepressed mutant have been described (35).

Growth conditions. S. typhimurium JP102 was grown aerobically in 100-liter batches in a New Brunswick Fermentcell in minimal medium containing 0.6% glucose, 0.68% KH₂PO₄, 1.42% Na₂HPO₄, 0.12% (NH₄)₂SO₄, 0.0246% MgSO₄·7H₂O, 0.0011% CaCl₂, and 1 ml of a trace element solution per liter (33). Temperature was maintained at 37°C; after 16 to 18 h of
growth, cells were harvested with a Sharples continu-
ous ultracentrifuge and stored as a cell paste at −20°C.
About 700 g (wet weight) of cells was obtained per
100-liter culture. *S. typhimurium* LT-2 was also grown
aerobically on the same minimal medium, containing
0.6% glucose in 1 liter of culture.

**Enzyme assays.** ADP-glucose pyrophosphorylase
was assayed in pyrophosphorylase and synthesis di-
rections as described previously (35). The reaction
mixtures contained: for assay A (activated pyrophos-
phorylation), 20 μmol of Tris-chloride buffer (pH 8.0),
2 μmol of MgCl₂, 100 μg of bovine plasma albumin, 0.2
μmol of ADP-glucose, 0.5 μmol of ³²P, (1 × 10⁶ to 3 × 10⁶
cpm/μmol), activator in the indicated concentra-
tions, and enzyme in a final volume of 0.25 ml; for
assay B (activated synthesis), 20 μmol of Tris-chloride
buffer (pH 8.0), 0.3 μmol of ATP, 0.1 μmol of [U⁻¹⁴C]-
glucose 1-phosphate (1.05 × 10⁶ cpm/μmol), 1 μmol of
MgCl₂, 100 μg of bovine plasma albumin, 0.24 μg of
yeast inorganic pyrophosphatase, activator, and en-
yzyme in a final volume of 0.2 ml. For assay C (unacti-
vated synthesis), in the absence of activator, the
amounts of ATP, glucose 1-phosphate, and MgCl₂
were increased to 1.5, 0.2, and 5.0 μmol, respectively.
One unit of enzyme activity equals 1 μmol of ATP
formed per min under the conditions of assay A in
the presence of 1.5 mM fructose buffer at 37°C.

**Determination of kinetic constants.** Kinetic
data were plotted as velocity versus substrate or effec-
tor concentration and were replotted as Hill plots (3,
17). Vₘₐₓ is determined from double-reciprocal plots.
Sₜₜ, Kₘ, Iₜₜ (corresponding to the concentration of
substrate, activator, and inhibitor, respectively, re-
quired for half-maximal velocity, activation, and inhi-
bition) and α (interaction coefficient) are determined
from the Hill plots (3, 17).

**Preparation of enzyme.** ADP-glucose pyrophos-
phorylase was purified from *S. typhimurium* JP102 by
the following procedure. (i) Cells (700 g) were sus-
pended in 3 volumes of 50 mM ice-cold glycylglycine
buffer (pH 7.2) containing 5 mM dithioerythritol, fil-
tered through two layers of cheesecloth, and passed
three times through a Manton-Gaulin homogenizer
at 7,000 lb/in². The temperature was kept below
15°C. The homogenate was disrupted ultrasonically in 500-
ml batches for 2 min with a Bronwill Biosonic III
probe to reduce the viscosity.

(ii) In the presence of 30 mM potassium phosphate
buffer (pH 7.0), 800-ml batches of the crude extract
were heated to 55°C for 5 min, cooled to 5°C, and
centrifuged (45 min, 16,500 × g). The pellet was ex-
tracted with 250 ml of the above glycylglycine buffer,
and to the combined washes and 16,500 × g superna-
tant, solid ammonium sulfate was added to 55% satu-
ration at 4°C. The precipitate obtained after centri-
figation of the resultant suspension (20 min, 16,500 ×
g) was suspended in sufficient 50 mM Tris-chloride
buffer (pH 7.2) containing 1 mM dithioerythritol and
15 mM potassium phosphate to give a workable slurry,
and was dialyzed for 12 h against 10 liters of the same
buffer containing 5% glycerol. The slurry was then
centrifuged (1 h, 100,000 × g), and the pellets were
extracted with 400 ml of the dialysis buffer and recen-
trifuged. The combined supernatants were dialyzed
again for 20 h against three changes of 10 liters of dia-
lysis buffer.

