

Cloning of the ADPglucose Pyrophosphorylase (*glgC*) and Glycogen Synthase (*glgA*) Structural Genes from *Salmonella typhimurium* LT2

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The structural genes of ADPglucose pyrophosphorylase (*glgC*) and glycogen synthase (*glgA*) from *Salmonella typhimurium* LT2 were cloned on a 5.8-kilobase-pair insert in the *SalI* site of pBR322. A single strand specific radioactive probe containing the N terminus of the *Escherichia coli* K-12 *glgC* gene in M13mp8 was used to hybridize against a *S. typhimurium* genomic library in λ 1059. DNA from a plaque showing a positive hybridization signal was isolated, subcloned into pBR322, and transformed into *E. coli* K-12 RR1 and *E. coli* G6MD3 (a mutant with a deletion of the *glg* genes). Transformants were stained with iodine for the presence of glycogen. *E. coli* K-12 RR1 transformants stained dark brown, whereas G6MD3 transformants stained greenish yellow, and they both were shown to contain a 5.8-kilobase-pair insert in the *SalI* site of pBR322, designated pPL301. Enzyme assays of *E. coli* K-12 G6MD3 harboring pPL301 restored ADPglucose pyrophosphorylase and glycogen synthase activities. The specific activities of ADPglucose pyrophosphorylase and glycogen synthase in *E. coli* K-12 RR1(pPL301) were increased 6- to 7-fold and 13- to 15-fold, respectively. Immunological and kinetic studies showed that the expressed ADPglucose pyrophosphorylase activity in transformed *E. coli* K-12 G6MD3 cells was very similar to that of the wild-type enzyme.

The biosynthesis of bacterial glycogen involves three enzymes: ADPglucose pyrophosphorylase (EC 2.7.7.27), glycogen synthase (EC 2.4.1.21), and branching enzyme (EC 2.4.1.18), which are encoded by *glgC*, *-A*, and *-B* genes, respectively, in *Escherichia coli* (12, 27-30). In this biosynthetic pathway, allosteric regulation has been shown to occur at the ADPglucose pyrophosphorylase level (12, 27-30). ADPglucose pyrophosphorylase has been isolated and characterized from a number of bacteria, and the nature of the activator has been shown to be related to the major route of carbon metabolism of the particular organism (12, 27-30).

Among the enteric bacteria, ADPglucose pyrophosphorylase is activated by fructose 1,6-bisphosphate (27), whereas ADP, AMP, and P_i are allosteric inhibitors (29, 30).

Okita et al. (25) have cloned the *glgA*, *-B*, and *-C* genes from *E. coli* K-12 into the *PstI* site of pBR322. The nucleotide sequence of the structural genes *glgA*, *-B*, and *-C* have been reported (2, 3, 13). The allosteric properties of ADPglucose pyrophosphorylase from *E. coli* K-12 were also studied in great detail with respect to its activator binding site, inhibitor binding site, and substrate binding site (14, 16) by means of a photoaffinity labeling technique with azido adenine nucleotides. The combination of nucleotide sequencing and active site labeling has enabled us to determine the location of the activator, inhibitor, and substrate binding sites.

As might be expected, the ADPglucose pyrophosphorylase of *Salmonella typhimurium* shows the same spectrum of allosteric activators and inhibitors as the *E. coli* ADPglucose pyrophosphorylase (31). Therefore, it was of interest to clone the glycogen biosynthetic genes from *S. typhimurium*,

since it may provide more insight in understanding how ADPglucose pyrophosphorylase is allosterically regulated at a structure-function level.

In the present study, the *glgC* and *glgA* genes from *S. typhimurium* were cloned into the *SalI* site of pBR322, and the expression of the enzymes ADPglucose pyrophosphorylase and glycogen synthase was observed in both *E. coli* and *S. typhimurium*. The cloned ADPglucose pyrophosphorylase was immunologically identical to the wild-type *S. typhimurium* enzyme, and the kinetic properties were very similar to those of the wild type. Physical map construction shows that the *glgC* and *glgA* genes are very close to one another. Their physical sizes are both about 1.3 kilobase pairs, thus allowing a coding capacity of protein of about 50 kilodaltons of subunit molecular mass for each enzyme (18).

MATERIALS AND METHODS

Bacterial and phage strains. The bacterial and phage strains used in this study are listed in Table 1.

