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Cloning and Expression of the *Escherichia coli glgC* Gene from a Mutant Containing an ADPglucose Pyrophosphorylase with Altered Allosteric Properties

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A mutant strain of *Escherichia coli* K-12, designated 618, accumulates glycogen at a faster rate than wild-type strain 356. The mutation affects the ADPglucose pyrophosphorylase regulatory properties (N. Creuzat-Sigal, M. Latil-Damotte, J. Cattaneo, and J. Puig, p. 647–680, *in* R. Piras and H. G. Pontis, ed., *Biochemistry of the Glycocide Linkage*, 1972). The enzyme is less dependent on the activator, fructose 1,6 bis-phosphate for activity and is less sensitive to inhibition by the inhibitor, 5′-AMP. The structural gene, *glgC*, for this allosteric mutant enzyme was cloned into the bacterial plasmid pBR322 by inserting the chromosomal DNA at the *Pst*I site. The glycogen biosynthetic genes were selected by cotransformation of the neighboring *asd* gene into an *E. coli* mutant also defective in branching enzyme (*glgB*) activity. Two recombinant plasmids, pEBL1 and pEBL3, that had *Pst*I chromosomal DNA inserts containing *glgC* and *glgB* were isolated. Branching enzyme and ADPglucose pyrophosphorylase activities were increased 240- and 40-fold, respectively, in the *asd glgB* mutant, *E. coli* K-12 6281. The *E. coli* K-12 618 mutant *glgC* gene product was characterized after transformation of an *E. coli* B ADPglucose pyrophosphorylase mutant with the recombinant plasmid pEBL3. The kinetic properties of the cloned ADPglucose pyrophosphorylase were similar to those of the *E. coli* K-12 618 enzyme. The inserted DNA in pEBL1 was arranged in opposite orientation to that in pEBL3.

The biosynthesis of α-1-4-glucosidic bonds of glycogen in bacteria occurs via synthesis of ADPglucose from ATP and glucose-1-phosphate and the subsequent transfer of glucose from ADPglucose to a glycogen or maltodextrin primer (16, 17). *Escherichia coli* mutants defective either in glycogen synthase (EC 2.4.1.21) or in ADPglucose pyrophosphorylase (EC 2.7.7.27) are not able to accumulate glycogen (6, 8, 16, 17). This strongly indicates that the above enzymes are essential for glycogen synthesis in *E. coli* and that glycogen synthesis in *E. coli* is regulated at the level of ADPglucose synthesis. ADPglucose synthesis has been shown to be activated by fructose-1,6-diphosphate and inhibited by AMP (16, 17, 19). Some mutants that accumulate glycogen at either a faster (6–9) or slower (18) rate than does the parent strain contain ADPglucose pyrophosphorylase affected in their regulatory properties.

Recently, the *E. coli* K-12 glycogen biosynthetic structural genes *glgA* (glycogen synthase), *glgB* (branching enzyme, EC 2.4.1.18), and *glgC* (ADPglucose pyrophosphorylase) have been cloned onto plasmid pBR322 (13), and the nucleotide sequence of *glgC* and the deduced amino acid sequence of its gene product have been determined (1). With chemical modification studies, the relative positions of the substrate binding sites (11a, 15) and the activator (14, 15) and inhibitor (Y. M. Lee, C. E. Larson, and J. Preiss, *in* J. J. L'Italien, ed., *Proceedings of the American Protein Chemists Symposium*, in press) binding sites have been identified.

To obtain further information on the nature of the amino acid substitution caused by the mutation and to gain new knowledge of the structure-function relationships between the regulatory sites, one of the ADPglucose pyrophosphorylase mutant regulatory genes was cloned. This report describes the cloning of a *glgC* gene from the overproducing glycogen mutant *E. coli* K-12 618. This mutant was shown to have an ADPglucose pyrophosphorylase gene affected in its regulatory properties which was designated *glgC16*. (6). The regulatory kinetic properties of this mutant enzyme in the ADPglucose synthesis direction have not been reported and thus are also described herein.

MATERIALS AND METHODS

Bacterial strains and media. The bacterial strains used in this study are listed in Table 1. LB medium contained 1.0% tryptone, 0.5% yeast extract, 1.0% NaCl, and 0.2% glucose. Enriched media contained 1.1% K_2HPO_4, 0.85% KH_2PO_4, 0.6% yeast extract, and 0.3 or 1.0% glucose. Solid medium was prepared by adding agar to a concentration of 1.5%. Drug selection plates contained either tetracycline (10 μg/ml) or ampicillin (20 μg/ml). All cells were grown at 37°C.

Restriction enzymes. All restriction nucleases were obtained from commercial sources. Buffer formulations used to digest the various DNAs in this study were prepared by the recommendation of the manufacturer.