(iii) The dialyzed ammonium sulfate fraction was
adsorbed on a DEAE-cellulose column (36 by 5 cm;
706-ml resin bed volume) equilibrated with 15 mM
potassium phosphate buffer (pH 7.5) containing 1 mM
dithioerythritol. The column was washed with 800 ml
of equilibration buffer, and the protein was eluted (250
ml/h, fractions) with a linear gradient of 7 liters con-
taining the equilibration buffer in the mixing chamber
and 0.1 M potassium phosphate (pH 7.0), 0.3 M KCl,
and 1 mM dithioerythritol in the reservoir. Fractions
containing the enzyme were pooled and concentrated
by precipitation with 60% ammonium sulfate. The
resultant precipitate was dissolved and dialyzed for 12
h against two changes of 5 liters of the same buffer as
in step (ii).

(iv) The dialyzed DEAE-cellulose fraction was di-
luted to about 3 mg of protein per ml with 20 mM
Tris-chloride buffer (pH 7.2) containing 0.2 M KCl, 1
mM dithioerythritol, 1 mM EDTA, and 10% glycerol
and absorbed on a P₃(6-amino,1-hexyl)-P₄(6-phos-
pho,1-hexyl)pyrophosphate Sepharose column (14
(30 ml) equilibrated with the same buffer that was used
in the mixing chamber and equilibration buffer plus
0.1 M potassium phosphate (pH 7.0) in the reservoir.
Active fractions were pooled, made 20% in glycerol,
and concentrated in an Amicon cell, using a PM30
membrane and pressure below 20 lb/in² to about 5 ml.
The protein solution was then dialyzed against 50 mM
Tris-chloride buffer (pH 7.2) containing 10 mM potas-
sium phosphate, 0.5 mM dithioerythritol, and 20%
glycerol and stored at −70°C.

Enzyme prepared in this way had a specific activity
of about 100 U/mg when measured in pyrophospho-
rylase direction in the presence of 1.5 mM fructose
diphosphate.

**Partial purification of S. typhimurium LT-2**
ADP-glucose pyrophosphorylase for immunolog-
ical studies. Bacterial cells (1 to 5 g) were sus-
pended in 10 ml of 50 mM glycylglycine buffer (pH 7.2)
containing 5 mM dithioerythritol and sonicated for 2 min
at 0°C. After addition of potassium phosphate to a
final concentration of 30 mM, the homogenate was
heated for 5 min at 55°C, cooled, and centrifuged (10
min, 30,000 × g). The enzyme was concentrated by
ammonium sulfate precipitation (30 to 60% saturation)
with ammonium sulfate (pH 7.5) containing 1 mM
dithioerythritol and 15 mM phosphate for 12 h and
stored at 0°C.

Polyacrylamide gel electrophoresis. The sys-
tem of O'Farrell was used for gel electrophoresis in
the presence of sodium dodecyl sulfate (SDS) (25). The
running gel was composed of 10% acrylamide, 0.1% SDS, and 0.375 M Tris-chloride buffer (pH 8.8). The stacking gel contained 2.75% acrylamide, 0.1% SDS, and 0.125 M Tris-chloride buffer (pH 6.8). Protein (5 to 50 μg) was applied, and electrophoresis was conducted at room temperature. Protein was stained by the procedure of Fairbanks et al. (11). Standard proteins used were bovine serum albumin (molecular weight, 68,000), hog pancreas α-amylase (molecular weight, 51,000), myoglobin (molecular weight, 17,000), and rabbit skeletal muscle phosphorylase (molecular weight, 100,000). Electrophoresis under nondenaturating conditions was performed as described by Davis (6), using acrylamide concentrations between 6 and 10%. The running gel contained 50 mM Tris-chloride buffer (pH 8.9), and the stacking gel contained 10 mM Tris-phosphate buffer (pH 7.2). Protein was stained with Coomassie blue (4), and ADP-glucose pyrophosphorylase activity was determined by an activity stain (27). Estimation of the molecular weight of the native protein was carried out by the method of Hedrick and Smith (16), using bovine plasma albumin (molecular weight, 68,000), aldolase (Boheringer Mannheim Corp., rabbit muscle; molecular weight, 160,000), and glucose 6-phosphate dehydrogenase (Sigma, baker’s yeast type V; molecular weight, 240,000) as standards.

