# ALLOSTERIC CONTROL OF THE ACTIVITY OF MALIC ENZYME IN ESCHERICHIA COLI*

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Ever since its discovery (Ochoa et al., 1948) the function of malic enzyme (malate: TPN oxidoreductase (decarboxylating); E.C. 1.1.1.40) in diverse organisms has remained largely enigmatic. In microorganisms, the general consensus of opinion seems to be that it has a role in the generation of pyruvate when the supply of $C_4$ compounds is abundant (Ashworth et al., 1965; Jacobson et al., 1966). Recent work of Katsuki et al., (1967) and Takeo et al., (1967) demonstrates that the TPN-specific malic enzyme in E. coli is induced in the presence of malate as the sole carbon source. Also, it has been shown by Jacobson et al., (1966) that the malic enzyme in Pseudomonas is repressed in the presence of acetate.

In the following report we show that the TPN-specific malic enzyme in E. coli is an allosteric protein whose activity is controlled by a negative feed-back inhibition by acetyl-CoA.

Experimental - Escherichia coli, strain K12, was grown in a mineral salts medium with 0.4% glycerol as the sole carbon source. The cells were harvested in the stationary phase of growth and disrupted in 0.05 M phosphate buffer, pH 7.0 by sonic oscillation. Cell debris was removed by centrifugation. The pH of the supernatant solution

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was adjusted to 5.2 and it was heated for 5 min. at 57°. The bulky precipitate was discarded and malic enzyme was precipitated in the cold by ethanol (20%-30%). Further purification was achieved by suspension of the enzyme in 0.02 M phosphate buffer, pH 5.2, adsorption on calcium phosphate gel, and elution by 0.2 M $(NH_4)_2SO_4$, pH 6.5. Routinely, this procedure gave an approximate 36-fold purification of the TPN-specific malic enzyme. Such preparations contained only small amounts of DPN-specific malic dehydrogenase and traces of DPN-specific malic enzyme (Katsuki et al., 1967), and glutamate dehydrogenase. The enzyme was dialyzed extensively before use against 0.05 M phosphate, pH 7.0, containing $10^{-4}$ M EDTA.

The malic enzyme was measured by following the rate of TPN reduction in an assay medium containing 10 mM sodium L-malate, 1 mM $MnCl_2$, 0.077 mM TPN and 0.05 M Tris-Cl, pH 7.5. Velocity is given as a change in absorbance at 340 mμ per min. at 22-24°.

Results and Discussion - As shown in Fig. 1a, malic enzyme is inhibited by acetyl-CoA. The inhibition curve is sigmoid, a characteristic which malic enzyme shares with large numbers of other allosteric enzymes (Monod et al., 1965). The inhibition is quite specific to acetyl-CoA. Under conditions (see legend to Fig. 1) where 0.25 mM acetyl-CoA gave approximately 50% inhibition, fructose diphosphate, phosphoenolpyruvate, AMP, ATP and CoA each tested at a concentration of 1.0 mM and fumarate and succinate each at 8.0 mM failed to affect enzyme activity. These facts not only point to the specificity of acetyl-CoA as an inhibitor but also indicate that acetyl-CoA perhaps does not bring about inhibition by chelating the $Mn^{++}$ ions essential for enzyme activity. The $K_m$ for $Mn^{++}$ is about $1.3 \times 10^{-5}$ M and in the experiment reported in Fig. 1 the $Mn^{++}$ concentration is nearly 100 x $K_m$.

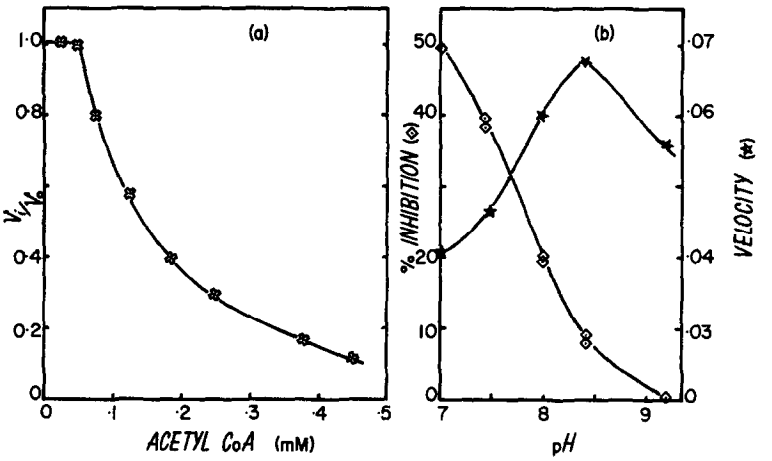


Fig. 1a. Inhibition of malic enzyme by Acetyl-CoA. The assay mixture contained, 2.5 mM malate, 0.03 mM TPN, 1.0 mM $MnCl_2$ and Tris-HCl, 0.1 M, pH 7.0, and varying amounts of the inhibitor.