Isolation of chromosomal DNA. *E. coli* K-12 618 DNA was isolated as previously described (13).

Cloning of the *E. coli* K-12 618 *glg* genes on pBR322. The chromosomal DNA of *E. coli* K-12 618 was exhaustively digested with *Pst*I and was ligated to *Pst*I-digested pBR322

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TABLE 1. *E. coli* strains

Strain	Genotype	Reference
K-12, G6MD3	Hfr *his thi* Str^s Δ(*malA-asd*)	20
K-12 6281	F$^-$ *glgB asd his thi*	11
K-12 RR1	F$^-$ *pro leu thi lacY* Str^r *hsdR hsdM* endonuclease I$^-$	2
B AC70R1-504	F$^-$ *glgQ glgC glpD*	5
K-12 356	*thr-1 leu-6 argB purE thi lacY1 malA xyl-7, ara-13, mtl-2, gal-6 fhuA2* Str	11

as previously described for the cloning of the *glg* genes of *E. coli* K-12 3000 (13). The ligated pBR322 containing the DNA fragments from *E. coli* K-12 618 was used to transform *E. coli* K-12 6281, a mutant deficient in aspartate semialdehyde dehydrogenase and branching enzyme and thus deficient in glycogen. Cells were then selected by growth on agar with enriched media containing tetracycline (20 μg/ml). Strain 6281 requires diaminopimelic acid for growth even in enriched media, and because of its absence, the *asd* gene, which is very near the *glg* genes (6), was selected. Two transformants were isolated which carried the *asd* gene and displayed the phenotype Tet^r Amp^s. The transformed cells were grown on petri plates with enriched medium containing 1% glucose and were stained with I_2 crystals for glycogen (8). Both were I_2 positive, suggesting that the transformed cells also contained *glgB*.

The plasmids designated as pEBL1 and pEBL3 were isolated and transformed into *E. coli* K-12 G6MD3, *E. coli* K-12 RR1, and *E. coli* B AC70R1-504. Transformation was done as previously described (13).

Gel electrophoresis. Restriction endonuclease digests were diluted with a 1/4 volume of XC dye (8 M urea, 2.5 mM disodium EDTA) and 0.05% each of bromophenol blue and xylene cyanol FF. DNA fragments were resolved by electrophoresis in 1% agarose gels or 7.5% polyacrylamide gels in 90 mM Tris–90 mM boric acid–2.5 mM disodium EDTA (3). The gels were run at 150 V for 2 to 3 h, stained with 5 μg ethidium bromide, and visualized by fluorescence under shortwave UV light.

Enzyme assays. ADPglucose pyrophosphorylase was measured in the pyrophosphorolysis and synthesis directions as previously described (19). Glycogen synthase (10) and branching enzyme (4) were assayed as previously described.

Protein determination. Protein was determined by the method of Lowry et al. (12).

Partial purification of *E. coli* K-12 618 ADPglucose pyrophosphorylase. All procedures except where noted were at 4°C. *E. coli* K-12 618 cell paste (8.5 g), obtained from growth on enriched medium with 0.3% glucose, was suspended in 34 ml of 0.05 M glycylglycine buffer (pH 7.0) containing 5 mM dithioerythreitol (DTE) and 1 mM EDTA and exposed to sonic oscillation to disrupt the cells. A 1 M potassium phosphate solution (pH 7.0) was then added to the suspension to a final concentration of 0.03 M P_i. The suspension was heated at 58°C for 5 min and then centrifuged at 30,000 × *g* for 10 min in a Sorvall centrifuge. The precipitate was washed with 5 ml of the above glycylglycine buffer, and the residue, after centrifugation, was combined with the heated supernatant. The combined supernatant fluids were adsorbed on a DEAE-Sepharose column (1.5 by 7 cm; resin bed volume, 12.4 ml) that was equilibrated with 0.05 M potassium phosphate buffer (pH 7.0) containing 5 mM DTE, 1 mM EDTA, and 10% glycerol. The column was then washed with 24 ml of 0.1 M potassium phosphate buffer (pH 7.5) containing 5 mM DTE and 10% glycerol. A linear gradient containing 60 ml of the above 0.1 M potassium phosphate buffer in the mixing chamber and 60 ml of 0.2 M potassium phosphate buffer (pH 7.0) containing 0.5 M KCl, 5 mM DTE, 1 mM EDTA, and 10% glycerol was used to elute the enzyme from the column. The fraction volume collected was 4 ml. The enzyme activity was eluted from the column after 40 ml. The fractions containing the enzyme activity were pooled, and solid $(NH_4)_2SO_4$ was added to a 0.7 saturation. The resultant precipitate, which contained the activity, was dissolved and dialyzed against 50 mM Tris buffer (pH 7.5) containing 1 mM EDTA, 0.5 mM DTE, and 10% glycerol.