Media and growth conditions. Luria broth and NZYM medium were prepared as described previously (21, 24). Ampicillin or tetracycline at 25 μ g/ml was added where indicated. Diaminopimelic acid was added at 50 μ g/ml for growing of *E. coli* K-12 G6MD3. Maltose was added at a final concentration of 0.2% to Luria broth for the growth of *E. coli* K-12 Q359 before infection by the λ 1059 genomic library. NZYM agar contained 1.5% agar, and NZYM top agar contained 0.7% agar. Enriched medium contained 1.1% KH₂PO₄, 0.85% K₂HPO₄, and 0.6% yeast extract. YT medium and YT soft agar were prepared as described previously (22).

Enzymes and chemicals. Restriction endonucleases were from Bethesda Research Laboratories, Inc., as were T4 DNA ligase and the large fragment of DNA polymerase I. All enzymes were used as recommended by the manufacturer. Isopropyl-D-thiogalactoside was from Sigma Chemical Co., and X-gal (5-bromo-4-chloro-3-indoyl-galactoside) was from Bethesda Research Laboratories, Inc. [α -³²P]dATP was from

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TABLE 1. Bacterial and Bacteriophage Strains

Strain	Genotype	Reference
<i>E. coli</i> K-12		
RR1	F ⁻ <i>hsd-20</i> (<i>r_B</i> ⁻ <i>m_B</i> ⁻) <i>recA13 ara-14 proA2 lacY1 galK2 rpsLS20</i> (Sm ^r) <i>xyl-5 mtl-5 supE44</i>	5
G6MD3	Hfr <i>his thi str ΔmalA → asd</i>	32
Q359	<i>r_K</i> ⁻ <i>m_K</i> ⁺ <i>su</i> ⁺ <i>Π φ80</i> ^r P2	11
JM103	<i>supE thi Δ(lac → proAB)</i> F' <i>traD36 proAB lacI^ΔM15</i>	22
<i>S. typhimurium</i>		
JP102		33
M13 mp8		23
λ1059		11

Amersham Corp. Sarkosyl NL30 was from CIBA-GEIGY Corp.

Construction of DNA hybridization probe. A DNA hybridization probe containing the N terminus of the *E. coli* K-12 *glgC* gene was constructed by the method of Hu and Messing (10) (Fig. 1). Plasmid pOP12 containing the *E. coli* K-12 *glgA*, *-B*, and *-C* genes (25) was digested with the restriction enzyme *HpaI*, and the 3.8-kilobase *HpaI* fragment was isolated. The 3.8-kilobase-pair *HpaI* fragment was then partially digested with *EcoRI* and ligated to M13 mp8 replicative form that had been double digested with *EcoRI* and *SmaI*. *E. coli* K-12 JM103 was then transformed with the ligated DNA and plated on YT medium with the isopropyl- β -thiogalactoside and X-gal as described by Messing (22). White plaques were picked and analyzed by rapid isolation of DNA from infected cells and restriction endonuclease digestions. A specific clone shown to contain the N terminus of the *E. coli glgC* gene in the *EcoRI* and *SmaI* site of M13 mp8 was chosen for single strand DNA isolation (22). The single strand DNA isolated from this clone served as template for DNA synthesis reaction in the preparation of radioactive DNA hybridization probe as described previously (10).

Screening of genomic library. A genomic library containing *S. typhimurium* LT2 DNA in λ1059 was a gift from the laboratory of S. Artz, Department of Bacteriology, University of California, Davis. The library was a partial digest of genomic DNA with the restriction enzyme *Sau3A*. The DNA restriction fragments of 14 to 24 kilobases were selected by sucrose density gradients and then were ligated to the λ1059 vector DNA that had been digested with *BamHI*. The titer of the library was 860 PFU/μl. A total of 1.6×10^4 plaques were screened, and 33 positive hybrids were found. *E. coli* K-12 Q359 was grown in LB-maltose medium and harvested at an absorbancy at 660 nm of 0.3 and was suspended in 0.25 volume of 0.01 M MgSO₄. One microliter of phage library was added to 0.2 ml of *E. coli* K-12 Q359 and incubated at 37°C for 10 min to allow adsorption. NZYM top agar (2.5 ml, prewarmed at 55°C) was then added, and the whole mixture was poured onto a NZYM agar plate. After incubation at 37°C for 8 to 10 h, the phages were transferred to nitrocellulose filters as described previously (4). The filters were soaked in 1.0 N NaCl-0.5 N NaOH for 30 s, then in 0.5M Tris hydrochloride (pH 7.4)-1.5 M NaCl for 30 s, and then in 3× SSC (1× SSC is 1.5 M NaCl and 0.15 M sodium citrate). After a wash in 3× SSC, the filters were dried and baked at 80°C for 2 h.