Carboxymethylation of proteins. The carboxymethylation procedure was that of Hirs (18) as modified for use in guanidine-hydrochloride (14). Protein (5 mg) was dissolved in 7 M guanidine-hydrochloride containing 0.2% EDTA to a final volume of 5 ml and reduced with 0.01 ml of mercaptoethanol with nitrogen as the gas phase (25°C, 3 h). Then 5 mg of [1-C]iodoacetic acid (specific radioactivity, 6 x 10⁶ cpm/μmol) was added, and the pH of the mixture kept at 8.5 with 10% ethanolamine. The reaction was judged complete as determined by no further decrease in pH. Then 25 mg of unlabeled iodoacetic acid, dissolved in 0.1 N NaOH, was added. The pH was adjusted to 7.0 with NaOH, acetic acid, and the mixture was dialyzed extensively against 5% acetic acid at 4°C for 60 h. The protein was concentrated by lyophilization.

Amino acid composition. Amino acid analyses were performed on carboxymethylated proteins hydrolyzed in 5.7 N HCl at 110°C for 24, 48, and 72 h. Before hydrolysis, samples were carefully degassed and sealed under vacuum. Norleucine was added as an internal standard. Quantitation of amino acids was carried out with a Durrum D-500 amino acid analyzer. Appropriate extrapolations were made to correct for loss of serine and threonine. All other values were uncorrected. Cysteine was determined as carboxymethylcysteine, and tryptophan was spectrophotometrically determined by the method of Edelhoch (9).

NH₂-terminal sequence determination. For determination of the amino-terminal sequence, automated Edman degradation (10) was performed, using a Beckman Sequenator model 890 C. The program used in sequence determination was Beckman program 830176, using diluted Quadrol as a buffer. The protein (4 mg) was prepared for sequence analysis by reduction and carboxymethylation with [¹⁴C]iodoacetic acid as described above. The lyophilized protein was dissolved in 0.5 ml of 70% formic acid and delivered to the spinning cup. The formic acid was removed by the vacuum steps and N₂ flush in the sample application subroutine program. Before automatic sequencing was begun, the protein was subjected to one heptfluorobutyric acid and butyryl chloride extraction step. Reagents used were Beckman Sequenator grade.

Amino acid phenylthiodyantoin derivatives and their trimethylsilyl derivatives were identified by gas-liquid chromatography (19, 30), using a Packard model 419 gas chromatography system. All residues were also identified by thin-layer chromatography (22) and counted for radioactivity. Arginine was identified as free amino acid after hydrolysis of the phenylthiodyantoin derivative (24). Residues were obtained at a repetitive yield of 96% with an extrapolated yield of 75%.

Digestion with carboxypeptidases. ADP-glucose pyrophosphorylase preparations (1.5 mg/ml) were digested for 12 h against 10 mM potassium phosphate buffer (pH 7.5). N-Ethylmorpholine–acetate buffer (pH 8.5) was added to a final concentration of 0.2 M. Samples (0.2 mg) were digested with carboxypeptidase A or B for 0.5 to 5 h, using a carboxypeptidase/protein ratio of 1:50 or 1:150 (wt/wt), respectively. Before digestion, carboxypeptidase A was washed with water and solubilized by the addition of 2 M NH₄HCO₃. The reaction was terminated by heating for 1 min at 100°C, and the samples were dried under nitrogen. Released amino acids were identified by thin-layer chromatography, using 1-butanol–acetone–1 N HCl (60:15:25, by volume) as a solvent. The plates were developed with either fluorescamine (12) or phenanthrenenequione (36). Quantitation of amino acids was carried out with the Durrum D-500 amino acid analyzer.

Immunological methods. Two rabbits were injected subcutaneously in the neck region, at intervals of 1 week (four times) and then 1 month (two times), with 0.35 mg of purified ADP-glucose pyrophosphorylase. For the injections, the protein (1.4 mg/ml) was mixed with equal amounts of Freund complete adjuvant. Serum (8 to 10 ml) was collected between 7 and 9 days after the third and all following injections and stored either at 0°C in the presence of 0.02% NaN₃ or at −70°C.

