THE ACTIVATION AND INHIBITION OF BACTERIAL ADENOSINE-DIPHOSPHOGLUCOSE PYROPHOSPHORYLASE*

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Recent reports have shown the presence of ADP-glucose pyrophosphorylase (reaction 1) (1) and ADP-glucose-glycogen Transglucosylase (reaction 2) (2) in cell-free extracts of Arthrobacter sp. NRRL B1973.

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

2) ADP-glucose + α-1,4-glucan $\longrightarrow$ α-1,4-glycosyl-glucan + ADP

Reaction 2 is quite specific; UDP-glucose, CDP-glucose, GDP-glucose, IDP-glucose and TDP-glucose cannot substitute for ADP-glucose. These two reactions provide a pathway for the biosynthesis of glycogen in Arthrobacter. The occurrence of these two reactions in other bacteria (Agrobacterium tumefaciens, Aerobacter aerogenes, Escherichia coli B., M. lysodeiktikus and Rhodospirillum rubrum) has also been observed[1] and thus reactions 1 and 2 could represent a common pathway of glycogen synthesis in bacteria. Recent results have indicated that the Arthrobacter ADP-glucose pyrophosphorylase is activated by either fructose-6-P (F-6-P), pyruvate, ribose-5-P or glucose-6-P and is inhibited by inorganic phosphate. The purpose of this communication is to present the evidence for the activation and inhibition of ADP-glucose pyrophosphorylase.

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[1] Shen, L., Greenberg, E. and Preiss, J. (Unpublished results)

METHODS

Preparation of ADP-Glucose Pyrophosphorylase--Arthrobacter sp. NRRL B1973 was grown in synthetic medium at 25° with glucose as the carbon source. After being harvested, the cells were suspended in four volumes of 0.05 M Tris-HCl buffer, pH 7.9, containing 0.005 M reduced glutathione (GSH). They then were broken in a French press at 20,000 psi and afterwards centrifuged for 15 minutes at 10,000 x g. The resultant supernatant fluid was centrifuged at 105,000 x g for 1 hour. The supernatant fluid obtained from this centrifugation was used as the source for the pyrophosphorylase. ADP-glucose pyrophosphorylase was purified 100-fold by protamine sulfate precipitation, ammonium sulfate fractionation, heat treatment (65°), and phosphocellulose chromatography. The enzyme preparation did not contain detectable amounts of phosphoglucomutase, UDP-glucose pyrophosphorylase or TDP-glucose pyrophosphorylase.

Assay of Pyrophosphorylase Activity--Enzymatic activity was determined by following the synthesis of ATP-P^{32} from ADP-glucose and PP_i^{32}. 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 8.3, 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% trichloroacetic acid. Unlabeled PP_i, 10 μmoles, was added to dilute the PP_i^{32}; then 0.1 ml of 150 mg/ml suspension of Norit A was added to adsorb the labelled ATP. The Norit suspension was centrifuged and the supernatant fluid was discarded. The charcoal was washed twice with 3 ml of 5% cold TCA and then once with 3 ml of cold distilled water. Following washing, it was suspended in 2 ml of an aqueous solution of 50% ethanol containing 0.1% NH_3. One ml of this suspension was dried in a planchet and counted in a gas-flow counter. One unit of enzymatic activity is defined as that amount which forms 1 μmole of ATP under the conditions of the assay. The 105,000 x g supernatant fluid, which contained 10 mg/ml of

protein, had a specific activity of 0.5 unit/mg of protein. Very little, or negligible incorporation of PP_i^{32} was observed if ADP-glucose was omitted from the reaction mixtures containing either the 105,000 x g supernatant fluid or purified enzyme.

To assay sugar nucleotide formation, the following assay method was employed. The reaction mixture, which was incubated for 10 minutes at 37°, contained 0.1 μmole glucose-C^{14}-1-P (specific activity, 6.1 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} while the formed sugar nucleotide is not degraded. 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. The extraction 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 PPO-dimethyl PoPoP-toluene solvent consisting of 6 g of 2,5-diphenyloxazole and 50 mg 1,4-bis-2-(4-methyl-5-phenyl-oxalzolyl) benezene-free 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 F-6-P or P_i 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 (3).

RESULTS

Figure 1 shows the effect of enzyme concentration on the rate of pyrophosphorolysis in the presence and absence of fructose-6-P or pyruvate.

The stimulation by fructose-6-P is 6-fold and by pyruvate, about 3-fold. All of the radioactive product formed by activation with fructose-6-P or pyruvate co-chromatographed with ATP 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 of 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)].

