TPNH AND PYRIDOXAL-5'-PHOSPHATE: ACTIVATORS OF ADP-GLUCOSE PYROPHOSPHORYLASE OF *ESCHERICHIA COLI* B[1]

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Received June 9, 1969

Summary: TPNH and pyridoxal-5-P have been found to be potent activators of *E. coli* B ADP-glucose pyrophosphorylase. The concentrations required for 50% maximal activation was 0.15 mM TPNH and 8 μM pyridoxal-5-P. These values compare favorably to the value of 0.13 mM found previously for the activator, fructose diP. Kinetic evidence is presented to suggest all three activators bind to the same site(s) on the enzyme. PLP-activated ADP-glucose synthesis is less sensitive to 5'-adenylate inhibition than is TPNH-activated synthesis. The physiological functions of these activations with respect to glycogen storage is discussed.

Previous reports (1-3) had indicated that ADP-glucose synthesis in *Escherichia coli* was under allosteric control. Fructose diphosphate and to lesser extents, other glycolytic intermediates activated ADP-glucose synthesis while 5'-adenylate, ADP and inorganic phosphate were inhibitors of ADP-glucose synthesis. These results suggested that ADP-glucose synthesis was regulated in part by the energy charge of the cell (4-6) and by the availability of an excess carbon source that would cause accumulation of glycolytic intermediates. Since ADP-glucose is the sole glucosyl donor for glycogen synthesis (7,8) control of ADP-glucose would in turn regulate the biosynthesis of the glucose polymer.

This report concerns itself with data indicating that in addition to fructose diP, both TPNH and pyridoxal-5'-P (PLP) are effective activators of ADP-glucose synthesis. The relationship of these activators to the control of glycogen synthesis is discussed.

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[1] Supported by a grant from the United States Public Health Service (AI05520).

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METHODS

Reaction mixtures contained the following in a volume of 0.20 ml: ATP, 0.3 μmole; glucose-C^{14}-1-P (specific activity 1 x 10^6 cpm per μmole), 0.1 μmole; $MgCl_2$, 1.0 μmole; Tris-chloride buffer, pH 8.5, 20 μmoles; bovine plasma albumin, 100 μg; and highly purified *E. coli* B ADP-glucose pyrophosphorylase (3). The concentrations of fructose diphosphate, TPN and PLP are listed in the figures and tables. ADP-glucose synthesis was measured as previously described (1). Pyridoxic acid-5-P was a gift from Dr. E. E. Snell of the University of California, Berkeley.

RESULTS

Fig. 1A shows the activator saturation curves for fructose diphosphate, TPNH and PLP. Concentrations giving 50% of the maximal stimulation ($A_{0.5}$) is 135 μM for FDP, 150 μM for TPNH and about 8 μM for PLP. Pyridoxal-5'-P is thus more effective than either TPNH and FDP. All the activation curves exhibit sigmoid kinetics. The plotting of the data according to the Hill equation (Fig. 1B) give values for $\bar{n}$, the interaction coefficient (or apparent order of reaction), of 1.85 for FDP, 2.2 for TPNH and 3.6 for PLP. The highest stimulation of ADP-glucose synthesis is observed with PLP. The presence of saturating concentrations of FDP in the reaction mixtures gives 90% of the stimulation observed with PLP, while the presence of TPNH gives about 2/3 the maximal stimulation observed with PLP.

Fig. 2 shows the effect of non-saturating concentrations of FDP and TPNH on the PLP saturation curve. Both FDP and TPNH enhance the activation effect of low concentrations of PLP. At higher concentrations of PLP inhibition by TPNH (∿15-20%) was observed. The inhibition by TPNH is most probably due to competition with PLP for the same activator sites since the maximal stimulation observed with TPNH is not as great as that observed with PLP. Inhibition is not observed with FDP and may not be expected as it is almost as effective as PLP as an activator. The presence of FDP and TPNH decrease the sigmoidicity

