AN ADP-GLUCOSE PYROPHOSPHORYLASE WITH LOWER APPARENT AFFINITIES FOR SUBSTRATE AND EFFECTOR MOLECULES IN AN ESCHERICHIA COLI B MUTANT DEFICIENT IN GLYCOGEN SYNTHESIS[1]

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SUMMARY

SG-14, a mutant strain of E. coli B that accumulates glycogen at one-half the rate observed for the parent strain, contains an altered ADP-glucose pyrophosphorylase with lower apparent affinities than that observed for the parent enzyme for its substrates, ATP and Mg^{2+} and for its effectors, fructose diphosphate, pyridoxal-P (PLP) and 5'adenylate. TPNH activates the native enzyme but not the mutant enzyme. In view of the reported physiological concentrations of ATP and Mg^{2+} and of the kinetic effects observed for FDP, PLP and AMP for the mutant enzyme, it is suggested that FDP is the important physiological activator for ADP-glucose pyrophosphorylase.

ADP-glucose pyrophosphorylase from E. coli has been found to be activated by fructose diP, TPNH and pyridoxal-P and inhibited by 5'adenylate (1-4). Since ADP-glucose is the sole glycosyl precursor for glycogen synthesis in E. coli (5,6), these phenomena have been postulated to be important in the physiological regulation of glycogen synthesis in Escherichia coli (1,3,7). Supportive evidence for this comes from published studies on glycogen mutants that overproduce glycogen and that contain ADP-glucose pyrophosphorylases that have higher apparent affinity for the activator molecules FDP, TPNH and PLP and lower apparent affinity for the inhibitor, 5'adenylate (7-10). This report describes preliminary studies of a modified ADP-glucose pyrophosphorylase present in an E. coli mutant that synthesizes glycogen at a lower rate than the parent strain. These studies suggest that fructose diphosphate is the most important activator of ADP-glucose and glycogen synthesis in E. coli when the organism is grown aerobically with glucose as a carbon source.

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METHODS

Measurement of ADP-glucose synthesis and pyrophosphorolysis has been previously reported (1). Reaction mixtures for assaying the E. coli B enzyme in the synthesis direction contained in a volume of 0.20 ml; ATP, 0.3 µmole, glucose C^{14}-1-P (specific activity, 5 to 10 x 10^5 cpm per µmole), 0.1 µmole; $MgCl_2$, 1.0 µmole; Hepes (N-2-hydroxyethylpiperazine-N'-2-ethane), pH 7.0, 20 µmoles; bovine plasma albumin, 100 µg; crystalline inorganic pyrophosphatase, 9 µg; activator at amounts indicated in figures and tables and purified E. coli B ADP-glucose pyrophosphorylase (1). When the SG-14 mutant ADP-glucose pyrophosphorylase activity was assayed, the amounts of ATP and $MgCl_2$ were increased to 1.0 and 3.0 µmoles, respectively.

Reaction mixtures measuring pyrophosphorolysis of ADP-glucose catalyzed by the E. coli B enzyme contained 10 µmoles of Tris-HCl, pH 8.5, 100 µg of bovine plasma albumin, 2.0 µmoles of $MgCl_2$, 0.2 µmole of ADP-glucose, 0.5 µmole of pyrophosphate-P^{32} (2.7 x 10^6 cpm/µmole), 2.5 µmoles of NaF, activator as indicated in Table 1 and enzyme in a volume of 0.25 ml. When the SG-14 enzyme was measured the amounts of ADP-glucose and $MgCl_2$ were increased to 0.9 µmole and 2.5 µmoles, respectively.

The mutant E. coli B strain, SG-14, was isolated as previously described (8) and both the parent (E. coli B) and mutant ADP-glucose pyrophosphorylases were purified essentially by the same procedure described previously for the E. coli B enzyme (1). The purified mutant SG-14 enzyme had a specific activity at 37° of 13.5 µmoles of ATP formed in 10 min per mg of protein in the presence of 2 mM FDP and 3.6 mM ADP-glucose, concentrations required for optimal pyrophosphorolytic activity for the mutant enzyme. The purified E. coli B enzyme had a specific activity of 14.5 µmoles of ATP formed per mg of protein in the presence of 1.2 mM FDP and 0.8 mM ADP-glucose.

