REGULATION OF NADP-LINKED ISOCITRATE DEHYDROGENASE ACTIVITY IN *ACINETOBACTER*

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1. Introduction

Within the last twenty years several investigators have reported the stimulation of NAD-linked isocitrate dehydrogenase (IDH) from a number of eucaryotic organisms by AMP or ADP, and it is believed that this effect plays a role in the overall regulation of the tricarboxylic acid cycle [1]. Similar stimulatory effects on the IDH of bacteria have not previously been reported, though nucleotide inhibition has been observed [2, 3].

A study of the IDH of *Acinetobacter lwoffi* has revealed the existence in this organism of two NADP-linked isoenzymes which we have termed IDH-I and IDH-II [4, 5]. The higher molecular weight isoenzyme, IDH-II, was found to be stimulated several-fold by low levels of glyoxylate or pyruvate, and preliminary experiments [6] also indicated some stimulation of this isoenzyme by AMP.

In this communication we report the marked stimulatory effects of AMP and ADP on IDH-II and show that the system displays the expected dependence on "energy charge" [7].

2. Experimental

A. lwoffi was cultured aerobically at 37° on nutrient broth. The cells were harvested at the end of logarithmic growth, washed, suspended in buffer of composition 20 mM tris, 10 mM $MgCl_2$, 1 mM EDTA, pH 8.0 and disrupted by ultrasonication. Nucleic acid was removed by treatment with protamine sulphate (1.3 mg per 10 mg protein) and the supernatant solution obtained after centrifugation was fractionated with ammonium sulphate. The material precipitating between 40% and 65% saturation was redissolved in a small volume of buffer (see above), dialysed against 50 mM phosphate, pH 8.0, 1 mM EDTA, 1 mM mercaptoethanol and then applied to a column of DEAE-cellulose equilibrated with this phosphate buffer at 4°. Elution was carried out with a phosphate gradient from 50 mM to 250 mM and 6-ml fractions were collected. As previously observed [5] IDH-I was eluted from the column before IDH-II. Fractions containing the separated isoenzymes were used in the experiments reported below.

Unless otherwise stated, IDH activity was measured spectrophotometrically at 340 nm and 25° in the presence of 20 mM tris, pH 8.0, 10 mM $MgCl_2$, 1 mM EDTA, 0.2 mM NADP and 5 mM D-isocitrate. The reaction was initiated by the addition of enzyme.

The effects of adenine nucleotides were examined by incorporating the appropriate concentrations of these into the assay mixture. The effects of energy charge were investigated by incubating appropriate mixtures of AMP + ATP (each 10 mM) with 10 μg of adenylate kinase (Sigma) at 37° for 15 min to reach equilibrium [7]. Aliquots of these mixtures were then incorporated into the assay medium to produce the required total nucleotide concentration (AMP + ADP + ATP).

3. Results and discussion

We first tested both IDH-I and IDH-II for sensitivity to adenine nucleotides. In the presence of 1 mM AMP or ADP the activity of IDH-II was stimulated five-fold and two-fold respectively, while 1 mM ATP

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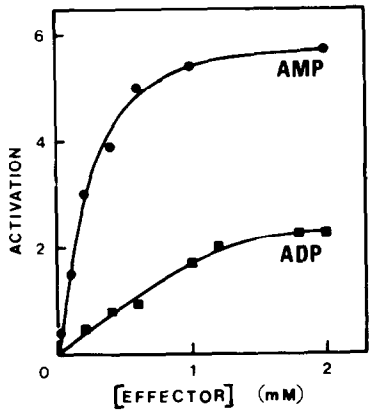

Fig. 1. Dependence of enzyme activation on AMP/ADP concentration. See text for definition of activation and assay conditions. • AMP. ▪ ADP.

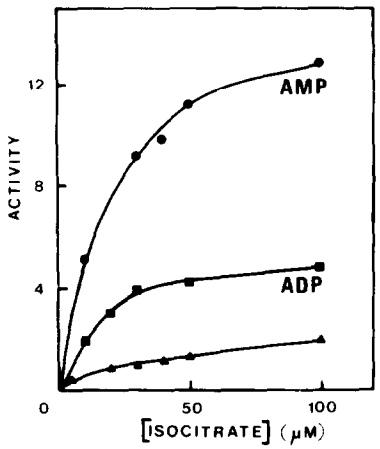

Fig. 2. Dependence of enzyme activity on isocitrate concentration in the absence and presence of AMP/ADP. ▲ no added nucleotide. • + 1 mM AMP. ▪ + 1 mM ADP.

exerted essentially no effect. By contrast, IDH-I remained unaffected by all three nucleotides. Fig. 1 shows the dependence of activation of IDH-II on the concentration of AMP or ADP. We define activation as the ratio of the additional activity measured in the presence of the activator to the activity in its absence.

