ALLOSTERIC ACTIVATION OF DPN-LINKED MALIC ENZYME FROM *ESCHERICHIA COLI* BY ASPARTATE

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Several hypotheses have been proposed to explain the metabolic function of malic enzyme. According to the recent reports (Theodore and Englesberg, 1964; Ashworth and Kornberg, 1966), the possibility of its functioning in the CO_2 fixation was excluded. The idea that it exerts its action in the generation of $TPNH_2$ is supported by some experiments with mammalian system (Young *et al.*, 1964; Ball, 1966). As the third possibility, malic enzyme is considered to control the level of C_4-dicarboxylic acids by decomposing malate into pyruvate and CO_2. Jacobson *et al.* (1966) have found that acetate, and C_3-compounds to a lesser extent, repressed the synthesis of malic enzyme in *Pseudomonas putida* and they made an interesting speculation that malic enzyme plays a role in the formation of acetyl-CoA from malate via pyruvate.

We previously reported that *E. coli* W has two malic enzymes requiring TPN and DPN, respectively, and suggested that they might have different functions from each other (Katsuki *et al.*, 1967). In this communication, we will report the allosteric activation of this DPN-linked malic enzyme by aspartate and also

point out the significance of the enzyme in the control of the C_4-dicarboxylic acid level in TCA cycle.

Experimental -- DPN-linked malic enzyme was prepared as described in the previous communication (Katsuki et al., 1967) with some modifications. The purification of the enzyme was carried out by DEAE-cellulose column chromatography instead of the calcium phosphate gel treatment. The protein was separated by linear gradient elution of KCl (0-0.5 M) in 0.05 M Tris-HCl (pH 7.9) containing 10 mM $MgCl_2$. The enzyme was purified about 200 fold over the crude extracts. The $DPNH_2$ oxidase activity in this preparation was less then 1/400 of the DPN-linked malic enzyme activity, and malate dehydrogenase, lactate dehydrogenase and TPN-linked malic enzyme were not detected.

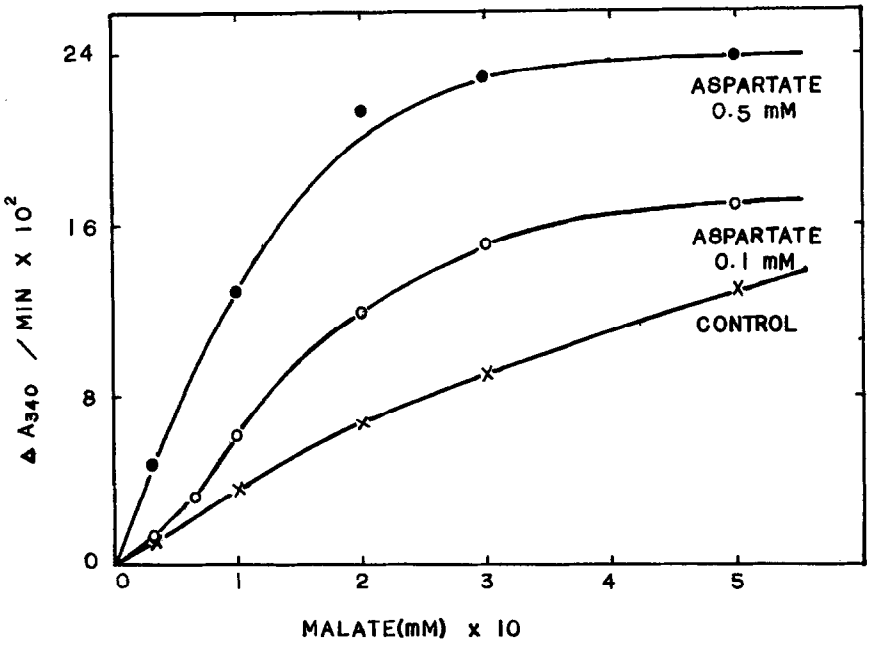


Fig. 1. Effect of malate concentration on enzyme activity in the presence of aspartate. The reaction mixture contained in a total volume of 3.0 ml, 30 μmoles of Tris-HCl buffer, pH 7.9, 1 μmole each of $MnCl_2$, $MgCl_2$ and DPN, the purified enzyme solution (22 μg of protein) and 0.3 or 1.5 μmoles of L-aspartate where indicated. The mixture was incubated at 30° in a cuvette for a Hitachi 124 spectrophotometer and the increase in absorbance at 340 mμ was recorded.

Results -- The establishment of aspartate and the related dicarboxylic acids as allosteric effectors of the phosphoenol pyruvate carboxylase (PEP carboxylase) (Nishikido et al., 1965; Maeba and Sanwal, 1965) which is known to replenish oxaloacetate by carboxylation prompted us to test the effect of these compounds on this purified malic enzyme since malic enzyme and PEP carboxylase, seemingly, function in the opposite direction to each other.

As can be seen in Fig. 1, the addition of L-aspartate stimulated the enzyme activity several times depending on the substrate concentration. Moreover, the results in Fig. 1 indicate that the substrate saturation curve becomes sigmoidal one characteristic of the allosteric enzyme when L-aspartate at low concentration is present in the reaction mixture. Replotting these data according to Lineweaver and Burk gave a marked curvature upwards (Fig. 2-a). A linear relationship was obtained if $1/v$ was plotted against $1/(\text{malate})^2$ (Fig. 2-b).

As shown in Fig. 3, a slight substrate-cooperativity was observed even in the absence of aspartate. The extent of cooperativity and aspartate stimulation varied with the change of pH value.