Prehybridization was carried out at 43°C in a solution containing 5× Denhardt solution (50× Denhardt solution

contains 1% Ficoll, 1% bovine serum albumin Pentax fraction V, 1% polyvinylpyrrolidone)-5× SSC-50 mM phosphate buffer (pH 6.8)-1% glycine-250 μg of sheared salmon sperm DNA per ml-50% formamide-0.1% sodium dodecyl sulfate. The filters were prehybridized overnight. After prehybridization, the prehybridization solution was replaced by a hybridization solution containing 1× Denhardt solution-5× SSC-50% formamide-20 mM phosphate buffer (pH 6.8)-100 μg of sheared salmon sperm DNA per ml-0.1% sodium dodecyl sulfate. A radioactive probe containing the N terminus of the *E. coli glgC* gene product (10⁶ cpm) was then added, and hybridization was allowed to proceed at 43°C for 40 h. The filters were then washed three times (15 min each) in 2× SSCP (1× SSCP contains 120 mM sodium chloride, 15 mM sodium citrate, 13 mM potassium dihydrogen phosphate, and 1 mM ethylenedinitrilotetraacetic acid, titrated to pH 7.2 with sodium hydroxide)-0.1% Sarkosyl at room temperature and four times in 0.2× SSCP-0.1% Sarkosyl at 50°C. The filters were blot dried and exposed to Kodak XAR-5 film at -70°C.

DNA preparation. DNA from the genomic library was purified by the method of Davis et al. (8). Plasmid DNA was prepared (3) and further purified on a cesium chloride gradient (21) as described previously.

Electrophoresis. DNA preparations were resolved on agarose slab gels in a 90 mM Tris-90 mM boric acid-2.5 mM disodium EDTA buffer at pH 8.2. After electrophoresis, the gel was stained with ethidium bromide at 5 μg/ml and visualized by fluorescence under longwave UV light.

Transformation. *E. coli* K-12 RR1 and *E. coli* K-12 G5MD3 were transformed by the method of Bolivar et al. (5). *E. coli* K-12 JM103 was transformed as described by Messing (2). *S. typhimurium* was transformed by the method of Lederberg and Cohen (15).

Enzyme assays. Cells harboring pPL301 were grown in enriched medium with 0.3% glucose and 25 μg of ampicillin

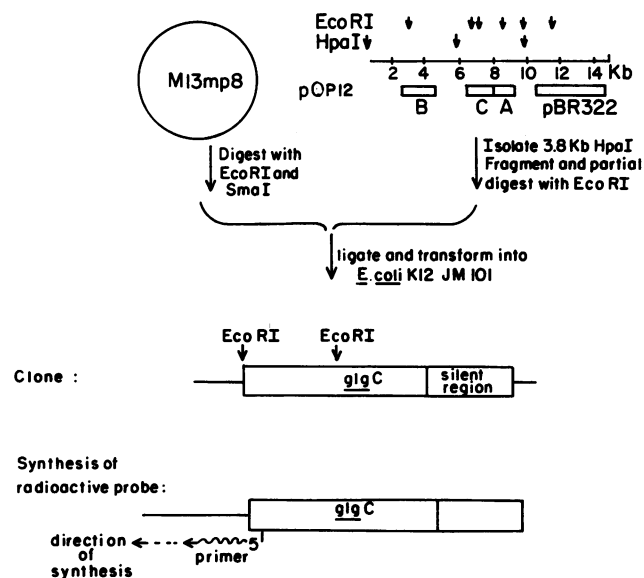


FIG. 1. Construction and synthesis of DNA hybridization probe. The 3.8-kilobase *HpaI* fragment from plasmid pOP12 was digested with *EcoRI* and then cloned into *EcoRI*-*SmaI*-digested M13 mp8RF. A clone containing the N terminus of *glgC* was chosen for single strand DNA isolation and subsequent radioactive probe synthesis by a DNA synthesis reaction as described in Materials and Methods.

per ml until the stationary phase and harvested for assaying of glycogen biosynthetic enzymes. The corresponding strains without pPL301 were grown in the same medium without ampicillin. ADPglucose pyrophosphorylase was assayed in the pyrophosphorolysis direction by measuring the formation of [³²P]ATP from ADPglucose and ³²PP_i as described by Steiner and Preiss (33). Synthesis of ADPglucose from [¹⁴C]glucose 1-phosphate and ATP was measured (17, 31) and glycogen synthase and branching enzyme were assayed (33) as described previously.

