Regulation of Adenosine Diphosphate Glucose Synthase from Escherichia coli

INTERACTIONS OF ADENYLATED ENERGY CHARGE AND MODIFIER CONCENTRATIONS*

(Received for publication, March 23, 1970)

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SUMMARY

The adenosine diphosphate glucose synthase (pyrophosphorylase) of Escherichia coli responds very sharply to variation in the energy charge of the adenylate pool. The response is in the right direction to insure that this enzyme, which catalyzes the first step unique to glycogen storage in E. coli, will have appreciable activity only under conditions of energy excess (high energy charge). The positive response to high energy charge is strongly enhanced by reduced triphosphopyridine nucleotide, fructose 1,6-diphosphate, 3-phosphoglyceraldehyde, and phosphoenolpyruvate. High concentrations of these metabolites seem appropriate as signals for energy storage.

Shen and Preiss (1), Shen et al. (2), and Greenberg and Preiss (3) have shown that adenosine diphosphate glucose is the glucosyl donor in glycogen synthesis by various bacterial species. Their extensive studies on the regulatory properties of ADP-glucose synthase (pyrophosphorylase) (ATP + glucose-1-P → ADP-glucose + PPi) from bacteria, algae, and green plants have recently been reviewed (4). Each of the ADP-glucose synthases studied (except those from two species of purple photosynthetic bacteria) was shown to be inhibited by AMP, ADP-glucose + PPI from bacteria, algae, and green plants. Their extensive studies on the regulatory properties of ADP-glucose synthase (pyrophosphorylase) of Escherichia coli respond very sharply to variation in the energy charge of the adenylate pool (5) in the direction appropriate for regulation of energy storage sequences (5, 6).

This paper demonstrates the energy charge response of ADP-glucose synthase from Escherichia coli, and the effects of other metabolite modifiers on that response.

METHODS

Preparation of Enzyme—E. coli B was grown at 37° in 15-liter cultures with aeration. The medium contained 10 g of glucose, 6 g of yeast extract, 11 g of K2HPO4, and 8.5 g of KH2PO4 per liter. Cells were harvested by centrifugation approximately when growth ceased. After being washed in 0.85% NaCl, 105 g of cell paste was resuspended in 420 ml of 50 mM Tris-chloride buffer, pH 7.5, containing 10 mM MgCl2 and 10 mM glutathione. Cells were disrupted sonically. The enzyme was purified 200-fold by protamine sulfate precipitation and elution, ammonium sulfate precipitation, heat treatment (70°) DEAE-Sephadex column chromatography, and a second ammonium sulfate fractionation, as described by Preiss et al. (7).

Assay—Formation of ADP-glucose was assayed as described previously (8). The reaction mixture, which was incubated for 10 min at 37°, contained 0.3 mM "C-glucose-1-P (specific activity, 106 cpm per pmole), 3 mM ATP (or an adenine nucleotide mixture of specified energy charge as indicated), MgCl2 as indicated, 50 mM Tris-chloride, pH 8.0, 50 μg of bovine serum albumin, 0.5 μg of yeast pyrophosphatase, and enzyme in a total volume of 0.2 ml. The reaction was terminated by immersion in boiling water for 40 sec. After cooling, 30 μg of E. coli alkaline phosphatase was added and the reaction mixture was incubated for 60 min at 37° to hydrolyze residual "C-glucose-1-P. A 0.1-ml sample of the reaction mixture was transferred to a DEAE-cellulose paper disc 3.5 cm in diameter. The disc was washed on a sintered glass filter with 450 ml of distilled water, placed in a scintillation vial with 10 ml of 0.4% Omniflor (New England Nuclear) in toluene and counted in a scintillation counter.

Desired values of the adenylylate energy charge were established by mixing appropriate amounts of ATP, ADP, and AMP, calculated on the basis of an equilibrium constant of 0.8 for the adenylylate kinase reaction.