The above purification procedure was also used to partially purify the ADPglucose pyrophosphorylases from *E. coli* K-12 356 (10 g) and *E. coli* B AC70R1-504(pEBL3) (6.8g), adjusting the conditions (volumes, resin bed volume, etc.) as needed. Heat treatment of the *E. coli* K-12 356 extract was done at 65°C since wild-type enzyme was more heat stable than the allosteric mutant enzyme.

RESULTS

Cloning of the *glgB* and *glgC* genes. Originally a number of attempts were made to clone the DNA of the mutant *E. coli* K-12 618 that contained the *glgC* gene by transforming *E. coli* K-12 strain G6MD3, which contains a deletion between *malA* and *asd* and is therefore lacking the *glg* genes. Since the *asd* gene is a neighboring gene, the *glg* genes could be selected for by selecting for the *asd* gene and then assaying for the *glgC* gene product. However, all attempts to obtain *asd*$^+$ transformants were not successful. This was in contrast to previous results (13), in which all of the *glg* and *asd*$^+$ genes were successfully cloned into pBR322 by use of the G6MD3 strain for transformation. Strain 6281 was thus used, since it is glycogen negative because of the lack of branching enzyme and it contains a defective *asd* gene (11, 20). Two

TABLE 2. Expression of the glycogen biosynthetic enzymes in strains containing pEBL1 and pEBL3[a]

Strain	Sp act (μmol/min per mg of protein) of:		
	ADPgglucose pyrophosphorylase	Glycogen synthase	Branching enzyme
E. coli			
6281	0.02	0.0063	0.02
6281(pEBL1)	0.41	0.018	2.1
6281(pEBL3)	0.81	0.031	4.8
G6MD3	<0.003	<0.003	<0.003
G6MD3(pEBL1)	0.28	<0.003	0.12
G6MD3(pEBL3)	0.36	<0.003	0.13
E. coli B			
AC70R1-504	<0.003	0.072	0.31
AC70R1-504(pEBL1)	2.8	0.072	9.0
AC70R1-504(pEBL3)	2.5	0.087	8.76
E. coli K-12			
618	0.033	0.14	0.44
356	0.04	0.11	0.34

[a] All strains were grown in 1 liter of enriched medium with 0.3% glucose. For growth of *E. coli* K-12 G6MD3, diaminopimelic acid (50 μg/ml) was added to the medium, and for growth of transformed cells, the medium contained tetracycline (20 μg/ml). Cells were then collected by centrifugation, suspended in 10 ml of 0.1 M glycylglycine buffer (pH 7.0) containing 5 mM DTE, and then disrupted by sonication. After centrifugation at 10,000 × *g* for 10 min, the supernatant fluids were assayed for the glycogen biosynthetic enzymes as descirbed in Materials and Methods.

transformants were isolated which carried the *asd* gene and displayed the phenotype Tet^r Amp^s, suggesting that pBR322 contains an insert in the *Pst*I site, rendering inactive the gene encoding Amp^r. The transformants pEBL1 and pEBL3 stained positive for glycogen, suggesting that they also contain the *glgB* gene.

There was a 20- to 40-fold increase in the level of the ADPglucose pyrophosphorylase and a 100- to 240-fold increase in branching enzyme activities when mutant 6281 was transformed with pEBL1 or pEBL3 (Table 2). Glycogen synthase activity was not affected.

E. coli K-12 G6MD3 is a deletion mutant having no *glg* genes. Transformation with either pEBL plasmid provided the organism with both ADPglucose pyrophosphorylase and branching enzyme activities but not with glycogen synthase activity.

The *E. coli* B strain, AC70R1-504, a mutant with a defective ADPglucose pyrophosphorylase gene but derepressed about five- to sevenfold with respect to the other glycogen biosynthetic activities, was also transformed with pEBL1 and pEBL3. Increases of about two- to sevenfold in branching enzyme and ADPglucose pyrophosphorylase activities were observed in this strain when compared with transformed 6281 cells. These transformed cells contained a 60- and 70-fold higher ADPglucose pyrophosphorylase activity and a 20- to 30-fold higher branching enzyme activity than either *E. coli* K-12 strain 618 or its parent strain 356.

In all of the transformations (Table 2), no effect was observed on glycogen synthase activity, indicating that the pEBL plasmids did not contain a functional *glgA* gene.

The data (Table 2) suggest that *glgC*, the structural gene for ADPglucose pyrophosphorylase, was also present in the pEBL insert, in addition to *glgB*, the branching enzyme structural gene.

Restriction enzyme mapping. It was of interest whether plasmids pEBL1 and pEBL3 were similar to pOP12, the plasmid that contains all of the glycogen structural genes (13). Thus, they were analyzed with the two restriction enzymes *Pst*I and *Eco*RI and compared with plasmid pOP12.