Protein assay. Total protein was assayed by the method of Lowry et al. (20).

Partial purification of ADPglucose pyrophosphorylase. *E. coli* K-12 G6MD3(pPL301) was grown to the stationary phase in enriched medium containing 1% glucose–50 μg of diaminopimelic acid per ml–25 μg of ampicillin per ml at 37°C in a 14-liter New Brunswick fermentor. The culture was mixed at 600 rpm and aerated at a rate of 15 liters per min. *S. typhimurium* JP102 was grown under the same conditions in enriched medium containing 1% glucose. The cells were stored at –20°C as a paste.

Ten grams of cells of either *E. coli* K-12 G6MD3(pPL301) or *S. typhimurium* JP102 was suspended in 50 ml of 50 mM glycolglycine buffer (pH 7.0) containing 5 mM dithioerythritol and sonicated for 4 min at 4°C. After the addition of potassium phosphate to a final concentration of 300 mM, the homogenate was heated at 60°C for 5 min in a water bath. It was then quickly cooled to 4°C and centrifuged at 18,000 × *g* for 15 min at 4°C. To the supernatant, ammonium sulfate was added to 55% saturation, and the mixture was gently stirred at 4°C for 20 min. It was then centrifuged for 30,000 × *g* for 15 min at 4°C. The precipitate was suspended in 13 ml of 0.05 M Tris hydrochloride (pH 7.2) containing 5 mM dithioerythritol and 15 mM potassium phosphate and dialyzed overnight against 2 liters of the same buffer containing 5% glycerol. The dialysate was centrifuged for 30 min, and the supernatant was applied to a DEAE-cellulose column (20-ml bed volume) that had been preequilibrated with 15 mM potassium phosphate buffer (pH 7.5) containing 1 mM dithioerythritol. The column was washed with 1 bed volume of the same buffer, and the proteins were eluted with a linear gradient of 0 to 0.3 M potassium chloride. Fractions that contained ADPglucose pyrophosphorylase activities according to the activated assay of ADPglucose pyrophosphorylase in the direction of pyrophosphorolysis were pooled and concentrated in an Amicon ultrafiltration apparatus with a PM-30 membrane. The concentrated enzyme were dialyzed against 2 liters of 50 mM Tris hydrochloride (pH 7.2) containing 1 mM dithioerythritol and 20% glycerol. The enzymes were stored at –20°C and used for kinetic experiments.

The specific activities of the partially purified preparations were as follows: for *E. coli* K-12 G6MD3(pPL301), 132 μmol of ATP formed per 10 min per mg of protein; for *S. typhimurium* JP102, 5.1 μmol of ATP formed per 10 min per mg of protein (pyrophosphorylase assay). A unit of enzyme activity is defined as 1 μmol of ATP formed in 10 min.

Neutralization reactions. The enzyme was diluted to 0.5 U/ml with 50 mM Tris hydrochloride (pH 8.0) containing 0.1 mg of bovine plasma albumin per ml. Antiserum against *S. typhimurium* JP102 ADPglucose pyrophosphorylase was diluted to 1:200 in 50 mM Tris hydrochloride (pH 8.0) containing 0.1 mg of bovine serum albumin per ml. Samples of different dilutions of antiserum were allowed to incubate with 0.05 U of ADPglucose pyrophosphorylase enzyme in a 100-μl volume for 20 min at room temperature. (Each

incubation reaction contained 10 μmol of Tris hydrochloride [pH 8.0], 0.05 U of enzyme, various amounts of antiserum, and water in a final volume of 0.1 ml.) Samples were then centrifuged for 2 min at room temperature, and 40 μl of the supernatant was used for assaying of ADPglucose pyrophosphorylase activity in the pyrophosphorolysis direction as described previously (33). Antibody to the *S. typhimurium* LT2 ADPglucose pyrophosphorylase was prepared as previously described (17).

Determination of kinetic constants. Kinetic data were plotted as velocity versus effector concentration and were replotted as Hill plots (6, 19). V_{max} was determined from double-reciprocal plots; $A_{0.5}$, $I_{0.5}$ (corresponding to the concentrations of activator, fructose 1,6-bisphosphate, and inhibitor, AMP, required for half-maximal or for 50% inhibition, respectively), and the Hill interaction coefficient n , were determined from Hill plots.