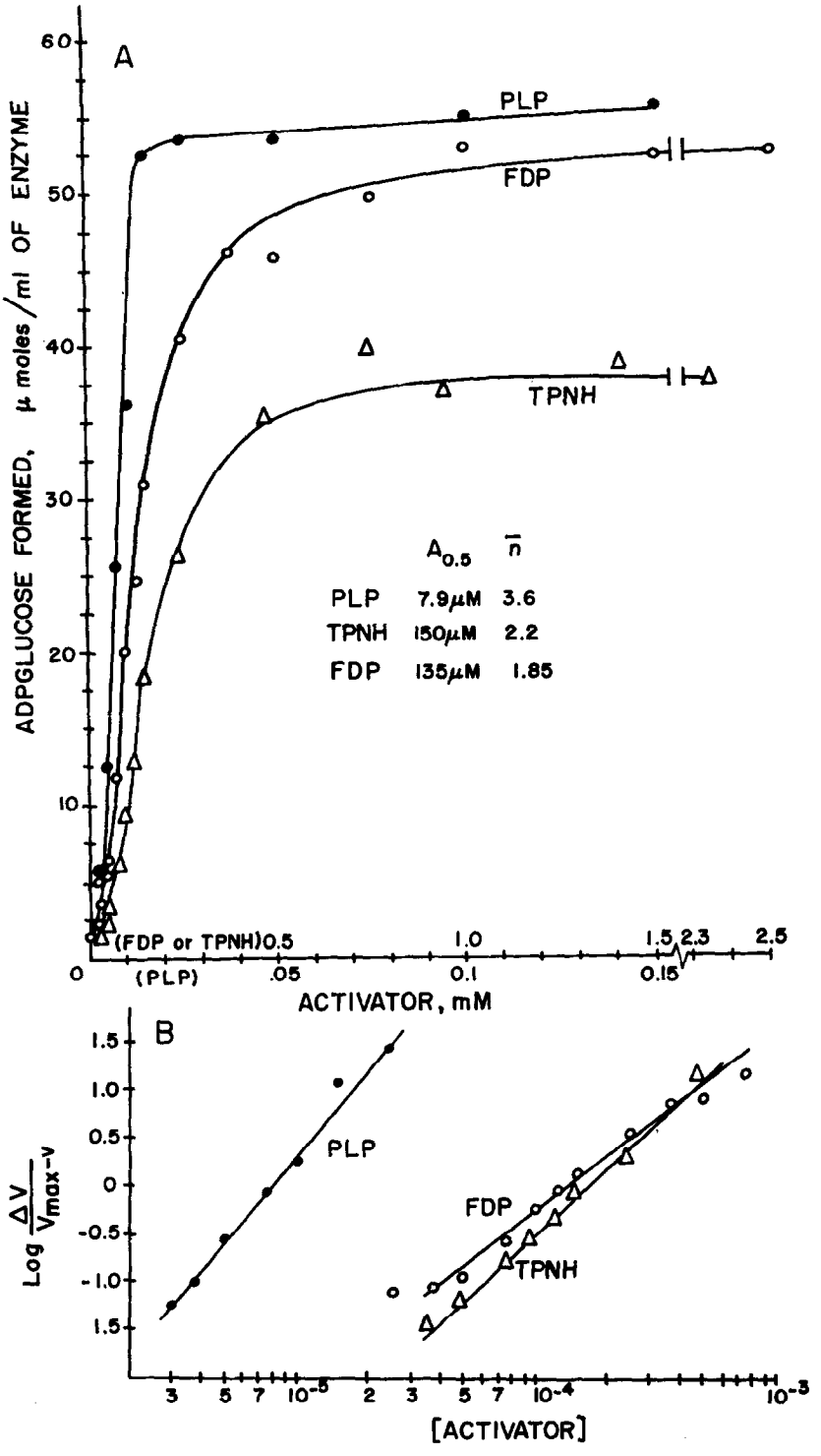

Figure 1.A. The effect of PLP, FDP and TPNH on ADP-glucose synthesis. The reaction mixture is the same as that described in the text. The concentration

of activators were varied as indicated in the figure. B shows a plot of the data according to the Hill equation (9-11). The values of V_{max} were estimated from reciprocal plots of rate vs. activator concentration. 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 of reaction mixtures containing no activator. $A_{0.5}$ is defined as the concentration of activator causing 50% of the maximal stimulation.