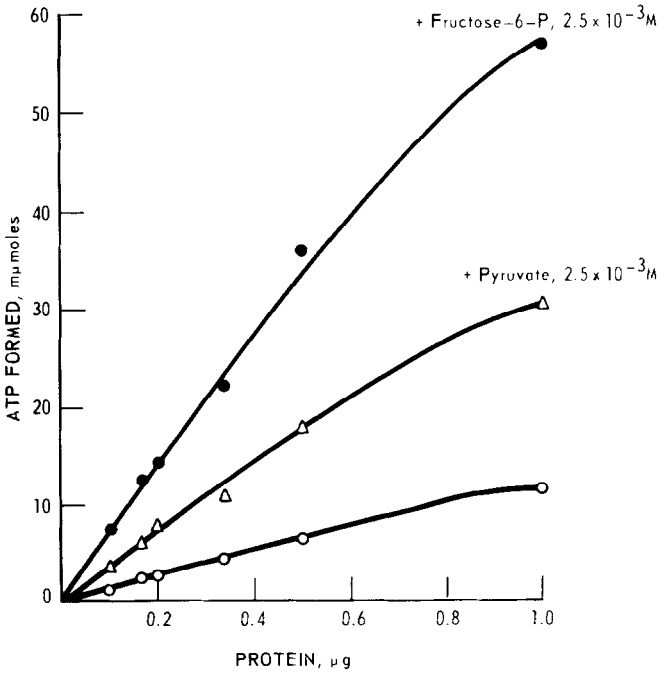

Fig. 1. Effect of enzyme and activator concentrations on pyrophosphorolysis of ADP-glucose.

Figure 2 shows the effect of activator concentration on the rate of ADP-glucose synthesis. F-6-P gave the best activation and its concentration for 50% maximal stimulation was 2.5×10^{-4} M. In order to determine whether the product synthesized during the stimulation was indeed ADP-glucose-C^{14}, portions of the reaction mixtures were chromatographed in solvent systems A, B, and C. In each solvent system the radioactive product co-chromatographed with ADP-glucose-C^{14}. Solvent systems A and C

separate ADP-glucose from glucose-1-P. Substrates which have been tested and do not activate are fructose-1,6 diphosphate, 3 phosphoglyceric acid, phosphoenol pyruvate, fructose-1-P, and glyceraldehyde-3-P.

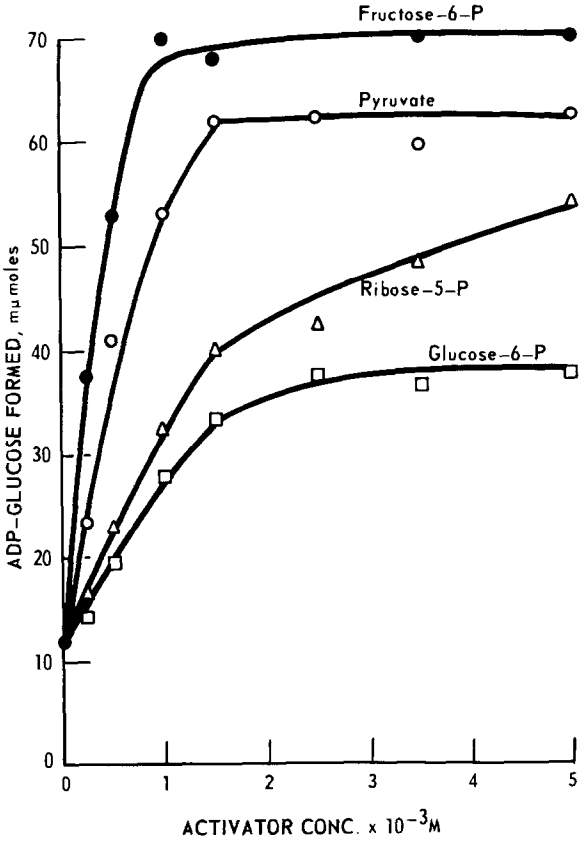

Fig. 2. Effect of activator concentration on ADP-glucose synthesis.

Table I shows that P_i inhibits the synthesis of ADP-glucose. However, the addition of F-6-P overcame this inhibition.

These observations might be related to the physiological control of glycogen synthesis in Arthrobacter. Increase of glycolytic intermediates would tend to increase the synthesis of ADP-glucose and then glycogen. On the other hand accumulation of P_i would inhibit ADP-glucose and therefore decrease glycogen.

Table I

Effect of Phosphate and Fructose-6-Phosphate on ADP-Glucose Pyrophosphorylase

Addition To Reaction Mixture	ADP-Glucose Formed
	mμmoles
None	10.8
P_i, 5×10^{-4} M	8.8
P_i, 1×10^{-3} M	6.1
P_i, 5×10^{-3} M	2.5
F-6-P, 2.5×10^{-3} M	66.
F-6-P, 2.5×10^{-3} M + P_i, 5.0×10^{-4} M	66.
F-6-P, 2.5×10^{-3} M + P_i, 1×10^{-3} M	64.
F-6-P, 2.5×10^{-3} M + P_i, 5×10^{-3} M	49.

The assay conditions are described in the text.

P_i and F-6-P are added where indicated.

Experiments on the mechanism of the activation and its physiological implications are in progress.

REFERENCES

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3). Sherman, J.R., Anal. Biochem. 5, 548 (1963).