In the *Pst*I digests, pEBL1 (Fig. 1) showed a pattern very similar to that observed with pOP12 (Fig. 2). However, the 0.9- and 1.6-kilobase (kb) fragments were missing. The *Pst*I digest for construction of pEBL1 was an exhaustive digestion, whereas the digestion to obtain pOP12 was not. Thus, the absence of the above fragments in pEBL1 is understandable. The digest pattern obtained with pEBL3 (Fig. 3) indicates that the *Pst*I fragment was arranged in an opposite orientation with respect to pBR322 than were the fragments in pOP12 and pEBL1. The fragment arrangements in the plasmids are given in Fig. 3. These are based on the patterns obtained after gel electrophoresis of the *Pst*I and *Eco*RI digests (1) and on the restriction endonuclease cleavage map obtained with pOP12 (13).

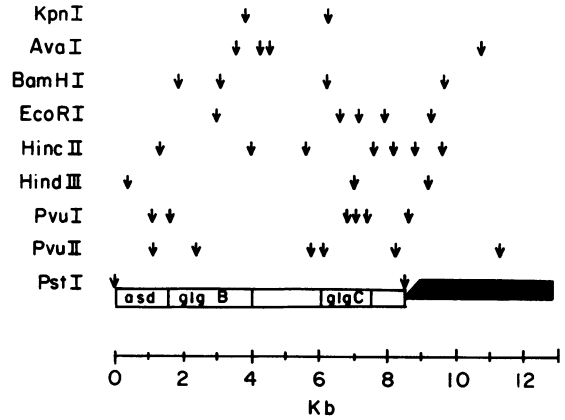

FIG. 1. Restriction endonuclease cleavage map of pEBL1. The plasmid was digested with nine restriction endonucleases which have one or two sites in pBR322 (21, 22). The map was constructed based on the size of the fragments obtained from the digest and comparison with the pOP12 restriction cleavage map previously deduced (13) (Fig. 2). The heavily shaded region is pBR322 DNA.

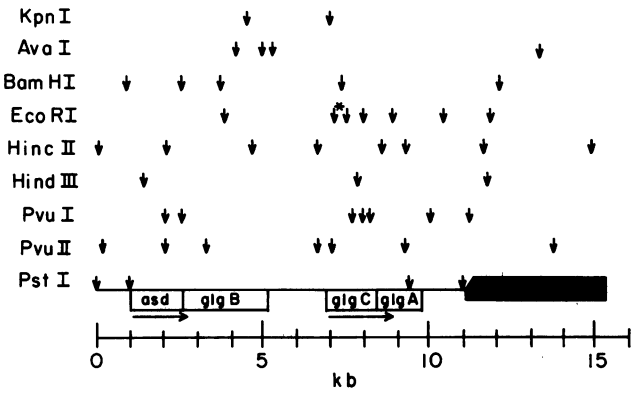

FIG. 2. Restriction endonuclease cleavage map of pOP12. The plasmid was digested with the same nine restriction nucleases used against pEBL1. The map was constructed by comparing single and double digests of the plasmid (13) and by using the known cleavage map of pBR322 (21, 22). The heavily shaded region is pBR322 DNA.

The restriction endonuclease cleavage map of pEBL1 with nine restriction nucleases is shown in Fig. 1, and it can be compared with the cleavage map obtained with pOP12 (Fig. 2). The maps are very similar, indicating that the *Pst*I digestion cloned the same chromosomal DNA area in both pEBL1 and pOP12. Note that the missing 0.9-kb fragment was upstream from the *asd* gene and that the missing 1.6-kb fragment was part of the *glgA* gene.

Kinetic characteristics of the ADPglucose pyrophosphorylase isolated from extracts of *E. coli* B AC70R1-504 transformed with pEBL3. To determine whether the cloned gene expressed an ADPglucose pyrophosphorylase similar to that isolated from allosteric mutant 618, the enzyme was

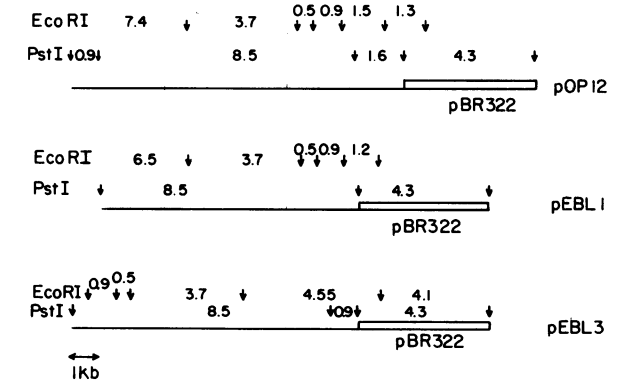

FIG. 3. *Pst*I and *Eco*RI nuclease fragments and arrangement based on a previous restriction endonuclease cleavage map obtained with pOP12 (13). pOP12 is about 15.3 kb, and pEBL1 is 12.8 kb, missing fragments of 0.9 and 1.6 kb. pEBL3 is 13.7 kb, missing only the 1.6-kb-*Pst*I fragment which is part of the glycogen synthase structural gene *glgA* (13).