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of the PLP saturation curve. The apparent order of reaction $\bar{n}$, for the PLP curve decreases from 3.3 to 1.7 and 1.9 in the presence of TPNH and FDP, respectively. The observations that less than saturating concentrations of either TPNH or FDP enhance the activation effect of low concentrations of PLP and that there is no additional stimulation by these compounds at saturating concentrations of PLP are consistent with the concept that these activators are

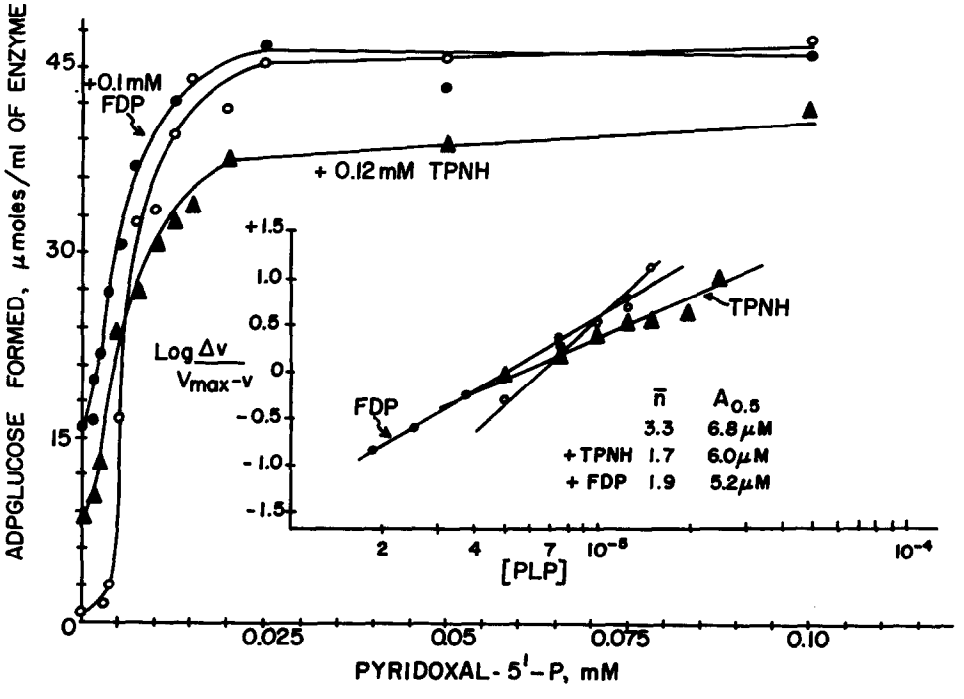

Figure 2. The effect of FDP and TPNH on the activation of ADP-glucose synthesis by PLP. The reaction mixture is the same as that described in the text. The concentrations of activators were varied as indicated in the figure. The inset shows a plot of the data according to the Hill equation (9-11). V_{max} values were estimated as indicated in Figure 1. ΔV is the increase in velocity due to addition of PLP, i.e., the velocity obtained upon addition of a certain amount of PLP to the reaction mixture minus the velocity of reaction mixtures containing no activator.

bound to the same sites on the enzyme. The decrease in the apparent order of reaction for PLP in the presence of TPNH or FDP as well as the slight inhibition of PLP activation that is caused by TPNH (Fig. 2) are also consistent with this view.