RESULTS

Cloning of *S. typhimurium* LT2 glycogen biosynthetic genes. DNA from a phage showing positive hybridization signal with the radioactive probe containing the N terminus of *E. coli* K-12 *glgC* gene was partially digested with several restriction endonucleases and resolved on an agarose gel. DNA from this phage was then digested with *SalI* and ligated with the *SalI*-linearized plasmid vector pBR322. Upon transformation of *E. coli* RR1 and *E. coli* K-12 G6MD3, colonies that were ampicillin resistant and tetracycline sensitive were picked and analyzed by rapid isolation of plasmid DNA and subsequent digestion by the restriction endonuclease *SalI*. They all showed the presence of a 5.8-kilobase-pair insert in the *SalI* site of pBR322 (Fig. 2). The plasmid was designated pPL301; a restriction map is constructed with respect to *AvaI*, *ClaI*, *HincII*, *HindIII*, *KpnI*, *PstI*, *PvuII*, and *SalI* (Fig. 3).

The clones were incubated overnight on enriched medium agar plates and stained with iodine for the presence of glycogen. *E. coli* K-12 G6MD3 stained yellow, whereas *E. coli* K-12 G6MD3(pPL301) stained greenish yellow. *E. coli* K-12 RR1 stained brown, and *E. coli* K-12 RR1(pPL301) stained dark brown (Table 2).

The clones were grown in enriched medium containing 0.3% glucose to the stationary phase and assayed for glycogen biosynthetic enzymes (Table 2). The specific activities of ADPglucose pyrophosphorylase and glycogen synthase in *E. coli* K-12 RR1 harboring pPL301 were increased 6- to 7-fold and 13- to 15-fold, respectively, when compared with enzymes from *E. coli* K-12 RR1. *E. coli* K-12 G6MD3, a mutant with a deletion in the *glg* genes, has no ADPglucose pyrophosphorylase, glycogen synthase, and branching enzyme activities. However, activities for ADPglucose pyrophosphorylase and glycogen synthase were restored in *E. coli* K-12 G6MD3 harboring pPL301. The iodine staining and the enzyme activities of the clones therefore show that the genes coding for ADPglucose pyrophosphorylase (*glgC*) and glycogen synthase (*glgA*) are cloned on the plasmid pPL301.

S. typhimurium JP102, a mutant of *S. typhimurium* LT2 derepressed in the levels of the glycogen biosynthetic enzymes (33), was also transformed with pPL301. The mutant had 5-fold more ADPglucose pyrophosphorylase activity and 2.4-fold more glycogen synthase activity than its parent strain. When the mutant was transformed with pPL301 the activities of ADPglucose pyrophosphorylase and glycogen synthase were increased 8.7- and 5.1-fold, respectively.

Characterization of pPL301. In the *E. coli* K-12 G6MD3 background, a *PvuII* deletion in the pPL301 plasmid totally

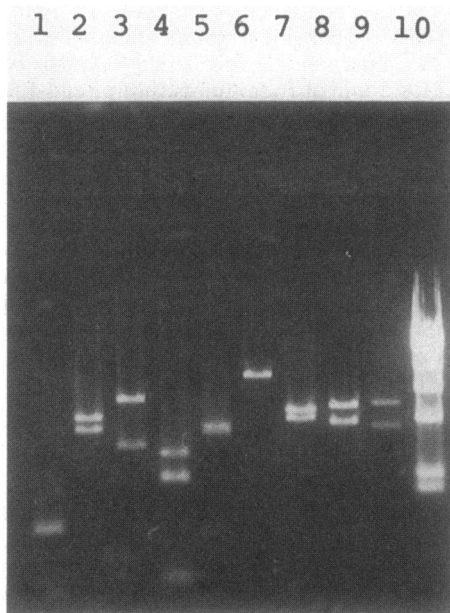


FIG. 2. Agarose gel electrophoresis of plasmid pPL301. Restriction endonucleases digestion of pPL301 was resolved on a 1% agarose gel with *Hind*III-digested lambda DNA as a molecular weight marker (lane 10). Lane 1 is a *Hinf*I digest of pBR322. Lanes 2 through 9 are digests of pPL301 with *Ava*I, *Cla*I, *Hinc*II, *Hind*III, *Kpn*I, *Pst*I, *Pvu*II, and *Sal*I, respectively.

eliminated *glgC* and *glgA* activities when compared with *E. coli* K-12 G6MD3(pPL301). Under the same condition, a *Hind*III deletion plasmid and a *Cla*I deletion plasmid showed glycogen synthase activity but not ADPglucose pyrophosphorylase activity (Fig. 3). Subsequent nucleotide sequencing (18) of a 1.5-kilobase-pair fragment from pPL301 showed significant homology with the nucleotide sequence of *E. coli*