RESULTS

When E. coli ADP-glucose synthase is assayed in the presence of a constant total adenylate level (3 mM), with the amounts of AMP, ADP, and ATP varied to provide a range of values of the energy charge, the curve of activity as a function of energy charge is sharply concave upward at a charge of 0.8, and becomes very steep at higher charge values (Figs. 1 and 2). The results of assays with no ADP and AMP are shown in these figures for comparison with results of the energy charge experiments. In the absence of activator (Fig. 1), 3 mM is not a saturating level of ATP, and the curve for ATP alone appears, in part because of its sigmoid character, to be roughly linear over the concentra-
Response of *E. coli* ADP-glucose (ADPG) synthase to variation in the adenylate energy charge in the absence of activators. The lower curve presents results obtained in the presence of an adenylate pool (ATP + ADP + AMP) of 3 mM, with concentrations of the individual nucleotides varied to produce the desired energy charge, as indicated on the lower horizontal coordinate. The upper curve, for comparison, reports results obtained in the absence of ADP and AMP, at ATP concentrations indicated on the upper horizontal scale. The reaction mixtures contained 25 mM MgCl₂ and 18.4 µg of enzyme and were otherwise as described in the text.

**Fig. 2.** Response of *E. coli* ADP-glucose (ADPG) synthase to variation in the adenylate energy charge in the presence of fructose diphosphate. Conditions as in Fig. 1, except that reaction mixtures contained 7.5 mM MgCl₂, 4.6 µg of enzyme, and 1 mM FDP.

The curve for the energy charge experiments is strikingly different, and reflects strong inhibition by ADP and AMP. Preiss et al. (7) have shown FDP¹ to be a powerful activator of *E. coli* ADP-glucose synthase. This activation is seen in Fig. 2, in which, in comparison with Fig. 1, the amount of enzyme has been reduced to one-fourth and the vertical scale compressed by a factor of 2. In the presence of 1 mM FDP, ATP is essentially saturating at a concentration of 1 mM. The energy charge response is extremely sharp.

¹ The abbreviation used is: FDP, fructose 1,6-diphosphate.
The response of the enzyme to variation in concentration of Mg²⁺ ion is altered by FDP, as seen in Figs. 3 and 4. Experiments in which the concentration of Mg²⁺ was varied at fixed energy charges of 0.9 and 1.0 have shown the reaction velocity to reach a maximal value at about 25 mM, and to remain at this level as the Mg²⁺ concentration is increased at least to 50 mM. In the presence of 1 mM FDP, maximal activity is seen at Mg²⁺ concentrations in the range of 5 to 9 mM at a charge of 1.0 and 7 to 10 mM at a charge of 0.9, with sharp decreases at higher concentrations of the cation. Interactions of Mg²⁺ with other modifiers were not studied further. For the sake of standardization and of conservatism in interpretation, experiments were run at approximately optimal Mg²⁺ levels; for example, 25 mM in Fig. 1 and 7.5 mM in Fig. 2.

As has been observed for several other regulatory enzymes, E. coli ADP-glucose synthase responds primarily to the ratios among the concentrations of ATP, ADP, and AMP (that is, to the energy charge of the adenylate pool), rather than to the absolute levels of the nucleotides. This response is demonstrated in Fig. 5, where each solid circle (●) represents an assay containing 67% more of each nucleotide than the assay represented by the corresponding open circle (○).

Interaction between adenylate energy charge and the concentration of FDP in the regulation of E. coli ADP-glucose synthase is illustrated in Fig. 6. Preiss (4) has recently reported that TPNH activates this enzyme. Fig. 7 shows interaction between TPNH and energy charge. When both FDP and TPNH were added simultaneously at the same levels as shown in Figs. 6 and 7, the resulting curves were nearly superposable on those of Fig. 6. It thus appears that in the presence of FDP the addition of TPNH causes little further activation.