A test of a number of pyridoxine derivatives showed that only pyridoxal-P and 4-pyridoxic acid-5-phosphate could serve as activators from this group. Pyridoxamine-P, pyridoxine-P, deoxypyridoxine-P, pyridoxal and pyridoxic acid

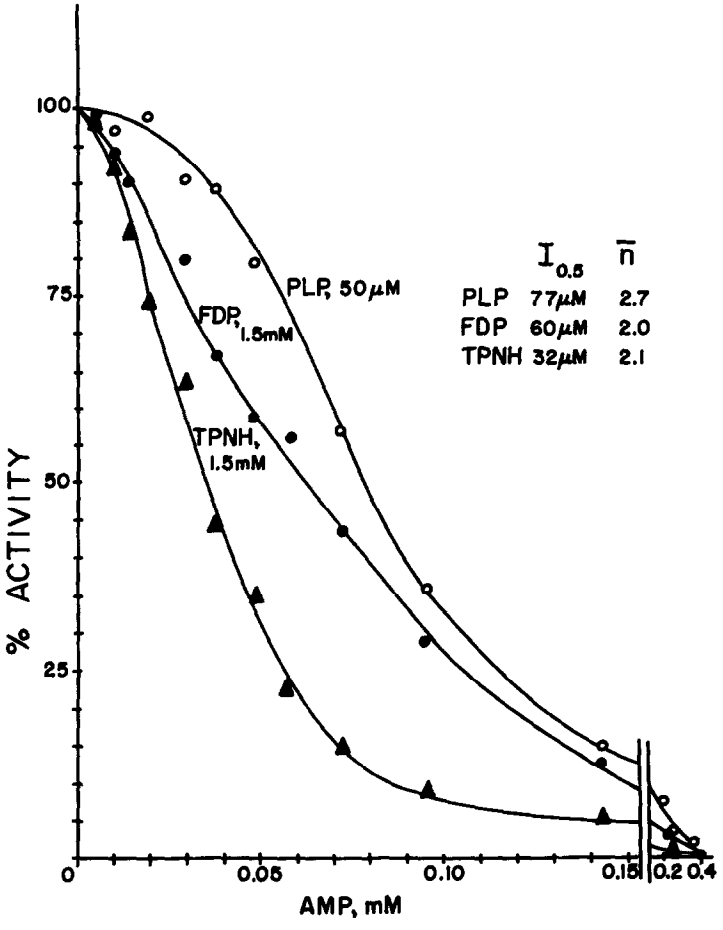

Figure 3. The effect of 5'-adenylate on ADP-glucose synthesis. The reaction mixture is the same as that described in the text. The concentration for activators used is listed in the figure. The values for 100% activity (mμmoles of ADP-glucose formed in 10 minutes) for reaction mixtures containing the different activators and no inhibitor were: FDP 11.7; TPNH 11.8; PLP 10.5. $I_{0.5}$ represents the concentration of 5'AMP causing 50% inhibition in the above conditions and $\bar{n}$ is the interaction coefficient obtained when plotting the data according to the Hill equation.

are relatively inactive. DPNH and DPN^+ are also inactive but TPN^+ does show some stimulating activity. However, the stimulation is only one-sixth that found for TPNH at saturating concentrations and higher concentrations of TPN are needed for half-maximal activation. Other compounds tested and found to be inactive are listed in a previous publication (1).

Fig. 3 shows the effect of the different activators on 5'-adenylate inhibition of the enzyme. The enzyme is more sensitive to AMP inhibition when TPNH is the activator and realtively less sensitive when PLP is the activator. Hill plots of the data give values for the concentration of AMP needed for 50% inhibition ($I_{0.5}$) in the presence of PLP, FDP, and TPNH of 77 μM, 60 μM, and 32 μM, respectively.

