INHIBITION OF NADP-LINKED MALIC ENZYME BY ATP IN *ACINETOBACTER*

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Received 17 January 1975

1. Introduction

Several hypotheses have been proposed to explain the metabolic function and regulation of malic enzyme (L-malate: $NADP^+$-oxidoreductase (decarboxylating) EC 1.1.1.40). In micro-organisms, the general consensus of opinion seems to be that it has a role in the generation of pyruvate and NADPH when the supply of C_4-dicarboxylic acids is abundant [1,2].

Acetyl-CoA, which derives from pyruvate, is an inhibitor of the malic enzyme from *Escherichia coli* [3] and *Acinetobacter calcoaceticus* [4]. The enzyme in *Pseudomonas putida* [2] but also in *Acinetobacter calcoaceticus* [5] is repressed during growth on acetate and *n*-alkanes, ensuring the content of C_4-dicarboxylic acids required for the tricarboxylic acid cycle. In this communication we report the inhibition of malic enzyme from *Acinetobacter calcoaceticus* by ATP.

2. Materials and methods

Ac. calcoaceticus was cultured aerobically at 30°C in minimal medium [6] with 2% succinate as the sole carbon source. The cells were harvested at the end of logarithmic growth, washed, suspended in buffer of composition 100 mM Tris–HCl, pH 8.0, 1 mM EDTA and disrupted by ultrasonication. Nucleic acids were removed by treatment with protamine sulphate. After centrifugation the supernatant solution obtained was fractionated with ammonium sulphate. The material precipitating between 35% and 50% saturation was redissolved in a small vol of buffer (see above), dialysed against 67 mM phosphate, pH 7.5, 1 mM EDTA and 1 mM mercaptoethanol, and then applied to a column of Sephadex G-200 (2.5 × 100 cm) equilibrated with this phosphate buffer at 4°C. Elution was carried out with the same phosphate buffer and 4 ml fractions were collected. The active peak fractions were pooled and concentrated. The specific activity of the enzyme, which was used for all the experiments reported here, was 6.22 μmol of NADPH formed/min/mg protein.

The activity of the malic enzyme was measured spectrophotometrically by following the formation of NADPH at 340 nm and 25°C. Unless otherwise stated, assay mixtures contained 0.1 M Tris–HCl, pH 7.9, 2.4 mM L-malate, 1 mM $NADP^+$ and 1 mM $MnCl_2$. The reaction was initiated by the addition of enzyme. Protein was determined by the method of Lowry et al. [7].

All chemicals were analytical grade reagents of the highest grades available.

3. Results and discussion

As shown in table 1, the malic enzyme is inhibited by nucleotides. Although the enzyme was sensitive to all five nucleoside triphosphates tested, ATP, CTP, GTP, ITP, and UTP; the most effective of these was ATP. ADP is also a powerful inhibitor. AMP and other nucleoside monophosphates have no effect on the rate of catalysis.

With ATP as a model inhibitor the inhibition curve is sigmoid (fig.1), a characteristic which malic enzyme shares with large numbers of other allosteric enzymes [8]. In this experiment the Mn^{++} concentration is nearly 50 × K_m (K_m for Mn^{++} = 0.019 mM).

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Table 1
Inhibition of malic enzyme reaction by nucleotides

Inhibition %	Nucleotides (3 mM)
> 60	ATP, ADP, ITP
40–59	CTP, GTP, UTP
20–39	CDP, GDP, UDP
< 3	AMP, 3′,5′-AMP, CMP GMP, IMP, UMP

The reaction mixtures contained (in μmol) in a final vol of 3 ml: Tris–HCl buffer, pH 7.9, 300; L-malate, 3.6; $NADP^+$, 1.5 $MnCl_2$, 3 and the indicated nucleotide. The reaction was started by the addition of the enzyme.

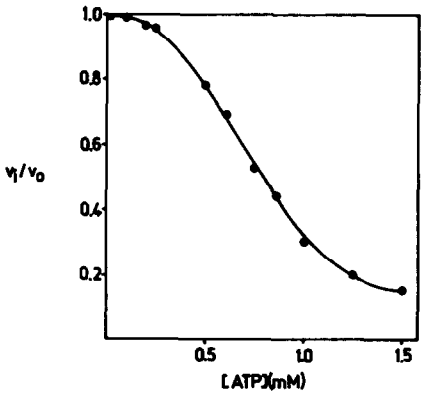

Fig.1. Inhibition of malic enzyme by ATP. Assays were carried out under standard conditions with 1 mM L-malate and 0.2 mM $NADP^+$. v_o and v_i refer to the activity in the absence or presence of ATP, respectively.

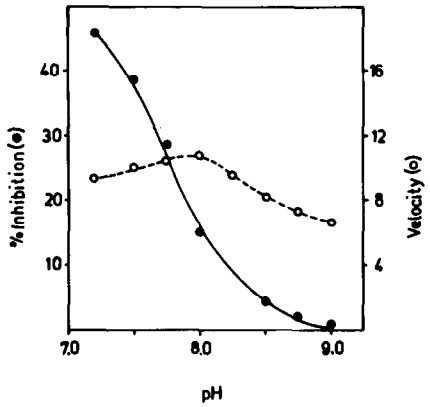

Fig.2. Effect of pH on the reaction velocity of the enzyme and its inhibition by 1 mM ATP. Assay conditions are given in Materials and methods, except for the concentrations of L-malate and $NADP^+$, which were 0.5 mM.