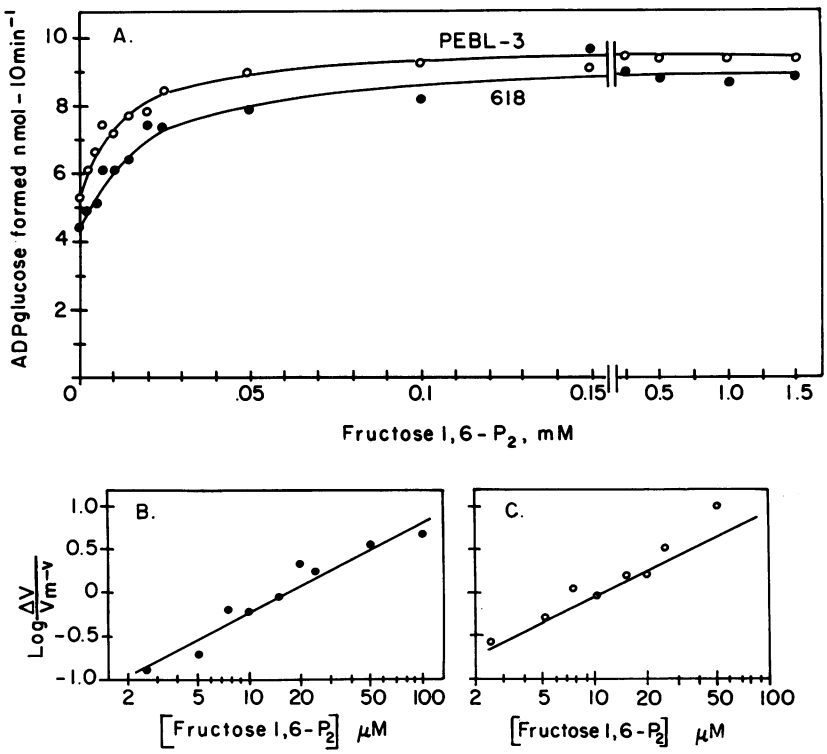

FIG. 4. (A) Activation of pEBL3 and strain 618 ADPglucose pyrophosphorylases by fructose-1,6-diphosphate. The reaction mixture (0.2 ml) contained 0.1 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (pH 7.0), 3.75 mM $MgCl_2$, 1.5 mM ATP, 5 mg of bovine serum albumin per ml, 0.5 mM [^{14}C]glucose-1-phosphate, and various concentrations of fructose-1,6-diphosphate. (B and C) Hill plots of the data.

partially purified from extracts of either transformed *E. coli* B AC70R1-504 or *E. coli* K-12 G6MD3. The enzymes from strain AC70R1-504 transformed with pEBL3, strain G6MD3 transformed with pEBL3, strain 618, and strain 356 were purified to specific activities of 15.5, 2.5, 0.47, and 0.29 μmol of ATP formed per min per mg of protein, respectively. All kinetic experiments described were with the enzyme isolated from the AC70R1-504 cells transformed with pEBL3. However, some of the kinetic experiments were repeated with the enzyme purified from *E. coli* G6MD3 transformed with pEBL3. In all cases, similar results were obtained.

Activation by fructose-1,6-diphosphate. The fructose-1,6-diphosphate activation curve for the enzyme from pEBL3-transformed strain AC70R1-504 (designated here as pEBL3 enzyme) and from strain 618 is shown in Fig. 4. The activation curve for the enzyme from parent strain 356 is shown in Fig. 5. About a 45-fold activation occurred with strain 356 ADPglucose pyrophosphorylase. The activation curve is sigmoidal in shape with a Hill slope of 1.7, and 50% maximal stimulation occurred at 62 μM. In contrast, the mutant strain 618 enzyme was quite active in the absence of the activator, and only a 1.8- to 2-fold activation occurred with fructose-1,6-diphosphate (Fig. 4). The curve is hyperbolic with a Hill slope of 1.0, and 50% maximal stimulation occurred at 15 ± 3.1 μM (the mean of six experiments). The pEBL3 enzyme gave the same kinetic constants for fructose-1,6-diphosphate activation, with 50% maximal stimulation occurring at 14.7 ± 3.3 μM (the mean of seven experiments).

