THE ACTIVATION OF ESCHERICHIA COLI ADP-GLUCOSE PYROPHOSPHORYLASE*

Jack Preiss, Laura Shen, and Marian Partridge

Department of Biochemistry and Biophysics
University of California
Davis, California

Received November 12, 1964

The biosynthesis of glycogen in bacteria occurs via the transfer of the glucose moiety to an α-1,4-glucan primer (reaction 1) (1).

(1) ADP-glucose + α-1,4-glucan → α-1,4-glucosyl-glucan + ADP

This reaction has been found to occur in cell-free extracts of Agrobacterium tumefaciens, Aerobacter aerogenes, Arthrobacter sp. NRRL B1973, Escherichia coli, Micrococcus lysodeiktikus, and Rhodospirillum rubrum (1-3). Thus glycogen synthesis in bacteria probably occurs via a transglucosylation from ADP-glucose to a glycogen primer. Synthesis of ADP-glucose was shown to be catalyzed by ADP-glucose pyrophosphorylase in plants (4) and in cell-free extracts of Arthrobacter (5) by reaction 2.

(2) ATP + α-glucose-1-P $\rightleftharpoons$ ADP-glucose + PP_i

The purpose of this communication is to present evidence for the activation of the E. coli ADP-glucose pyrophosphorylase by fructose-1,6-diphosphate (FDP), glyceraldehyde-3-P and phosphoenolpyruvate (PEP). They were found to stimulate the pyrophosphorolysis and the synthesis of ADP-glucose about 20 to 50 fold. Arthrobacter ADP-glucose pyrophosphorylase was shown previously to be activated by either fructose-6-P, ribose-5-P or pyruvate (5).

E. coli B was grown in synthetic, nitrogen limiting, medium at 37° with glucose as the carbon source (6). After harvest, the cells

* This work was supported by United States Public Health Service grant AI 05520.

were suspended in four volumes of 0.05 M Tris-HCl buffer, pH 7.9, containing 0.005 M reduced glutathione. They then were disrupted in a French Press at 20,000 psi and afterwards centrifuged for 15 minutes at 30,000 x g. The resultant supernatant fluid was used as the source of the pyrophosphorylase. ADP-glucose pyrophosphorylase was purified 44-fold by protamine sulfate precipitation, ammonium sulfate fractionation, and heat treatment (65°). The partially purified enzyme preparation did not contain detectable amounts of phosphoglucomutase, aldolase, or UDP-glucose pyrophosphorylase. These enzymes, however, were found in the crude supernatant fluid.

Enzymic activity was determined by following the synthesis of radioactive ATP from ADP-glucose and PP_i^{32}. The nucleoside triphosphate was separated from the labelled PP_i by the use of Norit (5,7,8). The reaction mixture, which was incubated for 10 minutes at 37°, contained 0.2 μmole ADP-glucose, 1.0 μmole PP_i^{32} (specific activity, 1.0 to 25 x 10^5 cpm/μmole), 30 μmoles Tris-HCl, pH 7.5, 3 μmoles $MgCl_2$, 5 μmoles KF and enzyme in a final volume of 0.5 ml. The reaction was terminated by the addition of 3 ml of cold 5% trichloracetic acid.

The following assay method was employed to assay sugar nucleotide formation. This reaction mixture, which was incubated for 10 minutes at 37°, contained 0.1 μmole glucose-C^{14}-1-P (specific activity, 6.6 x 10^5 cpm/μmole), 0.2 μmole ATP, 2 μmoles $MgCl_2$, 10 μmoles Tris-Cl buffer, pH 8.3, 0.9 μg crystalline yeast inorganic pyrophosphatase, and purified enzyme in a total volume of 0.20 ml. The reaction was terminated by heating the mixture in a boiling water bath for 30 seconds. Then 0.1 mg of *E. coli* alkaline phosphatase (Worthington) was added and the reaction mixture was incubated for 40 minutes. Alkaline phosphatase treatment converts the glucose-C^{14}-1-P to glucose-C^{14} plus P_i without degrading the sugar nucleotide that is formed. A portion of the alkaline phosphatase-treated mixture (0.1 ml) was then spotted on a 1 cm x 8 cm DEAE-cellulose paper strip. The strips were swirled for

one minute in 600 ml of deionized water to wash off the labelled glucose. The washing process was repeated 3 times with fresh portions of water. The strips were then dried with a hair drier and placed in scintillation vials containing a solution of 6 g of 2,5-diphenyloxazole and 500 mg 1,4-bis-2-(4-methyl-5-phenyloxalzolyl) benzene in a liter of reagent-grade toluene. The omission of ATP or the substitution of heat denatured enzyme for enzyme resulted in the presence of only 200 cpm on the paper strip. Addition of fructose-1,6-di-P to these reaction mixture "controls" still resulted in only 200 cpm on the paper strips. A similar assay using DEAE-cellulose paper for measuring kinase activity was described by Sherman (9).

