ACTIVATOR-INHIBITOR INTERACTIONS IN THE ADENOSINE DIPHOSPHATE GLUCOSE PYROPHOSPHORYLASE OF ESCHERICHIA COLI B

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Received April 10, 1967

Recently (1) the partial purification and some of the kinetic properties of an adenosine diphosphate glucose (ADPglucose) pyrophosphorylase

(I) ATP + glucose-1-P $\rightleftharpoons$ ADPglucose + PP_i

from Escherichia coli B were described. The activity of this enzyme was stimulated by a number of glycolytic intermediates, of which fructose-1, 6-diphosphate (FDP) was most effective. FDP increased the V_{max} of ADPglucose synthesis 7-fold. The enzyme was inhibited by 5'-AMP, ADP, and inorganic phosphate.

In bacteria, glycogen synthesis appears to be regulated at the level of synthesis of the glucosyl donor, ADPglucose (1, 2). The present report is concerned with activator-inhibitor interactions in the direction of synthesis of ADPglucose. These interrelationships may be of prime importance in the regulation of glycogen metabolism.

METHODS

The studies herein reported concern the activity in the direction of ADPglucose synthesis only. The assay of enzymatic activity has been previously reported (1).

*Supported by a grant from the United States Public Health Service (AI 05520).

Reaction mixtures contained the following in a volume of 0.20 ml: ATP, 0.3 μmole; glucose-C^{14}-1-P (specific activity, 8.0×10^5 cpm per μmole), 0.1 μmole; $MgCl_2$, 1.0 μmole; Tris-chloride buffer, pH 8.5, 10 μmoles; bovine plasma albumin, 100 μg; crystalline yeast inorganic pyrophosphatase, 9 μg; and E. coli B ADPglucose pyrophosphorylase (500-fold purified). The concentrations of fructose diphosphate and 5'-AMP or P_i present in the reaction mixtures are indicated in the figures and tables.

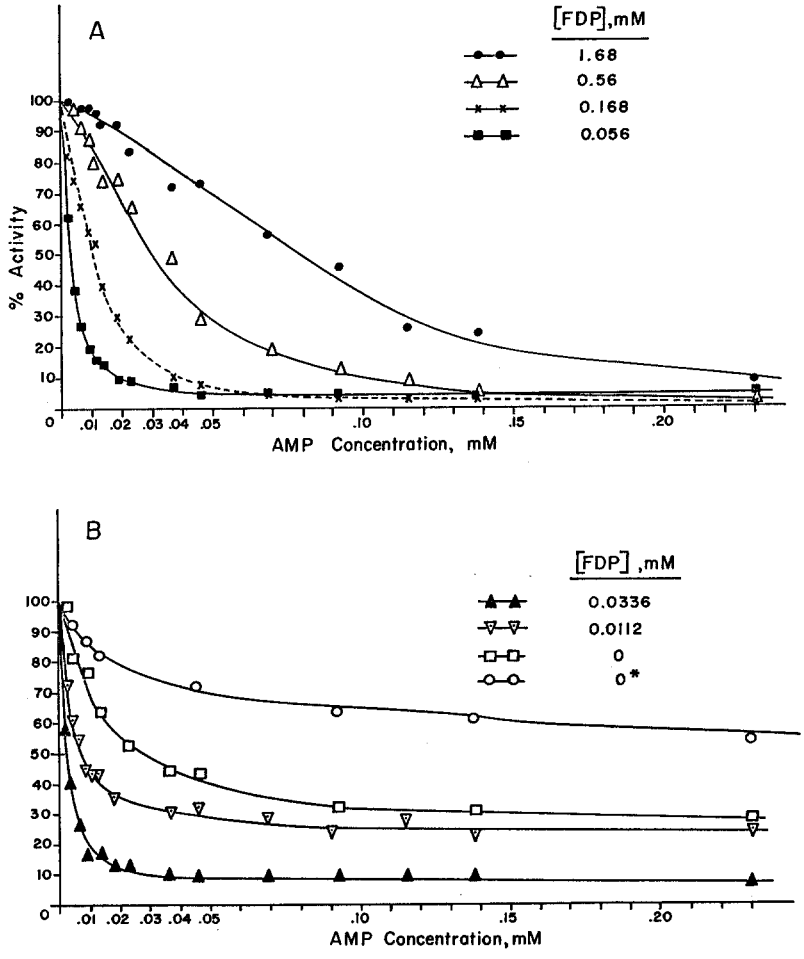

Fig. 1: Inhibition of ADPglucose synthesis as a function of 5'-Adenylate concentration. Each curve represents reaction mixtures containing different FDP concentrations as indicated in the figure. Curve -0-0- represents reaction mixtures containing no FDP and where the amounts of ATP, glucose-^{14}C-1-P, and $MgCl_2$ have been increased to 1.5 μmoles, 0.2 μmole, and 5.0 μmoles respectively. For each curve the velocity of the synthesis reaction in the absence of AMP has been normalized to 100.