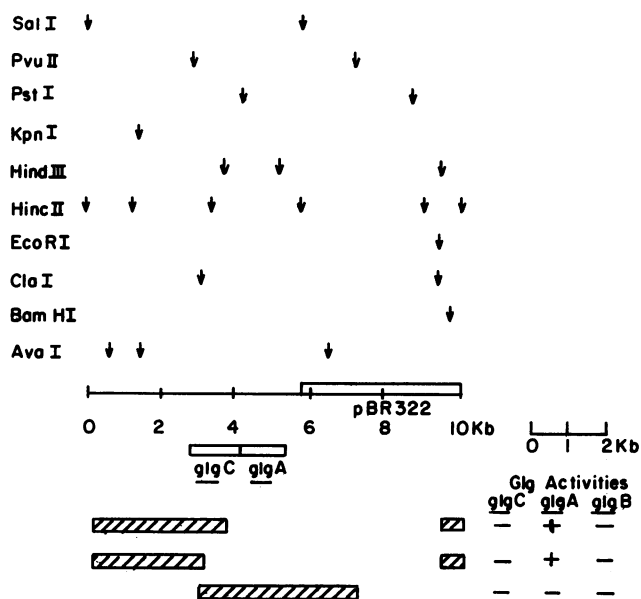


FIG. 3. Restriction map of pPL301. A physical map of pPL301 constructed with several restriction enzymes is shown with the location of *glgC* and *glgA* genes. (Hatched) Portion of the plasmid deleted, with the result of enzyme assays shown to the right.

TABLE 2. Enzyme activity and I_2 staining of bacteria

Bacterial strain	Sp act ($\mu\text{mol}/\text{min}$ per mg)		I_2 staining
	ADPglucose pyrophosphorylase	Glycogen synthase	
<i>E. coli</i>			
K-12 RR1	0.088	0.23	Brown
K-12 RR1(pPL301)	0.58	3.18	Dark brown
K-12 G6MD3	- ^a	-	Yellow
K-12 G6MD3 (pPL301)	0.38	1.59	Greenish yellow
<i>S. typhimurium</i>			
LT2	0.006	0.035	Brown
JP102	0.03	0.83	Dark brown
JP102(pPL301)	0.26	4.26	Black

^a -, Specific activity less than 0.003 $\mu\text{mol}/\text{min}$ per mg of protein.

K-12 *glgC* gene and hence suggest that the physical location of *glgC* gene is just in front of the *glgA* gene.

Immunological characterization. ADPglucose pyrophosphorylase from *S. typhimurium* JP102 and *E. coli* K-12 G6MD3(pPL301) were characterized with respect to their immunological properties. When antiserum against *S. typhimurium* JP102 ADPglucose pyrophosphorylase was reacted against partially purified ADPglucose pyrophosphorylase from *S. typhimurium* JP102 and *E. coli* G6MD3(pPL301) in Ouchterlony double-immunodiffusion gels (26), only one precipitin band was visible. No spurs were seen in the gels with the antibody tested against both proteins, and no precipitin bands were observed against the preimmune serum; this indicates that the two enzymes are antigenically identical.

ADPglucose pyrophosphorylase from plasmid pPL301 and *S. typhimurium* JP102 were also compared immunologically by specific inhibition of ADPglucose pyrophosphorylase activity with antibodies raised against the enzyme. The results are represented as microliters of serum per unit of enzyme in Fig. 4. Preimmune serum was used as a control and had no effect on either enzyme preparation. ADPglucose pyrophosphorylase purified from either *E. coli* K-12 G6MD3 containing pPL301 or *S. typhimurium* JP102 showed the same pattern of inhibition; the amount of antibody required

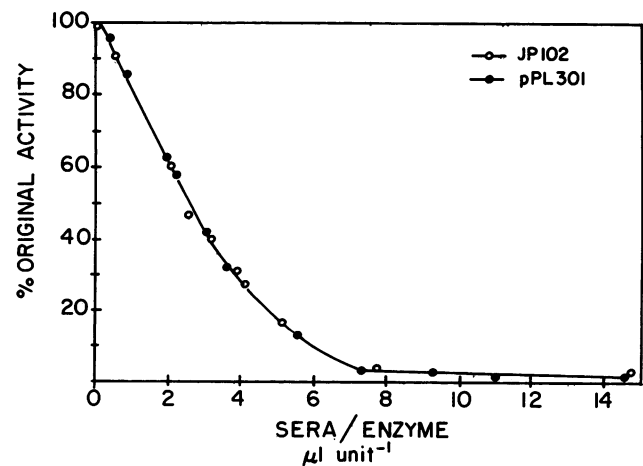


FIG. 4. Inhibition of activity of ADPglucose pyrophosphorylase from *S. typhimurium* JP102 and *E. coli* K-12 G6MD3(pPL301) with antibodies raised against ADPglucose pyrophosphorylase from *S. typhimurium* JP102.