RESULTS AND DISCUSSION

As reported earlier (8) mutant SG-14 accumulates only 55% and 75% the amount glycogen that is observed in the parent in minimal and enriched media,

TABLE 1

Comparison of E. coli B and SG-14 with Respect to Glycogen Accumulation and Properties of Their ADP-Glucose Pyrophosphorylases

Organism	Media	Glycogen Accumulation Rate		ADP-Glucose Pyrophosphorylase	
		μmoles/g-hour	mg/g	Pyrophosphorolysis	Synthesis
				μmoles/g-hour	
E. coli B	Enriched	23	20	270	292
	Minimal	32	20	306	326
SG-14	Enriched	12	14	248	56
	Minimal	21	11	291	71

The enriched and minimal media with glucose as a carbon source and the assays for glycogen accumulation are described in reference (8). Accumulation of glycogen is expressed as milligrams of anhydrous glucose per gram (wet weight) of cells and the value given is the maximal amount accumulated in the stationary phase of aerobic growth. The rate of glycogen accumulation was obtained by determining glycogen content of cells during the growth period and is expressed as the change of μmoles of anhydrous glucose per gram of cells (wet weight) per hour. ADP-glucose synthesis and pyrophosphorolysis was measured in crude extracts of cells prepared as described previously (8). The reaction mixtures for ADP-glucose synthesis and pyrophosphorylase are described in the methods. The concentration of the activator, FDP, used for pyrophosphorolysis of ADP-glucose was 1.2 mM and 2.0 mM for the E. coli B and SG-14 enzymes respectively. The concentration of FDP used for determing the ADP-glucose synthesis activity of the E. coli B and SG-14 enzymes was 1.5 and 4.0 mM, respectively These concentrations of activator were those giving optimal rates of pyrophosphorolysis and synthesis for both enzymes.

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respectively. The cells were grown aerobically with glucose as the carbon source (8). The rate of glycogen accumlation in these media by SG-14 is 50 to 65% of that observed for E. coli B (Table 1). SG-14 has normal amounts of ADP-glucose:α-glucan transferase and Table 1 shows that the amount of ADP-glucose pyrophosphorolytic activity in the parent and mutant strain extracts is about equal when assayed at optimal conditions. However, measurement of the ADP-glucose synthesizing activity indicates that the mutant extract contains only 23% of the activity observed in the parent strain extract. The enzymatic activities were assayed under conditions giving maximal activity of ADP-glucose synthesis for both extracts. Preliminary studies of the properties of the SG-14 enzyme also reveal that much higher concentrations of ATP and $MgCl_2$ and activator are needed to obtain optimal synthesis of ADP-glucose. The concentration of ATP and Mg^{2+}

required for 50% of maximal activity ($S_{0.5}$) is 4- to 5-fold higher for the SG-14 enzyme as compared to the E. coli B ADP-glucose pyrophosphorylase. Whereas the $S_{0.5}$ for ATP and Mg^{2+} are 0.39 mM and 2.38 mM respectively, for the E. coli B