Specificity of activation. The specificity of activation of the enzyme purified from extracts of *E. coli* 356, 618, and K-12 G6MD3 transformed with plasmid pEBL3 is shown in Table 3. The enzyme from the parent strain was relatively inactive in the absence of an activator, and the major activators gave

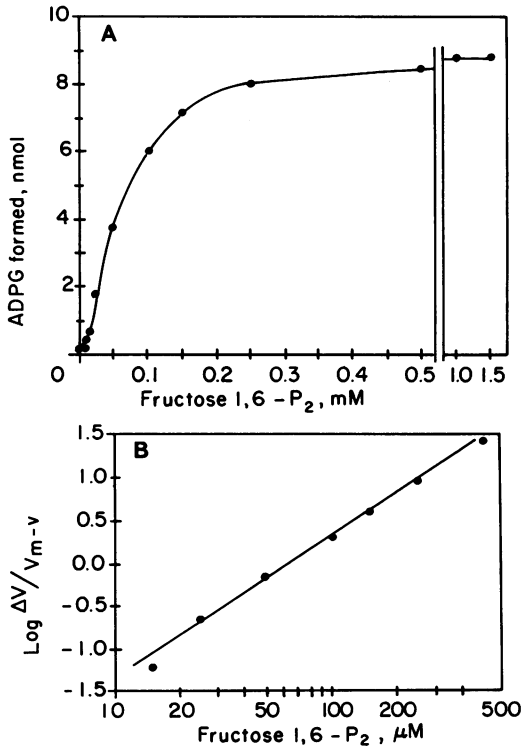

FIG. 5. (A) Activation of strain 356 ADPglucose pyrophosphorylase by fructose-1,6-diphosphate. The reaction mixture was the same as that described in the legend to Fig. 4, except the $MgCl_2$ was at 5 mM. (B) Hill plot of the data.

a 25- to 28-fold stimulation. In contrast, the strain 618 enzyme was only activated about two- to threefold by fructose-1,6-diphosphate, and thus was less dependent on activators for activity. The partially purified enzyme from *E. coli* K-12 G6MD3(pEBL3) was activated to the same extent as that from the *E. coli* K-12 618 mutant enzyme. The activator specificity seen for the pEBL-transformed cell enzyme was about the same as observed for the 618 enzyme, indicating again that the pEBL plasmid contained the strutural gene for the strain 618 enzyme. No activation of the 356, 618, or pEBL3 ADPglucose pyrophosphorylases was observed for the following metabolites at 1 mM: glucose 6-phosphate, fructose-6-phosphate, glyceraldehyde 3-phosphate, pyruvate, 6-phosphate-gluconate, acetyl coenzyme A, citrate, aspartate, oxaloacetate, 3,5'-cyclic AMP, and 3,5'-cyclic GMP.

AMP inhibition. The AMP inhibition of strain 356 ADPglucose pyrophosphorylase is shown in Fig. 6. A 50% inhibition occurred at 66 μM AMP in the presence of 1.0 mM fructose-1,6-diphosphate. The Hill plot shows a curve that is sigmoidal with a Hill slope of 1.9. In the absence of fructose-1,6-diphosphate, the enzyme became resistant to inhibition. Only 42% inhibition was seen at 2.5 mM AMP.

In contrast, strain 618 enzyme was more resistant to inhibition at 1 mM fructose-1,6-diphosphate (Fig. 7). About 0.86 mM AMP was required for 50% inhibition. The enzyme was sensitive, however, to AMP inhibition in the absence of fructose-1,6-diphosphate, with 50% inhibition occurring at 17 μM. The enzyme from the pEBL3-transformed cells was very similar to strain 618 enzyme with respect to AMP inhibition. A 50% inhibition occurred in the presence of 1 mM fructose-1,6-diphosphate at 0.91 mM and 17 μM in the absence of the activator, fructose-1,6-diphosphate. The pEBL3 and the 618 AMP inhibition curves are sigmoidal in the presence of 1.0 mM fructose-1,6-diphosphate, with Hill slopes of 1.9, and are hyperbolic in the absence of the activator.

Kinetic constants of the substrates. The kinetic constants for the substrates of the three ADPglucose pyrophosphorylases under study is shown in Table 4. In the presence of fructose-1,6-diphosphate, the wild-type strain 356 enzyme had an apparently higher affinity for the substrates in the presence of 1 mM fructose-1,6-diphosphate, since the concentration required for 50% maximal activity ($S_{0.5}$) for glucose-1-phosphate, ATP, and $MgCl_2$ was reduced about four- to fivefold. In the absence of the activator, the α-glucose-1-phosphate saturation curve exhibited negative cooperativity, with a Hill slope of 0.6. The ATP and $MgCl_2$ curves are sigmoidal, and the presence of activator had no effect on the shape of the curves. The V_{max} was stimulated four- to fivefold when the optimal substrate concentrations were used with or without the activator.