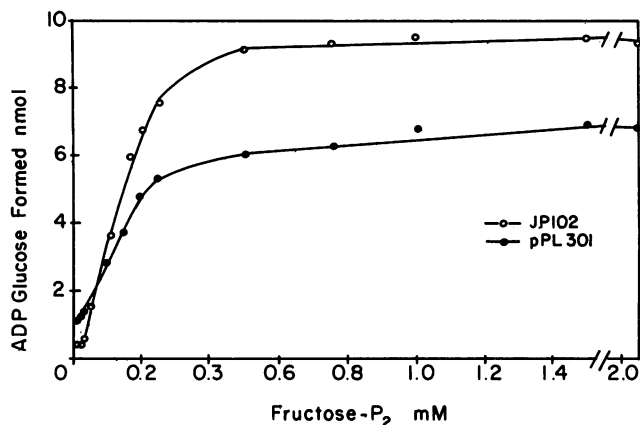


FIG. 5. Activation of ADPglucose pyrophosphorylase by fructose 1,6-bisphosphate. The reaction mixtures contained, in 0.2 ml, 0.1 μ mol of [14 C]glucose 1-phosphate (1.02×10^6 cpm/ μ mol), 20 μ mol of Tris hydrochloride (pH 8.0), 0.3 μ mol of ATP, 1 μ mol of $MgCl_2$, 100 μ g of bovine plasma albumin, 0.2 μ g of yeast inorganic pyrophosphatase, activator as indicated, and purified enzyme from either JP102 or G6MD3(pPL301).

to inhibit 50% of the activity was 2.5 μ l/U of enzyme for both the clone enzyme and the wild-type enzyme. Therefore, the ADPglucose pyrophosphorylase purified from *S. typhimurium* JP102 and *E. coli* K-12 G6MD3(pPL301) were immunologically identical.

Kinetic characterization of enzymes from pPL301 transformants. ADPglucose pyrophosphorylase from *S. typhimurium* JP102 and *E. coli* K-12 G6MD3(pPL301) were partially purified as described in Materials and Methods and characterized with respect to activation of activity by fructose 1,6-bisphosphate and inhibition by AMP. The cloned enzyme from pPL301 behaved similarly to the parent type enzyme from *S. typhimurium* JP102; they showed the same pattern of sigmoidal kinetics with $A_{0.5}$ values of 200 and 173 μ M for the cloned enzyme and the wild-type enzyme, respectively (Fig. 5). A Hill plot slope analysis (6, 19) of the activation curve yielded slope values of 1.8 and 1.9, respectively, for the cloned enzyme and the wild-type enzyme. Figure 6 shows the effect of AMP in inhibiting ADPglucose pyrophosphorylase activity of the cloned and wild-type enzymes. The $I_{0.5}$ values for the cloned enzyme and the wild-type enzyme were 70 and 65 μ M, respectively.

DISCUSSION

Cloning and expression of the *S. typhimurium glg* genes. The *E. coli* K-12 G6MD3 mutant has a deletion from the *malA* gene to the *asd* gene (32), and the *glg* genes are flanked by *asd* and *glpD* (1). Several attempts were made to clone the *S. typhimurium glg* genes by shotgun cloning of *S. typhimurium* LT2 genomic DNA into unique cloning sites of plasmid pBR322 (5) and pBR329 (7) and subsequent selection of *asd*⁺ or *glpD*⁺ transformants in *E. coli* K-12 G6MD3. However, none of these attempts was successful.

Previous studies in our laboratory has shown that there are vast similarities in the N-terminus amino acid sequence of ADP glucose pyrophosphorylase between *E. coli* and *S. typhimurium*; of the first 27 amino acids in the N terminus, 25 are identical, hence suggesting the possibility of great homology in their nucleotide sequence. The *glg* genes of *E.*

coli have been cloned in our laboratory (25); this has allowed us to use part of the *E. coli glg* gene as a DNA hybridization probe to hybridize against a genomic library of *S. typhimurium*. DNA from a plaque showing intense hybridization signals was isolated as described by Davis et al. (8) and subcloned into plasmid vector pBR322. The recombinant plasmid, pPL301, contains a 5.8-kilobase-pair insert in the *SalI* site of pBR322 and was shown to contain the *glgC* and *glgA* genes of *S. typhimurium* LT2.