Ouchterlony double immunodiffusion (26) was carried out on 0.8% agarose gels containing 0.9% NaCl, 0.01 M potassium phosphate (pH 7.2), and 0.02% NaN₃. Inhibition of enzyme by serum was performed by mixing enzyme (0.04 U/ml) and antiserum in 0.1 M Tris-chloride buffer (pH 8.0). After incubation for 20 min at room temperature, 0.01 ml of the mixture was assayed for enzymatic activity in the pyrophosphorylase direction in the presence of 1.5 mM fructose diphosphate. The antiserum generally used was that collected 12 weeks after the first injection. Preimmune serum was used as a control.

Sucrose density centrifugation. The determination of the molecular weight of ADP-glucose pyrophosphorylase was carried out as described by Martin and Ames (23). Purified enzyme (0.3 U) was mixed with 100 U of lactic dehydrogenase (Sigma, rabbit muscle) and 40 U of pyruvate kinase (Sigma, rabbit muscle type III) in a final volume of 0.1 ml of 50 mM HEPES (N-2-hydroxyethylpiperazine-N'-ethanesulfonic acid) buffer (pH 7.0). The mixture was layered on top of a linear sucrose gradient (4.15 ml, 5 to 25% sucrose) and centrifuged for 16 h (0°C, 34,500 x g),
using a Beckman L5-50 ultracentrifuge equipped with an SW60 rotor. Fractions of about 0.17 ml were taken from the gradient and assayed for activity of pyruvate kinase (2), lactic dehydrogenase (20), and ADPglucose pyrophosphorylase in pyrophosphorolysis direction in the presence of 1.5 mM fructose diphosphate.

RESULTS

Purification of enzyme. As indicated previously, strain JP102 has about a fourfold-greater amount of the glycoprotein biosynthetic enzymes than does the parent strain, S. typhimurium LT-2 (35), and therefore was used as the source of ADPglucose pyrophosphorylase.

Table 1 summarizes the purification of ADPglucose pyrophosphorylase from mutant JP102. Although the cell suspension was passed three times through the homogenizer, viscosity of the extract had to be reduced by ultrasonication. Heat treatment of the crude extract increased the total activity threefold, probably due to the inactivation of interfering reactions or of an inhibitor. A similar phenomenon had been previously observed with extracts from S. typhimurium LT-2 (34). The ammonium sulfate step, as well as increasing the specific activity fourfold, is time-saving, since it decreased the volume of the heat-treated fractions for ultracentrifugation.

After the first affinity column (AMP eluate), gel electrophoresis showed a main and a faint band, both of which were enzymatically active when assayed by an activity stain (27). By readсорption of the protein and elution with a phosphate gradient, it was possible to remove the faint band. To avoid losses, concentration of the enzyme after the second affinity column step was carried out by using an Amicon cell concentrator. The enzyme was found to be stable in the presence of 10 mM potassium phosphate (pH 7.0) and 20% glycerol.

Gel electrophoresis. Purified ADPglucose pyrophosphorylase (20 µg) run in the Tris-glycine system of Davis (6) with gel concentrations of 6 to 10% acrylamide showed one protein band, which coincided with the enzyme activity seen by using an activity stain (27). Determination of the molecular weight of the native protein by the method of Hedrick and Smith (16) gave a value of 195,000 ± 10,000. Sucrose density gradient centrifugation (16) of the pure ADPglucose pyrophosphorylase indicated a molecular weight of 190,000. When the protein was denatured and 5 to 50 µg was subject to slab gel electrophoresis according to O'Farrell (25), it migrated as a single, homogeneous band. The molecular weight of the subunit was determined to be 48,000. The amino acid composition of the enzyme is shown in Table 2.

Kinetic properties of ADPglucose pyrophosphorylase from S. typhimurium JP102. Fructose diphosphate has been found to be the physiological modulator of the ADPglucose pyrophosphorylase of S. typhimurium (34). Fructose-diphosphate as well as NADPH and pyridoxal phosphate have been found to be effective activators of other enteric ADPglucose pyrophosphorylases (34). Table 3 shows the activation of the purified ADPglucose pyrophosphorylase from JP102 by various effectors. The derepressed mutant showed activator specificity similar to that of the parent strain enzyme. Comparison of kinetic constants of activators, ATP, and inhibitors of ADPglucose pyrophosphorylase from S. typhimurium LT-2 and JP102 indicated that the enzymes from the mutant and parent organism were quite similar (Table 4).