RESULTS

FDP-AMP Interaction

The inhibition of ADPglucose synthesis was investigated in reaction mixtures containing various levels of the activator, FDP. In Fig. 1 (a) and 1 (b) the percentage activity is shown as a function of AMP concentration. In Table I, derived from the data of Fig. 1, v_o is the velocity obtained at the indicated FDP concentration and is relative to the velocity at saturating FDP concentration ($v_o = 100$), the K_i value is the concentration of AMP required for 50% inhibition, and the n value is the interaction coefficient or apparent order of reaction with respect to AMP (3). The K_i and n values for inhibitor are constants of the Hill equation (3) and were determined by plotting $\text{Log}\frac{v}{(V_{max}-v)}$ vs. Log (Inhibitor). The concentration of activator had a distinct modulating effect on the sensitivity of the rate of ADPglucose synthesis to AMP inhibition. Sub-saturating levels of FDP sensitized the enzyme to AMP inhibition. At very low levels of FDP, or in the absence of FDP, the rate was relatively insensitive to inhibition by AMP. It will be noted that the rate, over the range of AMP concentrations investigated, never became totally inhibited. Calculations showed that the absolute value of this "uninhibitable activity" was roughly constant, regardless of FDP concentration.

The concentration of FDP was extremely important in determining the extent of inhibition caused by a given concentration of AMP, especially in the range 0.005mM AMP to 0.10mM AMP.

n, the apparent order of the reaction with respect to AMP (Table I) continually decreased with decreasing concentrations of the activator. If n were to be taken as an interaction coefficient (3), this would indicate progressively decreasing cooperative interaction between sites binding AMP, even over the range of FDP concentration where the rate is becoming more sensitive to AMP inhibition. At very low FDP concentrations, or in the absence of FDP, n became less than 1. This would indicate that the binding of one molecule

of inhibitor hinders the binding of another molecule of inhibitor, instead of facilitating it. The kinetic implications of this "negative interaction between inhibitor sites" may be what is manifested by the percentage of activity not subject to AMP inhibition. In reaction mixtures containing no FDP but where the ATP concentration and $MgCl_2$ concentration have been increased to give maximal rates of ADPglucose synthesis in the absence of activator (Table 1 and Fig. 1), it was found that the enzyme could only be inhibited about 45%. Thus ATP and $MgCl_2$ concentrations may also play a role in reversal of AMP inhibition.

TABLE I

AMP Inhibition of ADPglucose Synthesis,
at Different Levels of the Activator, FDP

[FDP] mM	v_o	K_i mM	n	% of V_o resistant to AMP inhibition
1.68	100	0.07	1.75	-
0.56	98	0.03	1.65	-
0.17	65	0.011	1.48	2.0
0.056	35	0.0034	1.37	3.8
0.034	18.5	0.0032	1.25	7.0
0.011	5	0.0073	0.93	24
0	3.8	0.03	0.68	30
0*	14	0.35	0.50	55

*These reaction mixtures contained in 0.2 ml, 1.5 μmoles ATP, 0.2 μmole glucose-C^{14}-1-P, and 5.0 μmoles $MgCl_2$; all other components were present at the same concentrations as indicated in Methods. These are the conditions found for maximal activity in the absence of FDP.
V_o for reaction mixtures containing 1.68 mM FDP is 7.8 μmoles ADPglucose formed per minute per mg of protein.

Fig. 2 shows the effect of activator concentration on the rate of synthesis of ADPglucose. The presence of AMP increased the concentration of

FDP required for half-maximal activation, although there was little effect on the concentration of FDP required for optimal activation. It is evident that the percentage inhibition caused by a given concentration of AMP is greater at sub-saturating levels of FDP than at saturating FDP concentrations. 0.092 mM AMP inhibited V_{max} about 60%, but in the range 0-0.5 mM FDP it caused nearly complete inhibition of the rate.

