RESPONSE TO THE ADENYLATE ENERGY CHARGE BY ESCHERICHIA COLI ASPARTATE TRANSCARBAMYLASE AND ITS CATALYTIC SUBUNIT*

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Summary

Native Escherichia coli aspartate transcarbamylase activity is stimulated at high values of the adenylate energy charge. This response interacts with inhibition by the endproduct CTP in the way expected for a regulatory biosynthetic enzyme. Activity of the dissociated catalytic subunit, however, is strongly inhibited by ATP. Thus an appropriate response to adenylates appears to depend on subunit interactions in the native enzyme. Magnesium ion sharpens the energy charge response of the native enzyme, but decreases the effect of ATP on the catalytic subunit.

Introduction

Several regulatory enzymes that participate in biosynthetic sequences have been shown to respond simultaneously to the concentrations of product feedback inhibitors and to the adenylate energy charge (Atkinson and Fall, 1967; Klungsøyr, et al., 1968). Except at high concentrations of endproduct, such enzymes have steep positive responses to increasing energy charge in the region above a charge value of about 0.75. Aspartate transcarbamylase (ATCase), which catalyzes the first step unique to pyrimidine synthesis, is stimulated by ATP (Gerhart and Pardee, 1962); thus a positive response to high energy charge is to be expected. No effects of adenylates on dissociated catalytic subunits have been reported. An endproduct, CTP, inhibits the native enzyme (Gerhart and Pardee, 1962); the catalytic subunit has been variously reported to be unaffected (Gerhart and Pardee, 1964) and inhibited (Weitzman and Wilson, 1966) by CTP.

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This paper reports that the native enzyme responds to variation in energy charge and endproduct concentration in the manner expected for a regulatory biosynthetic enzyme, but that the response of the catalytic subunit is strikingly different.

Materials and Methods

Native aspartate transcarbamylase and its catalytic subunit were prepared from Escherichia coli as described by Gerhart and Holoubek (1967). ATCase activity was assayed by a radioactive method based on that described by Porter et al. (1969).* Compositions of assay mixtures are described in figure legends; the final assay volume was 0.25 ml. The reactions were stopped by addition of 0.1 ml of 0.7 M acetic acid. The reaction product, carbamylaspartate-^{14}C, was separated from L-aspartate-U-^{14}C by chromatography on Dowex 50.

Results and Discussion

The isolated catalytic subunit is inhibited by the individual components of the adenylate energy charge system and also by the feedback inhibitor of the native enzyme, CTP (Fig. 1). Phosphate can mask nucleotide inhibition of subunit activity. This effect may account for the apparent discrepancy in the literature concerning CTP inhibition of subunit activity, since Weitzman and Wilson (1966) assayed in Tris buffer, whereas Gerhart and Pardee (1962, 1964) used saturating levels of carbamyl phosphate and 40 mM phosphate. Relative inhibitory effects of the nucleotides on the activity of the catalytic subunit are generally in the order triphosphates > diphosphates > monophosphates, with ATP being the most inhibitory of the triphosphates. Carbamyl phosphate reduces the apparent affinity of the catalytic subunit for the nucleotide inhibitors.

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* We thank Dr. George Stark for kindly making the assay procedure available to us prior to publication.