NH₂-terminal sequence studies. The results of amino acid sequence analysis of the first 27 residues of ADPglucose pyrophosphorylase from JP102 are shown in Fig. 1. Residue 16 could not be positively identified, because identification of the corresponding phenylthiohydantoin derivative by gas chromatography did not result in a significant appearance of an amino acid, whereas thin-layer chromatography indicated glutamic acid. However, back-hydrolysis showed that residue 16 may be either glutamic acid or proline. Figure 1 also shows the corresponding sequence of the NH₂ terminal of ADPglucose.

![Table 1. Purification of ADPglucose pyrophosphorylase from S. typhimurium JP102](http://jb.asm.org/)

<table>
<thead>
<tr>
<th>Purification step</th>
<th>Vol (ml)</th>
<th>Total units (µmol/min)</th>
<th>Protein (mg/ml)</th>
<th>Sp act (U/µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude homogenate</td>
<td>2,530</td>
<td>605</td>
<td>18.5</td>
<td>0.0013</td>
</tr>
<tr>
<td>Heat treatment fraction</td>
<td>2,550</td>
<td>1,762</td>
<td>16.3</td>
<td>0.042</td>
</tr>
<tr>
<td>(NH₄)₂SO₄ precipitation</td>
<td>1,790</td>
<td>1,307</td>
<td>4.5</td>
<td>0.16</td>
</tr>
<tr>
<td>Chromatography on:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DBAE-cellulose</td>
<td>260</td>
<td>1,211</td>
<td>3.9</td>
<td>1.2</td>
</tr>
<tr>
<td>HDP-Sepharose (AMP eluate)</td>
<td>40</td>
<td>1,080</td>
<td>0.32</td>
<td>84.4</td>
</tr>
<tr>
<td>HDP-Sepharose (phosphate eluate)</td>
<td>6</td>
<td>761</td>
<td>1.2</td>
<td>106.0</td>
</tr>
</tbody>
</table>

*Enzyme was isolated from 700 g of cell paste as described in the text. Enzyme activity was measured in the pyrophosphorolysis direction in the presence of 1.5 mM fructose 1,6-diphosphate.

*Hexanediol 1,5-diphosphate.
ADPglucose pyrophosphorylase did not release amino acids as seen by thin-layer chromatography. Digestion with carboxypeptidase B and subsequent quantitation by amino acid analysis resulted in the release of 1.1 mol of arginine per

**Table 2. Composition of ADPglucose pyrophosphorylase from S. typhimurium JP102**

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Conc (mol/48,000 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspartic acid</td>
<td>45.2 ± 3.3</td>
</tr>
<tr>
<td>Threonine</td>
<td>15.5 ± 0.4</td>
</tr>
<tr>
<td>Serine</td>
<td>39.4 ± 1.1</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>43.6 ± 2.6</td>
</tr>
<tr>
<td>Proline</td>
<td>19.6 ± 0.6</td>
</tr>
<tr>
<td>Glycine</td>
<td>31.0 ± 1.6</td>
</tr>
<tr>
<td>Alanine</td>
<td>37.1 ± 2.7</td>
</tr>
<tr>
<td>Valine</td>
<td>34.0 ± 1.5</td>
</tr>
<tr>
<td>Methionine</td>
<td>12.3 ± 0.7</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>26.3 ± 0.4</td>
</tr>
<tr>
<td>Leucine</td>
<td>35.9 ± 1.6</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>15.1 ± 0.92</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>13.2 ± 0.3</td>
</tr>
<tr>
<td>Histidine</td>
<td>8.2 ± 0.1</td>
</tr>
<tr>
<td>Lysine</td>
<td>21.3 ± 1.7</td>
</tr>
<tr>
<td>Arginine</td>
<td>31.6 ± 1.7</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>5.6 ± 0.3</td>
</tr>
<tr>
<td>CM-cysteine</td>
<td>10.4</td>
</tr>
</tbody>
</table>

* Protein was reduced, carboxymethylated, and hydrolyzed for 24, 48, and 72 h as described in the text. The results are based on a 94% yield and are the average of two determinations on two different preparations of pure enzyme.