Figure 1 shows the effect of enzyme concentration on the rate of synthesis of ADP-glucose in the presence and in the absence of fructose-1,6-di-P. The stimulation produced by fructose-1,6-di-P was

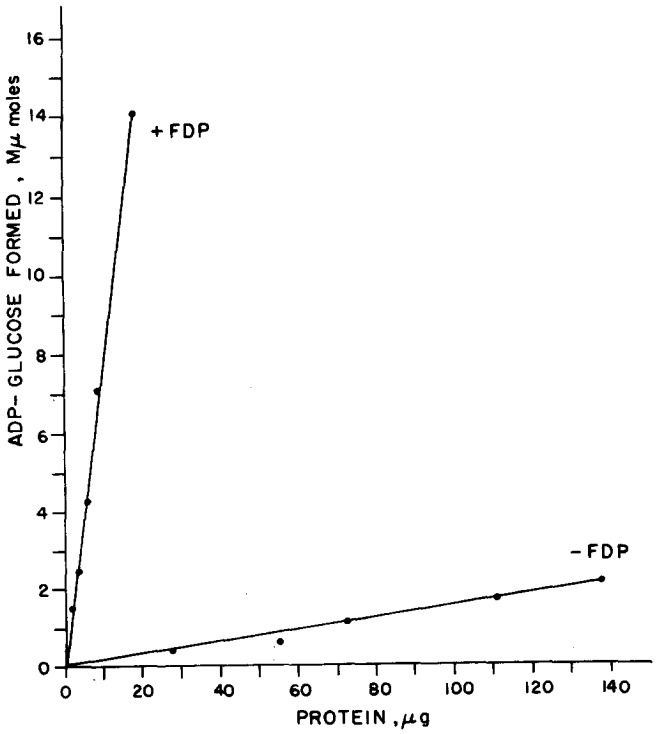

Figure 1. Effect of enzyme and activator concentrations on synthesis of ADP-glucose.

about 50-fold. Twenty-fold stimulations were observed with phosphoenolpyruvate or glyceraldehyde-3-P. Portions of these reaction mixtures were chromatographed in 3 solvent systems. [Solvent A: isobutyric acid, M NH_3, 0.1 M EDTA, pH 7.2 (10:6.0:0.16); Solvent B: 600 g ammonium sulfate in 1 liter of 0.1 M sodium phosphate, pH 6.8, and 20 ml of n-propanol; Solvent C: ethanol, M ammonium acetate, pH 3.8 (5:2)]. The radioactive product formed by the activation co-chromatographed with standard ADP-glucose in each solvent system.

Figure 2 shows the effect of activator concentration on the rate of ADP-glucose pyrophosphorolysis. The curves are sigmoidal in nature.

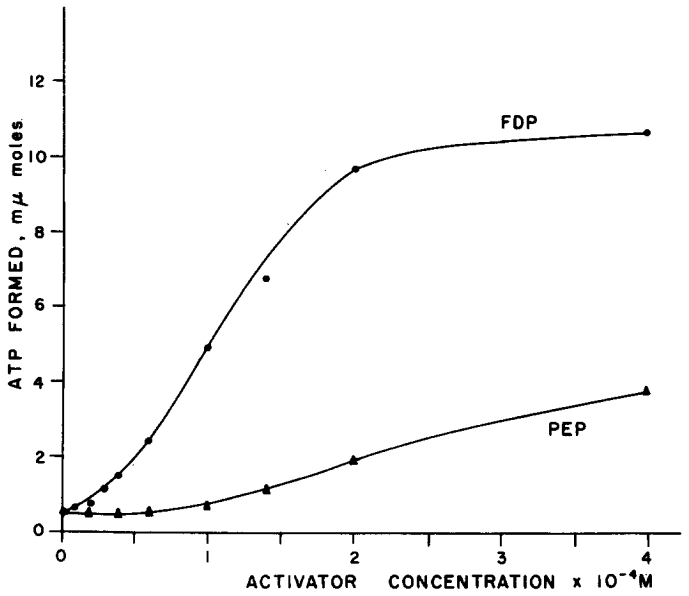

Figure 2. Effect of fructose-1,6-di-P (FDP) and phosphoenolpyruvate (PEP) concentrations on the synthesis of ADP-glucose.