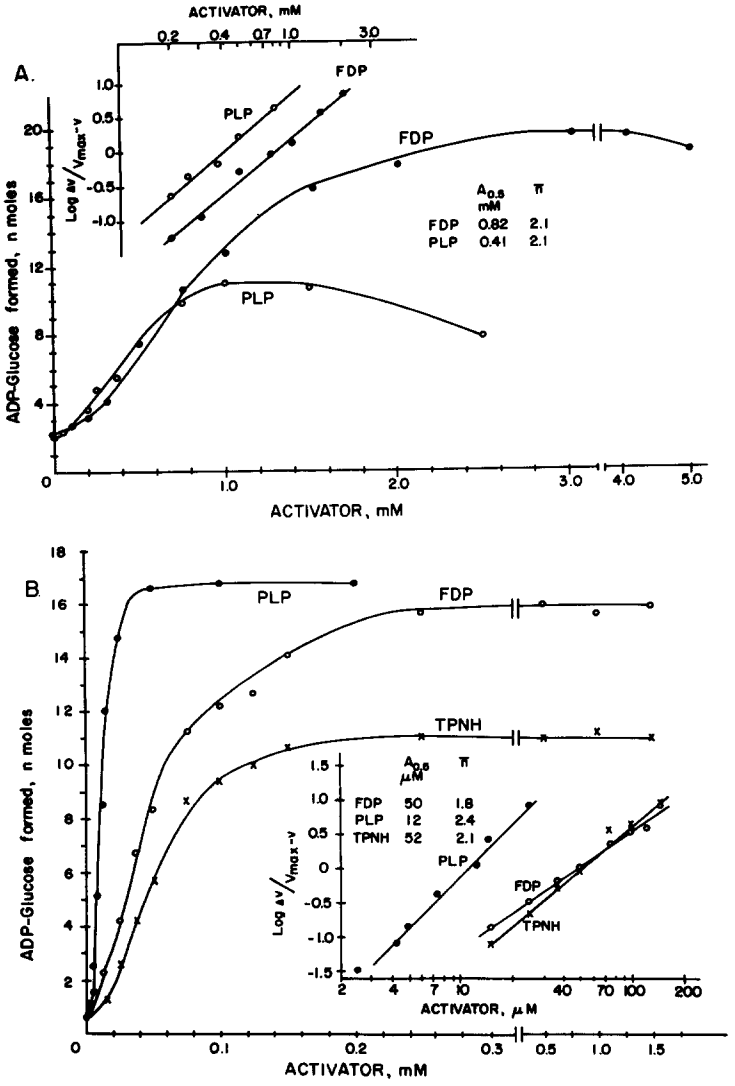

Fig. 1. Activation of ADP-glucose synthesis catalyzed by SG-14 (Part A) and E. coli B (Part B) ADP-glucose pyrophosphorylases. The conditions of the assay are described in the text and the activator concentrations are listed in the figure. The insets represent Hill plots of the data. Δv is the increase in velocity due to addition of activator, i.e., the velocity obtained upon addition of a certain amount of activator to the reaction mixture minus the velocity seen in reaction mixtures with no activator. V_{max} was obtained from reciprocal plots of v vs. activator concentration. $A_{0.5}$ is the designation for the concentration of activator giving 50% of the maximal activation.

enzyme in the presence of 1.5 mM FDP, the $S_{0.5}$ values for ATP and Mg^{2+} are 1.6 mM and 10.2 mM respectively for the SG-14 ADP-glucose pyrophosphorylase in the presence of saturating FDP (4.0 mM). Reports in the literature indicate that the ATP level in growing E. coli is about 2 to 6 mM (11,12) and the Mg^{2+} level is about 18 to 30 mM (13-16). Therefore, the SG-14 ADP-glucose pyrophosphorylase would essentially be saturated with respect to these substrates. The apparent affinity ($S_{0.5}$) for glucose-1-P for the E. coli B and SG-14 enzymes are about the same (0.036 mM).

Fig. one shows that 16-times more FDP is needed for 50% maximal stimulation ($A_{0.5}$) of the SG-14 enzyme than what is required for half-maximal stimulation of the E. coli B ADP-glucose pyrophosphorylase. Likewise the $A_{0.5}$ for PLP for the SG-14 ADP-glucose pyrophosphorylase is about 25-times higher than the $A_{0.5}$ observed for the E. coli B enzyme. Both PLP and FDP stimulate ADP-glucose synthesis catalyzed by the E. coli B enzyme to the same extent. However, the stimulation of the SG-14 enzyme seen with PLP is only one-half that elicited by FDP. Most notable is that TPNH cannot stimulate the SG-14 enzyme.