Physiological Function of the Activators. Glycogen accumulation occurs in *E. coli* just as soon as growth becomes limited. In the presence of an excess carbon source, such as glucose, it is conceivable that glycolytic intermediates would accumulate. Since the reducing power in the cell would no longer be needed for biosynthetic reactions, TPNH would also accumulate. The studies of Model and Rittenberg (12) have suggested that the hexose monophosphate shunt (HMPS) in *E. coli* is regulated by the availability of oxidized triphosphopyridine nucleotide. As bacterial growth ceases, the available TPN supply would be converted to TPNH which would accumulate because of its decreased utilization under these conditions. The HMPS pathway activity would then be decreased due to unavailability of the oxidized cofactor. If glucose is the carbon source its metabolism via the HMPS pathway would be diminished in the stationary phase and therefore it could be diverted towards glycogen synthesis. This view is consistent with the finding of Model and Rittenberg (12) that 28% of the glucose metabolized by *Escherichia coli* during active growth in minimal media is via the HMPS pathway while during stationary phase only 13 to 18% of the glucose is metabolized via this pathway. The accumulation of TPNH in the stationary phase would cause activation of the enteric bacterial ADP-glucose pyrophosphorylase activity which in turn would

TABLE I

ACTIVATION OF ADP-GLUCOSE SYNTHESIS

The reaction mixture is the same as that described in the text. The concentration of activator used is indicated in the table. $A_{0.5}$ refers to the concentration of activator required for 50% of the observed maximal stimulation and $\bar{n}$ is the Hill constant obtained from data plotted according to the Hill equation (9-11).

Activator	Conc. mM	ADP-glucose formed mμmoles	Stimulation -fold	$\bar{n}$	$A_{0.5}$ mM
None	-	0.32	-	-	-
FDP	1.5	14.8	46	1.9	0.13
Pyridoxal-5-P	0.05	18.0	56	3.3	0.008
4-Pyridoxic Acid-5-P	1.5	17.0	53	2.4	0.07
Pyridoxamine-5-P	1.3	0.56	1.7	-	-
Deoxy-Pyridoxine-5-P	1.5	1.5	4.7	-	-
Pyridoxine-5-P	1.5	0.30	1.0	-	-
Pyridoxal	1.5	0.30	1.0	-	-
TPNH	1.5	9.6	30	2.2	0.14
TPN^+	1.5	1.5	4.7	1.9	0.55
DPNH	1.5	0.32	1.0	-	-
DPN^+	1.5	0.29	0.9	-	-

stimulate glycogen synthesis.

Fructose diphosphate and other glycolytic intermediates that stimulate ADP-glucose synthesis may also be pertinent in the regulation of glycogen synthesis when the organism is grown on carbon sources such as tricarboxylic acid cycle intermediates, amino acids, or glycerol. When these carbon sources are in excess during limited growth, gluconeogenesis would ensue causing accumulation of the various glycolytic intermediates. These in turn would stimulate ADP-glucose synthesis leading to a stimulation of the flux of carbon towards glycogen synthesis.

At present it is difficult to rationalize the participation of pyridoxal phosphate in the regulation of ADP-glucose and glycogen synthesis. Present estimates of <u>total</u> pyridoxine compounds in bacterial cells is about 10^{-5} M (14). Presumably there is no reason for its concentration to fluctuate in different physiological conditions in the cell particularly when the cell is known to

accumulate glycogen. It may be that in the cell low concentrations of pyridoxal phosphate available for binding to the enzyme ensures a minimal amount of ADP-glucose synthesizing activity. However, glycogen accumulation readily occurs in nitrogen-limiting conditions (13). It is quite possible that under these conditions amino acid metabolism is minimal and there is much less need for pyridoxal-5-P. A greater availability of pyridoxal-P for binding to the ADP-glucose pyrophosphorylase may therefore occur when protein synthesis and amino acid metabolism is limited.

FDP, TPNH and PLP have been shown to be activators of other enteric ADP-glucose pyrophosphorylases (G. Ribereau-Gayons and A. Sabraw, unpublished results). Recent results (15-17) indicate that certain mutants of *E. coli* which accumulate more glycogen than the wild-type contain an ADP-glucose pyrophosphorylase that has higher affinity for FDP and a lower affinity for 5'AMP. Although the kinetics of the mutant enzymes with respect to the other activators, TPNH and PLP remain to be studied, it appears that the activation and inhibition phenomena observed *in vitro* are physiologically significant.

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