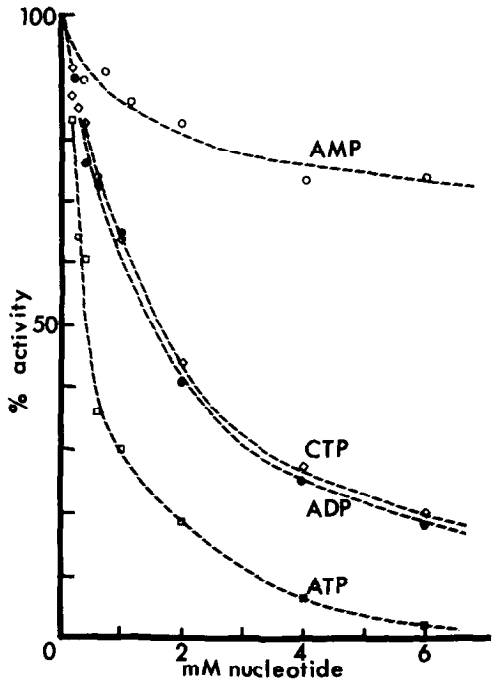

Figure 1. Inhibition of catalytic subunit by nucleotides. Assay mixtures contained 1 mM potassium L-aspartate, 90 μM dilithium carbamyl phosphate, 80,000 cpm of L-aspartic acid-U-^{14}C, 40 mM Tris-acetate, nucleotides as indicated, and 0.018 μg of enzyme, at pH 8.5 and 25°. Control activity (100%) corresponded to 3,200 cpm of ^{14}C incorporated into carbamylaspartate.

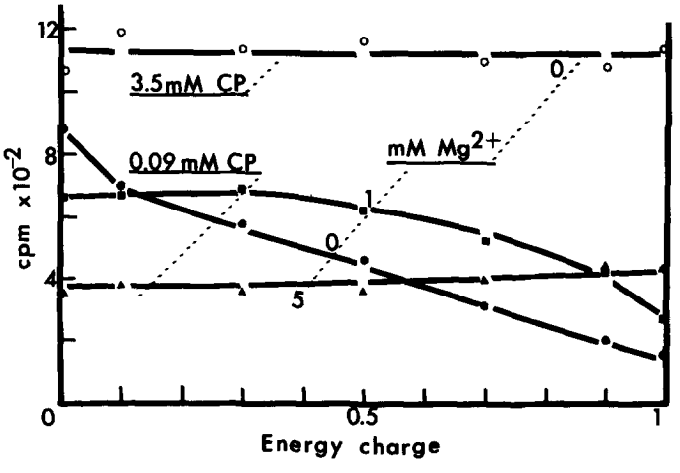

Figure 2. Response of catalytic subunit to adenylate energy charge. Aspartate, buffer, pH, and temperature were as in Figure 1. Carbamyl phosphate (CP) and magnesium acetate as indicated, 46,000 cpm of L-aspartic acid-U-^{14}C, and 0.014 μg enzyme were used. Desired values of energy charge were established by mixing appropriate amounts of AMP, ADP, and ATP (total concentration, 2 mM) as calculated on the basis of an equilibrium constant for the adenylate kinase reaction of 0.8. The vertical scale indicates incorporation of ^{14}C into carbamyl-aspartate.

Over a fairly wide range of carbamyl phosphate and magnesium acetate concentrations the catalytic subunit either does not respond to energy charge or its activity decreases with increasing charge (Fig. 2). Such responses to variation of energy charge are unlike those previously observed with biosynthetic enzymes (Klungsøyr, et al., 1968).

Native ATCase, in the absence of added divalent cation, exhibits an essentially linear four-fold increase in activity between energy charge values of 0 and 1 (curves not presented here). Magnesium ion at low concentrations enhances the activation by ATP but inhibits in the absence of ATP (Fig. 3). Within the stimulatory range, Mg^{2+} thus sharpens the energy charge curve by depressing activity at low charge and by increasing activation at high charge. At low values of energy charge and in the absence of adenylates, cation inhibition of native ATCase may be due either to complexing of substrates (Kleppe and Spaeren, 1966) or to direct interaction with the enzyme. If no adenylates are added the half-saturation concentration for Mg^{2+} inhibition remains approximately 0.6 mM when the aspartate concentration is varied from 1 to 3 mM or carbamyl phosphate concentration is varied from 0.4 to 1.8 mM. This observation suggests that such inhibition, at low substrate concentrations, may result from direct interaction of cation with enzyme.