Since the apparent affinity of the SG-14 enzyme for its activators is considerably lower than what is observed for the E. coli B enzyme one might expect much lower rates of glycogen accumulation in SG-14 than what is actually observed. However, as shown in Fig. 2 the SG-14 ADP-glucose pyrophosphorylase is much less sensitive to inhibition by 5'adenylate than the parent enzyme, particularly in the range of 5'AMP concentration of 0 to 0.2 mM. At a saturating concentration of FDP (4.0 mM), only 8% inhibition of the SG-14 enzyme is observed at 0.2 mM 5'AMP, while 0.2 mM adenylate gives 78% inhibition at a saturating concentration of FDP for the E. coli B enzyme (1.5 mM). At a lower concentration of FDP which gives 60% of maximal velocity (1.1 mM), 0.2 mM adenylate inhibits the SG-14 enzyme 24% while at a concentration of FDP giving about 55% maximal velocity for the E. coli B enzyme (0.05 mM), 0.2 mM adenylate gives greater than 95% inhibition. Thus although the modification of the SG-14 enzyme causes it to have a lower apparent affinity for its activators, it also renders the enzyme insensitive

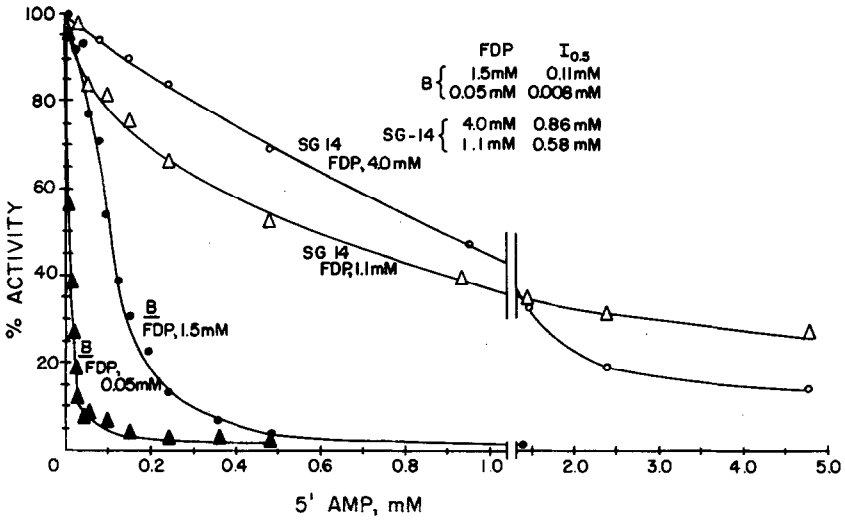

Fig. 2. Inhibition of the SG-14 and E. coli B ADP-glucose pyrophosphorylases. ADP-glucose synthesis was measured and 100% activity represents the synthesis rate in the absence of inhibitor under each set of conditions. $I_{0.5}$ is the designation for the concentration of inhibitor giving 50% inhibition under the conditions of the experiment.

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to 5'adenylate inhibition. These two effects appear to compensate for each other and allows SG-14 to accumulate glycogen at about one-half the rate of the parent strain.

Since TPNH is not an activator of the SG-14 ADP-glucose pyrophosphorylase and the concentrations of PLP needed for activation are considerably higher than the physiological concentrations of PLP known to exist in E. coli B (6 to 8 μM; W. B. Dempsey, private communication), it appears that the most important physiological activator for ADP-glucose synthesis is FDP; at least under the conditions where the organism is grown aerobically with glucose as the carbon source. Under certain conditions with glucose as the carbon source, the concentrations of FDP have been found in E. coli to be about 0.6 to 1.2 mM (17). These concentrations would allow SG-14 to have an adequate rate of synthesis of glycogen. However, it is conceivable that under other conditions of growth that TPNH and pyridoxal-P would play more significant roles in the regulation of glycogen synthesis. Further work on this aspect remains to be done.

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