This may indicate that the affinity of the enzyme for activator is increased with increasing concentrations of activator. All of the radioactive product formed during the pyrophosphorolysis of ADP-glucose co-chromatographed with ATP in solvents A,B and C. The determination of the fructose-1,6-di-P concentration in the reaction mixtures with

Table I. Activation of the synthesis of ADP-glucose. The concentration of the activators was 2×10^{-3} M. The enzyme concentration was 75μg.

Activator	ADP-glucose formed
	mμmoles
None	2.1
Acetyl CoA	3.9
Acetyl-P	5.1
P-gluconate	3.1
Fructose-1,6-Di-P	57.
Glucose-6-P	4.6
Fructose-6-P	1.9
Glyceraldehyde-3-P	50.
Phosphoenolpyruvate	46.
Ribose-5-P	2.3

aldolase, triose phosphate dehydrogenase, DPN, and arsenate showed that its concentration did not change during the formation of ADP-glucose from ATP and glucose-C^{14}-1-P. Table I shows the specificity of the activation. Of the compounds tested fructose-1,6-di-P, phosphoenolpyruvate, and 3-phosphoglyceraldehyde appeared to be the best activators. Fructose-6-P and pyruvate, compounds which activated the Arthrobacter ADP-glucose pyrophosphorylase (5), did not activate the E. coli enzyme. Other compounds tested and having no effect were 2,3-diphosphoglycerate, glucose-6-P, glucose, fructose-1-P, $NaHCO_3$, and succinate. The UDP-glucose, TDP-glucose and GDP-mannose pyrophosphorylases which were present in the 30,000 x g supernatant fluid were not activated by fructose-1,6-di-P. However, the possibility remains that these pyrophosphorylases may be activated by other compounds.

A study of the Aerobacter aerogenes ADP-glucose pyrophosphorylase revealed that it is also activated by fructose-1,6-di-P,

phosphoenolpyruvate and glyceraldehyde-3-P[1]. On the other hand, ADP-glucose pyrophosphorylase from Rhodospirillum rubrum or Agrobacterium tumefaciens is activated by fructose-6-P, pyruvate, and ribose-5-P[1]. Thus, at least two types of bacterial ADP-glucose pyrophosphorylases can be distinguished on the basis of their activation by different groups of compounds.

These activations might be related to the control of glycogen synthesis in bacteria. An increase of certain glycolytic intermediates conceivably could activate the synthesis of ADP-glucose. The glucosyl moiety of the ADP-glucose could then be transferred to the glycogen chains. In both Arthrobacter and E. coli extracts the levels of ADP-glucose pyrophosphorylase activity are lower than the ADP-glucose: glycogen transglucosylase activity. Thus, synthesis of ADP-glucose probably is the limiting reaction in bacterial synthesis of glycogen. It is possible then that an increase in synthesis of ADP-glucose would increase glycogen synthesis.

REFERENCES

1. Greenberg, E. and Preiss, J., J. Biol. Chem., in press (1964).
2. Shen, L., Ghosh, H.P., Greenberg, E., and Preiss, J., Biochem. Biophys. Acta., 89, 370 (1964).
3. Preiss, J., Shen, L., and Wood, E., Abstracts of the 6th International Congress of Biochemistry VI, 89, New York, 1964.
4. Espada, J., J. Biol. Chem., 237, 3577 (1962).
5. Shen, L. and Preiss, J., Biochem. and Biophys. Research Commun., in press (1964).
6. Holme, T. and Palmisteirna, Acta Chem. Scand., 10, 1557 (1956).
7. Crane, R.K. and Lipmann, F., J. Biol. Chem., 201, 235 (1953).
8. Preiss, J. and Wood, E., J. Biol. Chem., 239, 3119 (1964).
9. Sherman, J.R., Anal. Biochem., 5, 548 (1963).

[1] E. Greenberg, M. Partridge, and J. Preiss, unpublished results.