Transformants of *E. coli* K-12 G6MD3 and *E. coli* K-12 RR1 harboring pPL301 were stained with iodine for the presence of glycogen. *E. coli* K-12 G6MD3(pL301) transformants stained greenish yellow and *E. coli* K-12 RR1 (pL301) transformants stained dark brown, whereas the controls *E. coli* K-12 G6MD3 stained yellow and *E. coli* K-12 RR1 stained light brown. A mutant of *E. coli* designated as 6281 which lacks branching enzyme stained greenish yellow with iodine and hence suggested that our clones may not contain *glgB*. Subsequent enzyme assays of *E. coli* K-12 G6MD3(pPL301) showed ADPglucose pyrophosphorylase and glycogen synthase activities but not branching enzyme activity (unpublished results). Moreover, in *E. coli* K-12 RR1(pPL301), there are elevated levels of ADPglucose pyrophosphorylase and glycogen synthase activities but not branching enzyme activity (unpublished results) when compared with *E. coli* K-12 RR1 itself (Table 2). Thus, the *S. typhimurium glgC* and *glgA* genes are cloned in the plasmid pPL301 and are expressed in *E. coli* and in *S. typhimurium*.

The physical mapping of *S. typhimurium glgC* and *glgA* genes on pPL301 showed that the two genes are physically linked and thus confirmed the genetic mapping results reported by Steiner and Preiss (33). The physical size of *glgC* gene is 1.3 kilobase pairs, which has the coding capacity for a protein of about 50 kilodaltons (18). This is in accordance with the result reported by Lehmann and Preiss (17). Glycogen synthase from *E. coli* has been purified to homogeneity, and the subunit molecular weight is 52,000 as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (9). The physical size of the *glgA* structural gene is about 1.3 kilobase pairs and has the coding capacity for a protein of 50,000 daltons. This suggests that the subunit molecular

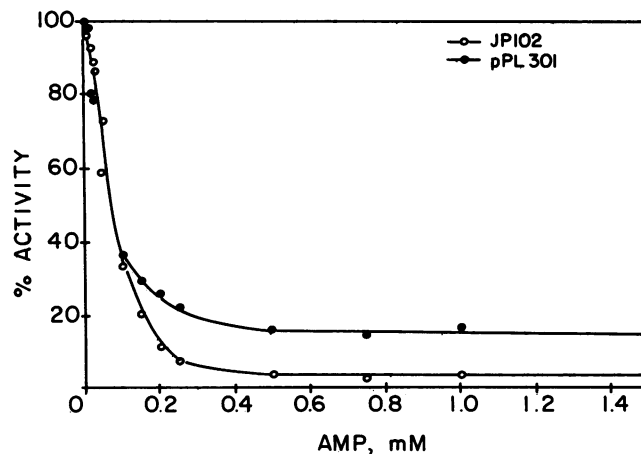


FIG. 6. Inhibition of ADPglucose pyrophosphorylase by AMP. The reaction mixtures as described in the legend to Fig. 5, except that the concentration of fructose 1,6-bisphosphate was 1 mM. The enzymes used were from *S. typhimurium* JP102 and from G6MD3 transformed with pPL301.

weights of ADPglucose pyrophosphorylase and glycogen synthase of *S. typhimurium* and *E. coli* are very similar.

ADPglucose pyrophosphorylase from *S. typhimurium* JP102 has been shown to behave kinetically and immunologically the same as the enzyme from *S. typhimurium* LT2 (17, 31, 33). We have partially purified ADPglucose pyrophosphorylase from *S. typhimurium* JP102, a derepressed mutant of ADPglucose pyrophosphorylase and compared it with the same enzyme from *E. coli* K-12 G6MD3(pPL301). Our results showed that the enzymes were immunologically and kinetically very similar.

The cloning of the *S. typhimurium* *glgA* and *glgC* genes onto pBR322, a high-copy-number plasmid, has elevated manifold the production of glycogen synthase and ADPglucose pyrophosphorylase, respectively. It will enable us to purify these enzymes in large quantity, to study in greater detail the protein chemistry of these enzymes from *S. typhimurium* and compare it with that of enzymes from *E. coli* and other organisms. Hence it should provide more insight into the nature of the amino acids involved in the allosteric and catalytic activities of the enzyme. Further work could also be directed to the studying of genetic regulation of glycogen biosynthesis both in vivo and in vitro at the molecular level with the isolated genes.

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