We next examined the effects of AMP and ADP on the rate dependences of IDH-II on substrate concentrations. Figs. 2 and 3 show the dependences on iso-

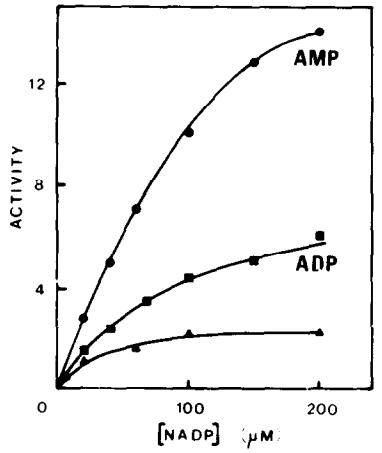

Fig. 3. Dependence of enzyme activity on NADP concentration in the absence and presence of AMP/ADP. ▲ no added nucleotide. • + 1 mM AMP. ▪ + 1 mM ADP.

citrate and NADP concentrations in the absence and presence of 1 mM AMP or ADP. These results, together with double reciprocal plots, indicate that although some change of the apparent K_m values does occur the stimulation is primarily an effect exerted on the V_{max}.

Atkinson [8] has discussed systems such as the present one in which enzyme activity is regulated by the various levels of AMP, ADP and ATP. He has proposed that it is preferable to examine activity at a constant total nucleotide concentration (AMP + ADP + ATP) while varying the "energy charge". This is a measure of the energy content within a fixed total nucleotide pool and is defined as half the average number of anhydride-bound phosphate groups per adenine moiety, or

$$\frac{[ATP] + 0.5[ADP]}{[ATP] + [ADP] + [AMP]}$$

Examination of the effects of energy charge may conveniently be carried out by allowing adenylate kinase to act on various proportions of AMP + ATP and then introducing the equilibrium mixtures of AMP + ADP + ATP into the enzyme assay. Fig. 4 shows the dependence of IDH-II activation on the energy charge at three different total nucleotide concentrations. The curves obtained are precisely of the form expected for

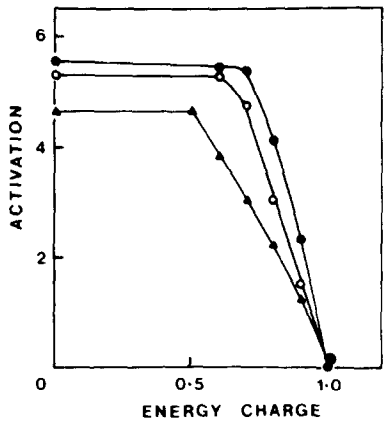

Fig. 4. Dependence of enzyme activation on energy charge. See text for experimental details. Total nucleotide concentrations: ▲ 1 mM; ○ 2 mM; ● 3 mM.

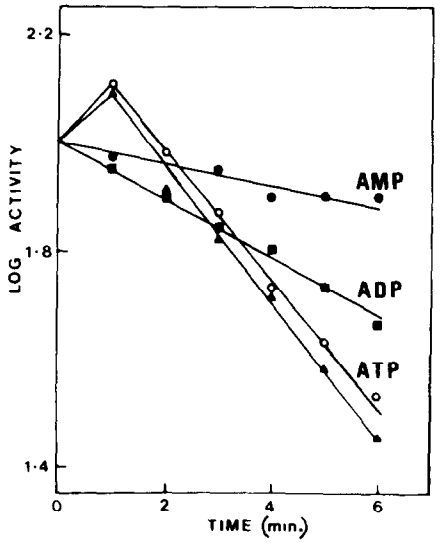

Fig. 5. Thermal inactivation of isocitrate dehydrogenase at 42° in the absence and presence of adenine nucleotides. ▲ no added nucleotide. ● + 1 mM AMP. ■ + 1 mM ADP. ○ + 1 mM ATP.

the regulation of an enzyme concerned, as is IDH, in ATP regeneration. The enzyme is seen to be fully activated over a wide range of energy charge but exhibits a sharp fall in the upper end of the range. Such a response permits sensitive control of enzyme activity by the energy charge. Our results resemble closely those obtained for yeast NAD-linked IDH [8].

It is not unreasonable that the stimulatory action of AMP and ADP arises from a structural or conformational change of the enzyme molecule. We have sought evidence for this by examining the thermal inactivation of the enzyme in the presence and absence of adenine nucleotides. The results are presented in fig. 5. ATP had little effect, but AMP and ADP produced a marked alteration of the pattern of thermal inactivation in two ways. First, the initial increase in activity produced after a brief heat treatment of enzyme alone was not observed in the presence of AMP or ADP. Secondly, ADP and particularly AMP reduced the rate of thermal inactivation. These results are consistent with a nucleotide-induced alteration of enzyme conformation and are in line with the kinetic observations reported above.

Our results indicate for the first time a regulatory behaviour of a bacterial NADP-linked IDH comparable with that of NAD-linked IDHs of higher organisms. Such regulation may play an important role in the energy metabolism of the cell.

Acknowledgement

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