The strain 618 and pEBL3 enzymes had the same kinetic constants for the substrates. In contrast to the strain 356 enzyme, fructose-1,6-diphosphate had no effect on the $S_{0.5}$ for glucose-1-phosphate and $MgCl_2$ and decreased the $S_{0.5}$ for ATP about two- to threefold. The ATP curves also appeared to be less sigmoidal than those seen for strain 356 enzyme. The V_{max} was only stimulated about twofold by the presence of activator.

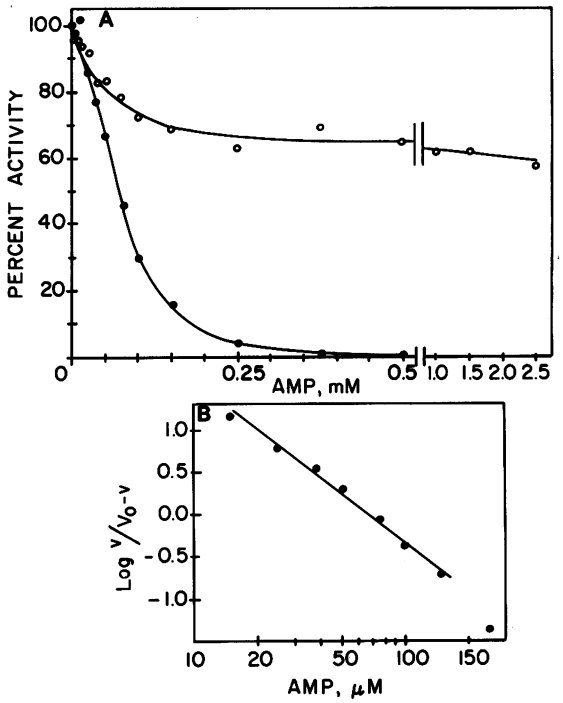

FIG. 6. (A) Inhibition of the strain 356 ADPglucose pyrophosphorylase by AMP. The reaction mixtures were the same as that described in the legend to Fig. 4, except that the fructose-1,6-diphosphate concentration was 1.0 mM (●) or 0 (○). When the fructose-1,6-diphosphate was omitted, the concentrations of ATP, glucose-1-phosphate, and $MgCl_2$ were increased to 5, 1, and 25 mM, respectively. Also, four times as much enzyme was added. The amount of ADPglucose formed in the absence of inhibition was 9.6 (●) and 6.5 (○) nmol. (B) Hill plot of the data where fructose-1,6-diphosphate is present.

TABLE 3. Activator specificity of the ADPglucose pyrophosphorylases isolated from *E. coli* K-12 356 and 618 and *E. coli* B AC70R1-504(pEBL3)

Strain activator[a]	ADPglucose synthesized[b]:		
	Strain 356	Strain 618	pEBL3
None	0.04	0.43	0.49
Hexanediol-1,6-diphosphate	1.10	1.05	1.03
Fructose-1,6-diphosphate	1.0	1.0	1.0
NADPH	0.79	0.86	0.76
Pyridoxal-phosphate	1.10	1.05	0.90
3-Phosphate-glycerate	0.15	0.76	0.74
Dihydroxyacetone-phosphate		0.67	0.76
2-Phosphate-glycerate	0.64	0.90	0.86
Phosphate-enol-pyruvate	0.62	0.90	0.80
PRPP	0.83	0.87	
2-Keto-3-deoxy-6-phosphate-gluconate	0.33	0.81	
Ribulose-1,5-diphosphate	0.81	1.05	0.8
1,3-Glycerol-diphosphate	0.54	0.90	0.66

[a] The reaction mixtures for measurement of ADPglucose synthesis were the same as those described in the legends to Fig. 4 and 5, except that 1 mM activator was tested. PRPP, 1-pyrophosphoryl-ribose-5-phosphate.

[b] The data are expressed as relative activities with the value obtained with fructose-1,6-diphosphate as the activator arbitrarily set at 1.0. The amounts of ADPglucose synthesized with fructose-1,6-diphosphate as the activator were 8.6 nmol, 9.3 nmol and 9.6 nmol for the *E. coli* K-12 356, *E. coli* K-12 618, and *E. coli* B AC70R1-504(pEBL3) enzymes, respectively.