Fig. 1b. Effect of pH on the velocity of the enzyme and inhibition by 0.25 mM Acetyl-CoA. Assay conditions are given in Fig. 1a.

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When malate was used as a variable substrate in the presence of 20 x $K_m$ of TPN ($K_m$ for TPN = 0.0075 mM) and 100 x $K_m$ of $Mn^{++}$, the initial velocity plot was hyperbolic (Fig. 2) but it became sigmoidal in the presence of concentrations of acetyl-CoA higher than 0.25 mM. Owing to the sigmoidality of the plots it is difficult to ascertain whether acetyl-CoA competes with malate, but it is considered to be unlikely (Fig. 2). In contrast to malate, the hyperbolic rate-concentration curves with TPN as the variable substrate do not significantly deviate from a hyperbola in the presence of 0.25 mM acetyl-CoA. The question arises whether inhibition by acetyl-CoA is due to its binding on a specific, allosteric site. In an effort to desensitize the enzyme, we, therefore, tested the effect of pH of the assay medium on the inhibition by acetyl-CoA. These results are presented in Fig. 1b.

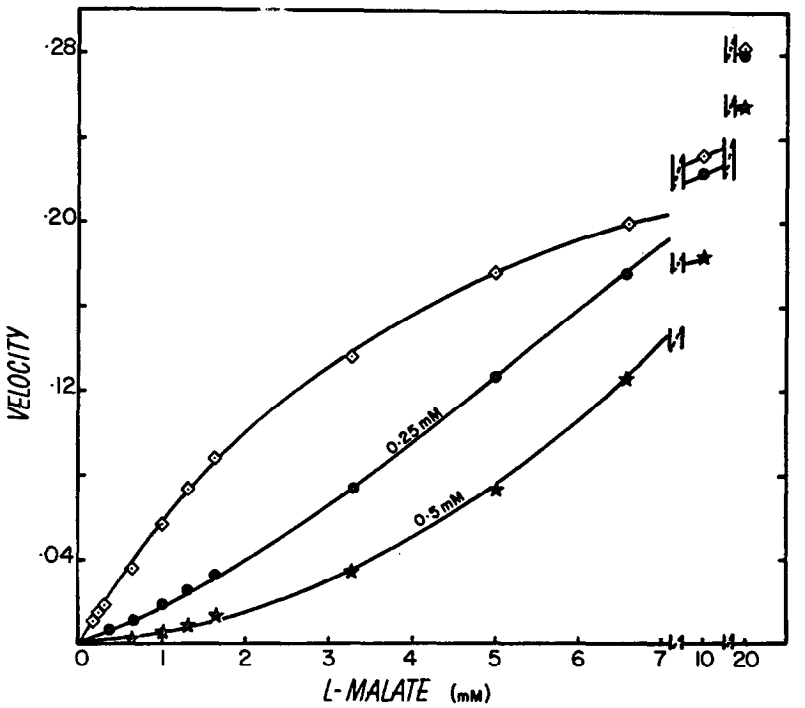


Fig. 2. Initial velocity plots of the enzyme with malate as the variable substrate in the absence and presence of two concentrations of Acetyl-CoA. The concentrations of non-varied substrates were: TPN, 0.15 mM and $MnCl_2$, 1.0 mM. Tris-Cl, 0.05 M, pH 7.5, was used as buffer.

It will be noted that 50% inhibition caused by 0.25 mM acetyl-CoA at a fixed concentrations of the substrates at pH 7.0 is reduced to zero at a pH of 9.3. The activity of the enzyme itself in the absence of inhibitor is slightly higher at pH 9.3 as compared to pH 7.0 (Fig. 1b). It thus seems likely that the inhibitor binding site is quite distinct from the substrate binding sites.

The kinetics of malic enzyme resembles that of the threonine deaminase from Salmonella (Maeba and Sanwal, 1966). Indeed, in recent years a number of other allosteric enzymes have been described (see, Maeba and Sanwal, 1966) which follow Michaelis-Menten kinetics only in the absence of inhibitors but not in their presence.

Without invoking any subunit interactions to explain sigmoidality (Monod, et al., 1965; Koshland et al., 1966) such plots (in the presence of allosteric effectors) can easily be explained on the basis of partial inhibition, i.e., on the assumption that the enzyme-inhibitor complex binds the substrates but the rate constants for the breakdown of ES and EIS are different. The equations derived for such cases (see, Maeba and Sanwal, 1966) should be equally applicable with minor modifications to the kinetics of malic enzyme.

The results presented above lead to the conclusion that the physiological function of the TPN-specific malic enzyme is to generate pyruvate, possibly under conditions when malate or other $C_4$ compounds (which can easily generate malate) are relatively abundant in the cell. Acetyl-CoA, according to this line of reasoning would be the end-product of the pathway leading from malate to pyruvate, and inhibition of the malic enzyme would be equivalent to a negative feed-back inhibition.

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