**Table 3. Activation of ADPglucose pyrophosphorylase from S. typhimurium JP102 by various metabolites**

<table>
<thead>
<tr>
<th>Effector</th>
<th>ADPglucose formed (nmol/10 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0.3</td>
</tr>
<tr>
<td>3-Phosphoglycerate</td>
<td>0.5</td>
</tr>
<tr>
<td>2-Phosphoglycerate</td>
<td>1.8</td>
</tr>
<tr>
<td>PLP, 0.05 mM</td>
<td>9.4</td>
</tr>
<tr>
<td>NADP, 1 mM</td>
<td>0.9</td>
</tr>
<tr>
<td>NADPH, 1 mM</td>
<td>8.6</td>
</tr>
<tr>
<td>Fructose-diphosphate</td>
<td>9.2</td>
</tr>
<tr>
<td>Ribose 5-phosphate</td>
<td>0.5</td>
</tr>
<tr>
<td>Glucose 1,6-diphosphate</td>
<td>1.0</td>
</tr>
<tr>
<td>3-Phosphoglyceraldehyde</td>
<td>0.95</td>
</tr>
<tr>
<td>Glycerol 1,3-diphosphate</td>
<td>4.2</td>
</tr>
<tr>
<td>6-Phosphogluconate</td>
<td>0.6</td>
</tr>
</tbody>
</table>

* Purified enzyme was assayed in the synthesis direction in the presence of 1.5 mM effector, unless otherwise indicated.

**Table 4. Kinetic parameters of ADPglucose pyrophosphorylase from S. typhimurium LT-2 and JP102**

<table>
<thead>
<tr>
<th>Effector</th>
<th>Vmax (μmol min⁻¹ mg⁻¹)</th>
<th>S0.5 ATP</th>
<th>α</th>
<th>S0.5 ATP</th>
<th>α</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>22.9</td>
<td>2.0</td>
<td>1.6</td>
<td>2.4</td>
<td>1.5</td>
</tr>
<tr>
<td>Fructose 1,6-di-phosphate</td>
<td>91.6</td>
<td>0.50</td>
<td>2.0</td>
<td>0.48</td>
<td>2.2</td>
</tr>
<tr>
<td>NADPH</td>
<td>76.6</td>
<td>0.36</td>
<td>1.6</td>
<td>0.37</td>
<td>1.5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Effector</th>
<th>Vmax (μmol min⁻¹ mg⁻¹)</th>
<th>A0.5 (mM)</th>
<th>α</th>
<th>A0.5 (mM)</th>
<th>α</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fructose 1,6-di-phosphate</td>
<td>91.6</td>
<td>0.12</td>
<td>2.4</td>
<td>0.10</td>
<td>2.3</td>
</tr>
<tr>
<td>NADPH</td>
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<td>2.4</td>
<td>0.11</td>
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<td>2.3</td>
<td>0.31</td>
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<th>Inhibitor</th>
<th>I50 (mM)</th>
<th>α</th>
<th>I50 (mM)</th>
<th>α</th>
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<tr>
<td>AMP (0.25)</td>
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<td>1.5</td>
<td>0.028</td>
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<tr>
<td>PO43⁻ (0.25)</td>
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<td>0.93</td>
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<td>PO43⁻ (1.0)</td>
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<td>2.0</td>
<td>1.7</td>
<td>2.5</td>
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* Concentration of ATP at which 50% maximal velocity is attained.
* Numbers in parentheses indicate concentration (millimolar) of fructose 1,6-diphosphate.

**Fig. 1. NH2-terminal sequence of ADPglucose pyrophosphorylases from S. typhimurium JP102 (A) and Escherichia coli B (B).** The S. typhimurium amino acid sequence was determined as described in the text. Residue 16 could not be positively identified by gas chromatography; thin-layer chromatography indicated glutamic acid, and back-hydrolysis of the phenylthiohydantoin derivative showed glutamic acid or proline. The E. coli B amino acid sequence was previously determined (14, 29).
48,000 g. All other amino acids were found in amounts less than 0.1 mol/48,000 g.