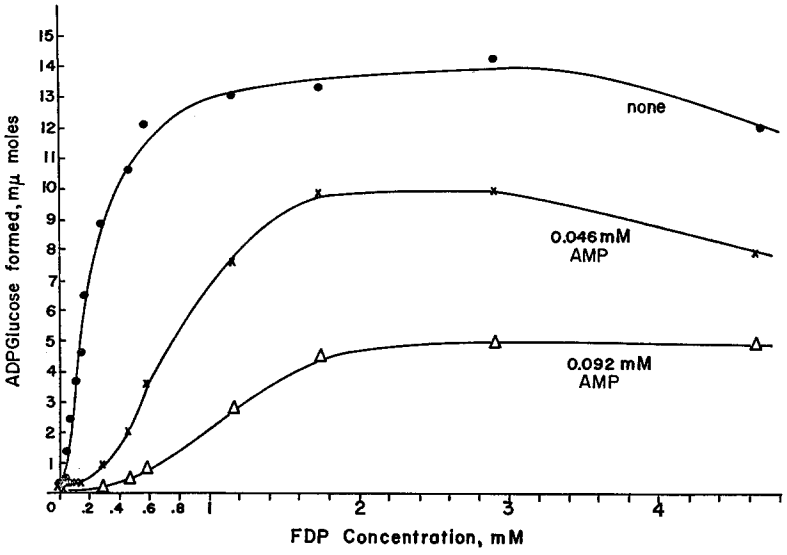

Fig. 2: Effect of 5' Adenylate concentration on the activation of ADPglucose synthesis by fructose diphosphate. The concentrations of AMP in the reaction mixture are indicated in the figure.

FDP-P_i Interaction

Essentially the same pattern of activator-inhibitor interaction as the above was found for the inhibition of ADPglucose synthesis by inorganic phosphate (P_i). In Fig. 3 percentage activity is shown as a function of P_i concentration; each curve represents a different activator concentration. Table II was derived from the data of Fig. 3. Sub-saturating levels of the activator sensitized the enzyme to inhibition by P_i.

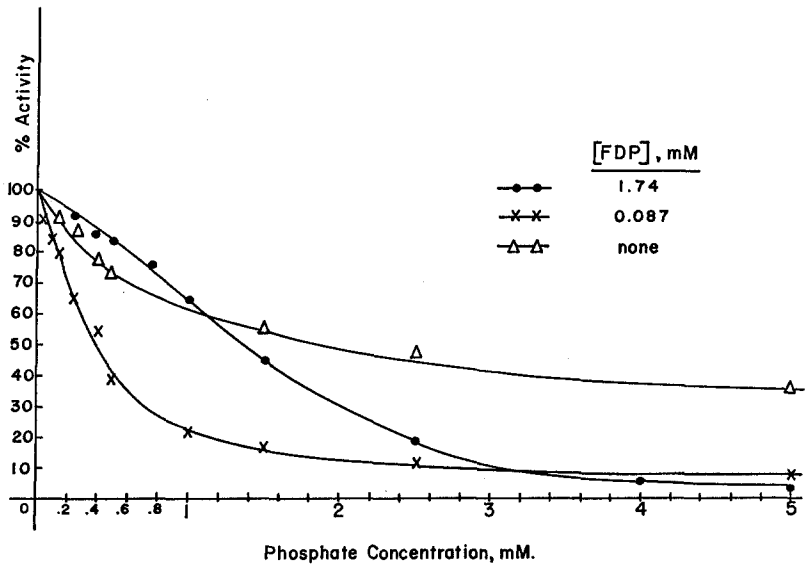

Fig. 3: Inhibition of ADPglucose synthesis by inorganic phosphate. Each curve represents reaction mixtures containing different FDP concentrations which are indicated in the figure.

TABLE II

P_i Inhibition of the Synthesis of ADPglucose, at Different Levels of the Activator, FDP

[FDP] mM	Relative v_o	K_i mM	n
1.74	100	1.25	2.0
0.087	27.0	0.40	1.45
0	3.6	1.8	0.88

DISCUSSION

The activator FDP thus appears to have at least two important functions; (a) it activates the E. coli ADPglucose pyrophosphorylase, by increasing the V_{max}, and by increasing the affinity of the enzyme for its substrates (1);

and (b) it modulates the sensitivity of the enzyme to inhibition by the energetically important intermediates AMP and P_i. Unsaturating amounts of the activator serve to make the enzymatic activity very sensitive to inhibition by AMP and P_i.

The sum of the adenine nucleotides in a bacterial cell presumably is approximately constant. ATP is a substrate as well as an activator of the ADPglucose pyrophosphorylase (1). The concentration of FDP relative to the concentrations of ATP, AMP, and P_i may be the prime factor in the physiological regulation of glycogen metabolism in E. coli.

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