The response of the native enzyme to energy charge in the presence of 1 mM Mg^{2+} and various concentrations of the product feedback inhibitor, CTP, (Fig. 4) is generally similar to the interaction pattern observed with other regulatory biosynthetic enzymes in the presence of their feedback modifiers (Atkinson and Fall, 1967; Klungsøyr, et al., 1968). The possible physiological significance of such interactions has been discussed (Atkinson, 1968; Klungsøyr, et al., 1968); presumably they allow synthesis to occur only when the endproduct is needed (its concentration is low) and the cell's energy balance is favorable.

The striking differences between the native enzyme and the catalytic

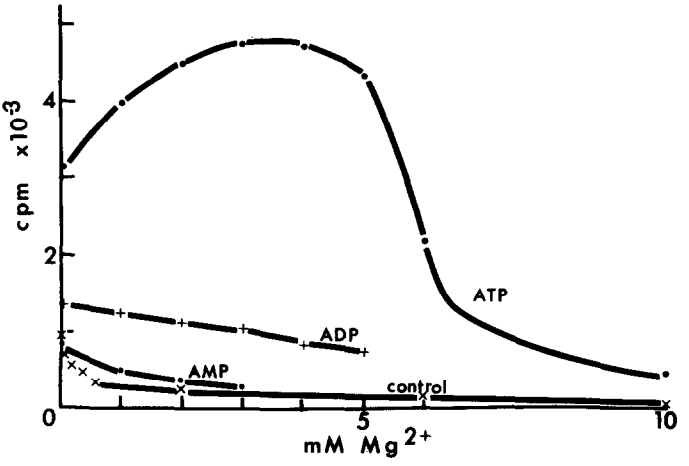

Figure 3. Response of native ATCase to variation in Mg^{2+} concentration in the presence of saturating levels of individual adenylates. Conditions as in Figure 1 except that adenylate concentration was 5.8 mM, dilithium carbamyl phosphate concentration was 1.8 mM, and 25,000 cpm of L-aspartic acid-U-^{14}C and 0.023 μg of native enzyme were used.

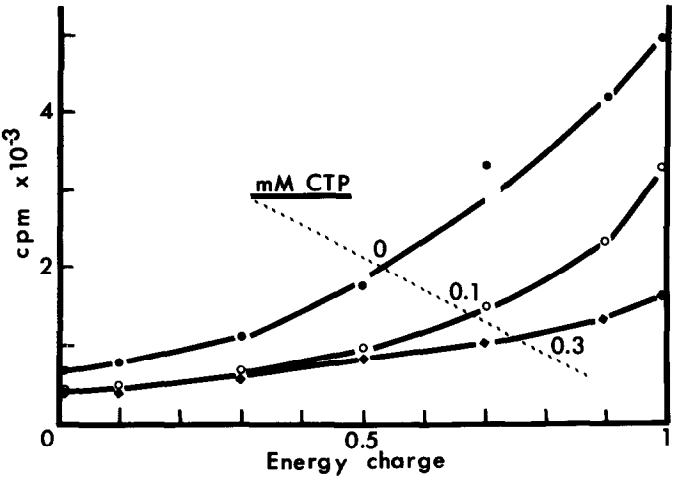

Figure 4. Response of native ATCase to adenylate energy charge in the presence of feedback inhibitor, CTP. Aspartate, buffer, pH, and temperature were as in Figure 1. Assay mixtures contained CTP as indicated on the figure, 42,000 cpm L-aspartic acid-U-^{14}C, 3.5 mM dilithium carbamyl phosphate, 1 mM magnesium acetate, and 0.023 μg of native enzyme. Total adenylate concentration was 2 mM. Similar results were obtained when 90 μM carbamyl phosphate was used.

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subunit in response to variation in the adenylate energy charge indicate that subunit interactions in ATCase not only increase sensitivity to CTP,

but also play an important role in tailoring the general kinetic behavior of the enzyme to fit the metabolic needs of the cell.

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