DISCUSSION

The *glgC* (ADPglucose pyrophosphorylase) and *glgB* (branching enzyme) genes of the *E. coli* K-12 allosteric mutant 618 were cloned. Two plasmids of opposite orientation in pBR322, which contain the mutant *glgC* gene, as well

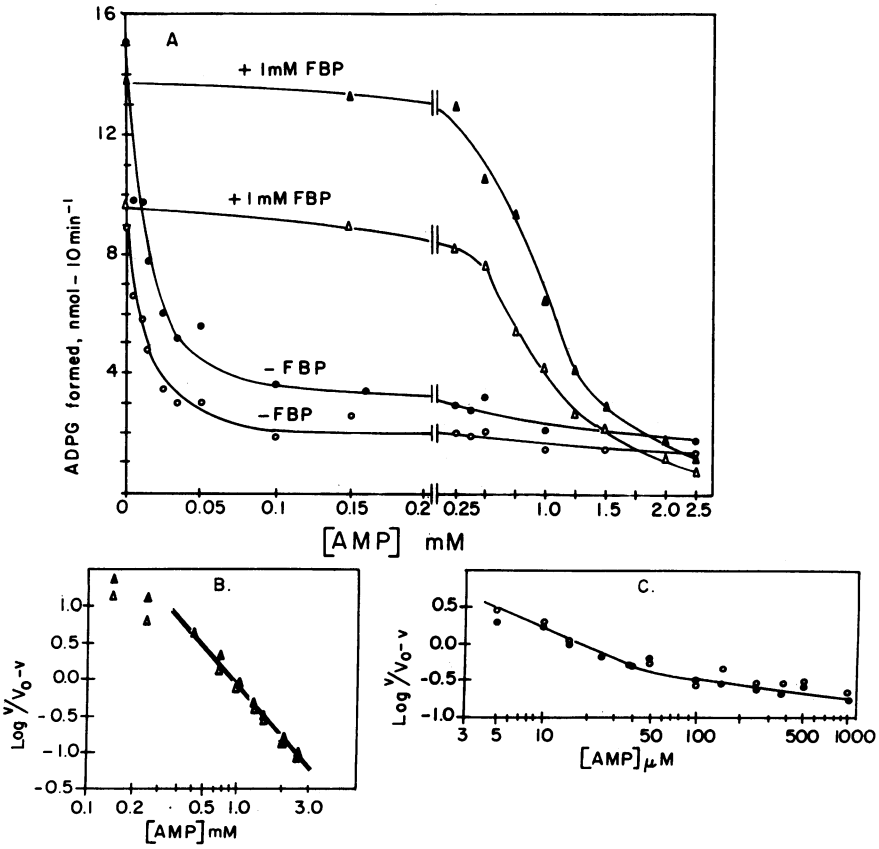

FIG. 7. Inhibition of 618 and pEBL3 ADPglucose pyrophosphorylases by AMP. The reaction mixtures were the same as that described in the legend to Fig. 4, except that the fructose-1,6-diphosphate concentration was 1.0 mM or 0. Symbols: ▲ and ●, experiments with the pEBL3 enzyme; △ and ○, experiments with the *E. coli* K-12 618 enzyme.

as the *glgB* gene, were isolated. One of the plasmids, pEBL1, was studied in some detail with respect to restriction nuclease mapping, and the expression product of the *glgC* gene of the other plasmid, pEBL3, was studied and shown to have the same kinetic properties in the synthesis direction as observed for mutant strain 618 ADPglucose pyrophosphorylase.

The sequence of the *E. coli* K-12 ADPglucose pyrophosphorylase gene has been determined (1). Thus, DNA sequencing of the *glgC* gene of the mutant strain will enable us to determine the amino acid substitution that has occurred because of the mutation and the nature of the change that alters the allosteric properties of the ADPglucose. Determination of the site of the amino acid change will enable us by in vitro and site-directed mutagenesis (23, 24) to obtain more information on the structure and function of the allosteric site(s). It would be of interest to determine the nature of the amino acid replacement, in view of previous studies indicating the location of the allosteric activator binding site (14, 15), the substrate binding site (11a, 14, 15), and the inhibitor binding site (Lee et al., in press).

TABLE 4. Kinetic constants for the 618, 356, and pEBL3 ADPglucose pyrophosphorylase substrates and $MgCl_2$

Substrate	Fructose-1,6-diphosphate (1 mM)	mM $S_{0.5}$ (Hill slope constant)		
		Strain 618	Strain 356	pEBL 3
α-Glucose-1-phosphate	+	0.024 (1.0)	0.02 (1.0)	0.02 (1.0)
	−	0.021 (1.0)	0.11 (0.6)	0.02 (1.0)
ATP	+	0.11 (1.7)	0.28 (2.2)	0.12 (1.5)
	−	0.34 (1.7)	1.35 (2.2)	0.35 (1.7)
$MgCl_2$	+	1.6 (4.7)	2.6 (4.6)	1.7 (3.3)
	−	1.7 (4.4)	14.0 (4.4)	1.7 (4.1)

[a] $S_{0.5}$ is the concentration at which 50% maximal velocity is observed. The reaction mixtures used to obtain the various kinetic constants were the same as those described in the legends to Fig. 4 and 5. In the absence of the activator, the concentrations of ATP, glucose 1-phosphate, and $MgCl_2$ in the reaction mixture were raised as indicated in the legend to Fig. 6 when the various kinetic parameters of the strain 356 enzyme were measured.

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