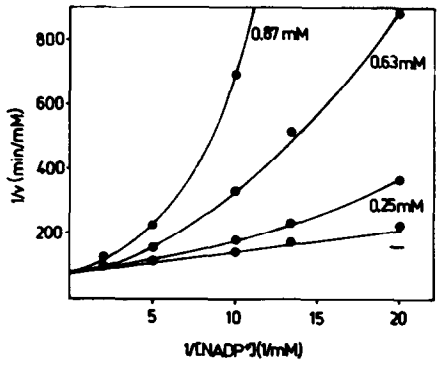

Fig.3. Inhibition of malic enzyme by ATP with $NADP^+$ as the variable substrate at pH 7.9. The L-malate concentration was 1 mM. The concentrations of ATP used are indicated under the curves.

It will be noted that 44% inhibition caused by 1 mM ATP at fixed concentrations of the substrates at pH 7.2 is reduced to zero at pH 9.0 (fig.2). The activity of the enzyme itself in the absence of inhibitor is slightly higher at pH 7.2 as compared to pH 9.0.

In the absence of inhibitors the double reciprocal initial velocity plots of both of the substrates, $NADP^+$ (fig.3) and L-malate (fig.4), were linear. With ATP as the inhibitor, however, the initial velocity plots for $NADP^+$ (fig.3) and malate (fig.4) became nonlinear.

Although the levels at which ATP is an effective inhibitor, 0.2–1.25 mM, appear to be within the generally accepted physiological range, it is difficult to distinguish between a direct effect on the enzyme, namely, binding at an inhibitor site, or an indirect effect produced by a nonenzymatic interaction between the metallic cofactor and ATP. Subsequent experiments (table 2), however, showed that preincubation of the enzyme with ATP (2 mM) for 30 min results in significantly greater ratios of inhibition than those obtained when measurements are initiated immediately after adding ATP, although the amounts of $MnCl_2$ and the substrates in either case are the same. Table 2 also shows that the effect

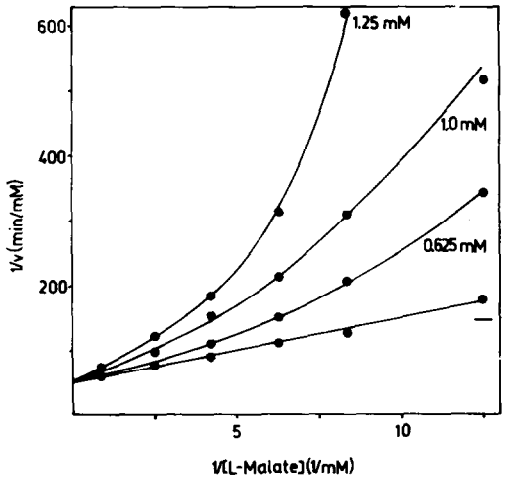

Fig.4. Inhibition of malic enzyme by ATP with malate as the variable substrate at pH 7.9. The $NADP^+$ concentration was 0.5 mM. The concentrations of L-malate used are indicated under the curves.

of ATP addition to the enzyme preincubated with Mn^{++} for 30 min, is essentially the same as without prior incubation of the enzyme with the cation. It may be noted that in all assays the amounts of Mn^{++} and ATP are constant in each case. The marked inhibition noted if the enzyme is exposed to ATP before the addition of Mn^{++} to the reaction mixture, indicates that ATP has a direct inhibitory effect on the enzyme which is quite distinct from Mn^{++} complexing.

Similar inhibitory effects of nucleoside triphosphates on the NADP-linked malic enzyme of bacteria have not previously been reported. However, an inducible NAD-linked malate dehydrogenase (decarboxylating) from *Streptococcus faecalis* was shown to be subject to a product inhibition by nucleoside triphosphates [9] and also the NAD-specific malic enzyme of *Escherichia coli* is inhibited by ATP (and some other nucleoside triphosphates) in allosteric manner [10].

The reasons for the inhibition of malic enzyme by ATP are not completely clear. The inhibition may serve as a regulatory mechanism providing the organism with an effective means of controlling malate catabolism during growth. A control mechanism of this sort would be necessary if the

Table 2
Effect of ATP on malic enzyme activity

Treatment	Inhibition (%)
Assayed immediately after addition	45
Preincubated with $MnCl_2$ for 30 min.	49
Preincubated with ATP for 30 min.	71

Assays were run at alkaline pH and substrate concentrations as described in Materials and methods.

bacteria are to maintain a balance between biosynthesis and energy production. By a high content of ATP the inhibition of malic enzyme may be advantageous because under such conditions there may not be a high demand for pyruvate by the energy-generating pathway of the tricarboxylic acid cycle. This argument finds support from the earlier observations in other micro-organisms that one form of pyruvate kinase [11,12] and pyruvate dehydrogenase [13] are both activated by AMP, a compound whose level is expected to be quite low when the ATP: ADP concentration is high [14].

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