Immunology. When anti- 

S. typhimurium

JP102 ADPglucose pyrophosphorylase serum was reacted against the purified enzyme from JP102 in double-immunodiffusion gels (26), only one precipitin band was visible. Partly purified ADPglucose pyrophosphorylases from 

S. typhimurium

LT-2 was prepared as previously described (34) and also reacted with the antiserum in double-immunodiffusion gels. When at least 0.15 U of enzyme activity was placed on the gel, a single precipitin band formed and was cross-reactive with the band produced with the purified enzyme from JP102. In contrast, the purified ADPglucose pyrophosphorylase from 

E. coli

B (14) formed a cross-reactive precipitin with the 

S. typhimurium

enzyme antiserum which contained a spur. Preimmune serum did not form precipitin bands with either enzyme. Figure 2 shows the effect of antibody on the activity of the ADP-glucose pyrophosphorylases from 

S. typhimurium

JP102, 

S. typhimurium

LT-2, and 

E. coli

B. The amounts of antibody required to inhibit 50% of the enzyme activity of purified ADP-glucose pyrophosphorylases from JP102 and 

E. coli

B, of enzyme in a crude extract of JP102, and of a partially purified ADPglucose pyrophosphorylase fraction from 

S. typhimu-

rrium

LT-2 were about 5.0, 13, 5.2, and 5.5 μl of serum per unit of enzyme, respectively. Preimmune serum, used as a control, had no effect on any of these enzymes. These results indicate that the two 

S. typhimurium

enzymes are antigenically similar, whereas the 

E. coli

B enzyme has some antigenic dissimilarities when compared with the 

S. typhimurium

enzymes.

**DISCUSSION**

These results indicate that the ADPglucose pyrophosphorylase from 

S. typhimurium

LT-2 mutant JP102 is very similar if not identical in all tested respects with the enzyme from the parent strain, 

S. typhimurium

LT-2. Therefore, the mutant JP102 appears to synthesize the wild-type parent enzyme but in greater amounts. Kinetic studies showed that the two enzymes are similar with respect to kinetic constants for the allosteric effectors, fructose diphosphate, NADPH, AMP, ADP, P_i, and the substrate, ATP. Furthermore, the immunological reactions of both enzymes toward antibody produced from the mutant JP102 enzyme were identical in double-immunodiffusion gels and in neutralization of activity experiments.

The purified enzyme from mutant JP102 has been shown to have a subunit molecular weight of about 48,000. Both gel electrophoresis and sucrose gradient density ultracentrifugation experiments suggest that the native molecular weight is 190,000 to 195,000, indicating that the native ADPglucose pyrophosphorylase is a tetramer. Amino-terminal sequence studies showed only one sequence and suggest that the subunits are similar if not identical. Moreover, the release of no amino acids by carboxypeptidase A and the release of 1 mol of arginine per 48,000 g of enzyme by carboxypeptidase B are also consistent with similar subunits of 48,000 molecular weight.

Although some antigenic dissimilarities are noted between the 

E. coli

B and 

S. typhimurium

enzymes, there is a cross-reaction between the antibodies produced against each enzyme (this report: 14). Both enzymes have the same activator specificity, with the major activators being fructose diphosphate, pyridoxal phosphate, and NADPH, and are very sensitive to AMP inhibition (13, 34). Both enzymes have similar subunit and native molecular weights, and the amino acid compositions are similar (14). The similarity extends also to the amino-terminal sequence. Of the first 27 amino acids in the sequence, there are only two positive differences (residues 8 and 9) and perhaps a third (residue 16). The changes at residues 8 and 9 are conservative, since there is a change of arginine for histidine and valine for leucine. The conservation of the amino terminal of these two enzymes takes on added significance since it has been shown that a lysine residue, 37 amino acids from the amino-terminal valine, is involved in the binding of the allosteric activator (15, 28, 29).

The similarity of the ADPglucose pyrophosphorylase of 

E. coli

and 

S. typhimurium

correlates with the results regarding the two proteins being very similar.
lates also with the close evolutionary relatedness of these two organisms (1). The availability of antiserum prepared against the E. coli and S. typhimurium enzymes will enable more extensive immunological comparisons of the ADP-glucose pyrophosphorylases of other organisms of the Enterobacteriaceae having the same activator specificity as the enzyme from the above two organisms as well as from other related bacteria. This will be the subject of another report.

ACKNOWLEDGMENTS

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LITERATURE CITED