No stimulation of the enzyme was observed with the compounds such as L-asparagine, L-glutamate, DL-alanine, L-arginine, L-cystine, fumarate, succinate, citrate, isocitrate, α-ketoglutarate and oxaloacetate (5×10^{-4} M). Unlike the DPN-linked enzyme, the activity of TPN-linked enzyme, so far tested, was not affected by aspartate.

Unexpectedly, acetyl-CoA inhibited the activity only to about the same order of magnitude as some nucleotides such as CoA and ATP, though the inhibition was significant.

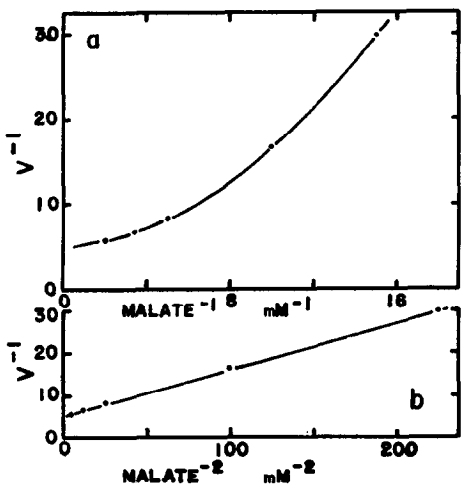


FIG. 2

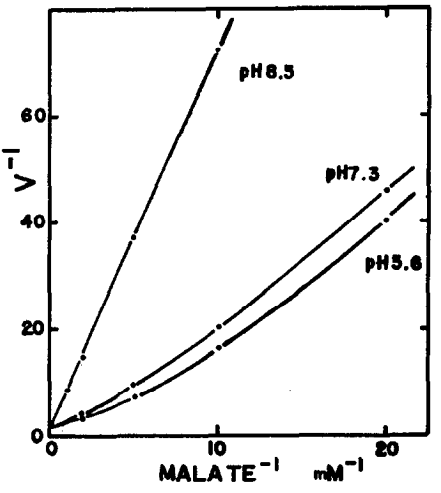


FIG. 3

Fig. 2. Relationship between malate concentration and velocity in the presence of 0.1 mM L-aspartate. The reciprocal of the reaction velocities are plotted against (a) 1/(malate) and (b) $1/(\text{malate})^2$.

Fig. 3. Substrate cooperativity of the enzyme at various pH in the absence of aspartate. The experimental conditions were the same as those shown in Fig. 1, except that different buffer solutions were used at indicated pH (Tris-HCl for pH 8.5 and 7.3: acetate for pH 5.6).

The specific effect of L-aspartate on the DPN-linked enzyme was also confirmed by the experimental results shown in Table 1. L-Aspartate among several compounds tested significantly protected the enzyme against heat inactivation. This protecting effect by aspartate was not found in the case of the TPN-linked enzyme.

Discussion -- The results obtained suggest that the DPN-linked malic enzyme plays its role for the decomposition of malate. The TPN-linked enzyme, though it catalyzes the same reaction, seems to have another physiological role. These

TABLE I

Protection of Enzyme Against Heat Inactivation

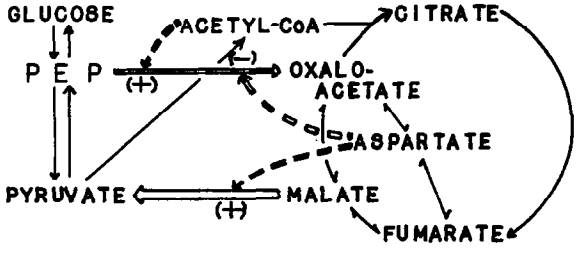
Additions	Activity survived (%)
None	27.8
L-Malate	37.7
L-Aspartate	58.4
Succinate	31.5
Pyruvate	31.5
L-Glutamate	37.7

The incubation mixture contained 200 μmoles of Tris-HCl buffer, pH 6.9, 2 μmoles of $MnCl_2$, the supernatant (12 mg of protein) obtained by the centrifugation at 10^5 xG and the subsequent dialysis, and 10 μmoles of addition compounds as potassium salt in final volume of 2.2 ml. The activities of the enzyme before and after the incubation for 4.5 minutes at 51°C were measured and the survived activities were calculated.

situations are somewhat similar to those of two isocitric dehydrogenases in mammals (Chen and Plaut, 1963; Goebell and Klingenberg, 1963) and fungi (Sanwal et al., 1963) and of two glutamate dehydrogenases in Thiobacillus novellus (LéJohn, 1967) requiring TPN and DPN, respectively. Only the DPN-linked enzymes in both cases were demonstrated to be an allosteric enzyme concerning in the regulation for the decomposition of isocitrate and glutamate.

Previously, with the same strain of E. coli, it was reported from this laboratory that PEP carboxylase, an allosteric enzyme, is stimulated by acetyl-CoA and strongly inhibited by aspartate, fumarate and malate (Nishikido et al., 1965). Obviously PEP carboxylase plays its role in replenishing oxaloacetate for the maintenance of the activity of TCA cycle taking the balance between C_4-dicarboxylic acids and acetyl-CoA. On the other hand, aspartate, which is accumulated when the level of C_4-dicarboxylic acids is high, stimulates the decomposition of C_4-compounds to

C_3-compounds by affecting the activity of DPN-linked malic enzyme. However, it remains unsolved whether the DPN-linked malic enzyme is concerned in the control for the conversion of C_4-compounds to acetyl-CoA via pyruvate. These situations are summarized in the scheme.



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