Preiss et al. (7) reported that a plot of the rate of the E. coli ADP-glucose synthase reaction as a function of ATP concentration is sigmoid, and that Hill plots of this function have a slope of 1.7 to 1.8. We have repeated this observation. Response curves for all of the modifiers of this enzyme are also sigmoid, as seen in Fig. 8. (On this scale the sigmoidicity of the upper...
FDP and TPNH curves is barely apparent.) This figure also indicates that the interactions between adenylate energy charge and other modifiers are quite complex. The four activators shown are all highly effective at an energy charge of 1, but only FDP stimulates significantly at a charge of 0.85. TPNH activates only weakly at this charge value, and both 3-phosphoglyceraldehyde and phosphoenolpyruvate are ineffective. Change in energy charge affects both the $V_{\text{max}}$ and the $M_{\text{a.s}}$ (concentration required for half-maximal response) of the activators. Hill plots show, however, that the apparent order of the responses to FDP and TPNH remain approximately 2 at both charge values, despite the large $V_{\text{max}}$ and $M_{a.s}$ changes seen in Fig. 8. For FDP, values of slope ($m$) and $M_{a.s}$ from these Hill plots are: at a charge of 1.0, $m = 1.9$, $M_{a.s} = 85 \mu M$; at a charge of 0.85, $m = 2.3$, $M_{a.s} = 1.4 \mu M$. For TPNH, at a charge of 1.0, $m = 2.1$, $M_{a.s} = 85 \mu M$; at a charge of 0.85, $m = 2.2$, $M_{a.s} = 1.35 \mu M$. Although these parameters are, within experimental error, identical for the two activators, it is evident from Fig. 8 that $V_{\text{max}}$ for TPNH changes much more drastically with change in energy charge than does $V_{\text{max}}$ for FDP.

**DISCUSSION**

The response of *E. coli* ADP-glucose synthase to variation in energy charge is evidently in the right direction for an enzyme of an energy storage sequence (5, 6). Activation by glycolytic intermediates and by TPNH also seems reasonable for an enzyme that regulates energy storage. Biosynthesis, broadly speaking, is the conversion of intermediates in primary metabolic pathways into the building blocks for macromolecular synthesis (and into the macromolecules themselves), largely at the expense of ATP and TPNH. When a source of carbon and energy is available but biosynthesis is restricted for any reason, such as nitrogen limitation, both TPNH and ATP (energy charge) will tend to increase. Storage of polysaccharides is obviously appropriate under these conditions, and the properties of ADP-glucose synthase seem adapted to ensure that it will occur.

In general, it also seems reasonable that the concentrations of glycolytic intermediates, such as fructose diphosphate, 3-phosphoglycerate, and phosphoenolpyruvate, should rise under conditions of excess metabolic energy; thus activation of ADP-glucose synthase by these intermediates seems appropriate.

Energy storage compounds such as glycogen should presumably be made at a significant rate only when the cell's supply of energy exceeds current demands. Hence it may be predicted that enzymes that regulate energy storage sequences should show significant activity only at very high values of energy charge. ADP-glucose synthase is the first enzyme participating solely in energy storage that has been studied with respect to energy charge. It thus seems appropriate that, in the presence of TPNH or fructose diphosphate, this enzyme exhibits the sharpest energy charge response yet observed for any enzyme of an ATP-utilizing (biosynthetic or energy storage) sequence. Under some conditions this rate increases by about 20-fold when the energy charge is increased from 0.8 to 1.0 (Fig. 2, the 5 mM MgCl$_2$ curve of Fig. 4, the 0.5 mM fructose diphosphate curve of Fig. 6, and the 0.5 and 1.5 mM TPNH curves of Fig. 7). In the presence of lower levels of the activators (TPNH and fructose diphosphate) the curves are at least equally steep, and the rise is concentrated even closer to the 1.0 charge coordinate. It should be noted that Figs. 6 and 7 cover only the upper half of the charge range, from 0.5 to 1.0. If the whole range were shown, the steepness of the curves would be more apparent. For comparison, typical curves for a biosynthetic enzyme at very low and at high concentrations of its end products are shown in Fig. 7. If the concentration of TPNH were around 100 to 500 $\mu M$, for example, and the energy charge were 0.85, a biosynthetic enzyme that behaved (at very low end product concentration) as shown by the upper broken curve would be highly active, while under these same conditions ADP-glucose synthase would be almost totally inactive. Thus in normally growing cultures, biosynthetic sequences would be expected to outcompete energy storage. If, however, growth and protein synthesis stop for any reason while a source of carbon and energy is available, the resulting accumulation of end products should cause the average response of regulatory enzymes in biosynthetic sequences to approximate that indicated by the lower broken curve. If now the energy charge rises to 0.9 or slightly higher, and especially if the concentration of TPNH increases as a consequence of decreased demand for this cofactor in biosynthesis, it is to be expected that energy storage, as exemplified by the ADP-glucose response, should proceed much faster than biosynthesis. Thus the type of interaction illustrated in Fig. 7 would seem to insure that the cell will make glycogen when, and only when, the storage of energy would be metabolically advantageous.
REFERENCES

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