REGULATION OF α-KETOGLUTARATE DEHYDROGENASE ACTIVITY IN *ACINETOBACTER*

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Received 4 March 1972

1. Introduction

α-Ketoglutarate plays a dual role in the tricarboxylic acid cycle. It may either be oxidised through the cycle via α-ketoglutarate dehydrogenase (α-KGDH) and hence yield energy to the cell, or be withdrawn from the cycle under the action of glutamate dehydrogenase and thus give rise to the glutamate family of amino acids.

In our studies of the regulation of other tricarboxylic acid cycle enzymes in *Acinetobacter* we have found that citrate synthase may be controlled by the levels of NADH and AMP [1] and that isocitrate dehydrogenase may also be subject to adenylate control [2]. In view of these findings it was of interest to examine the effects of NADH and adenine nucleotides on the activity of α-KGDH from *Acinetobacter*. The results reported here show that the regulatory effects of these 'signal' metabolites on the enzyme are consistent with its role in energy metabolism.

2. Experimental

The enzyme was prepared in the following way. *Acinetobacter* sp., strain 4B, was grown aerobically at 37° in nutrient broth or salts-acetate (50 mM) medium. The cells were harvested at the end of logarithmic growth, washed, resuspended in buffer of composition 20 mM Tris-HCl, pH 8.0, 10 mM $MgCl_2$, 1 mM EDTA and disrupted by passage twice through a French press. Cell debris was removed by centrifugation at 25,000 *g* for 10 min and the preparation was then centrifuged at 200,000 *g* for 40 min to remove NADH oxidase activity. The clear supernatant solution so obtained was used without further purification.

The activity of the α-KGDH enzyme complex was measured spectrophotometrically by following the formation of NADH at 340 nm and 25°. Assay mixtures contained 0.15 M Tris-HCl, pH 8.0, 0.1 mM coenzyme A, 0.5 mM NAD, 0.2 mM thiamine pyrophosphate, 2.5 mM cysteine and α-ketoglutarate as indicated in the figures. The reaction was initiated by the addition of enzyme. When other substances were tested for their effect on activity they were

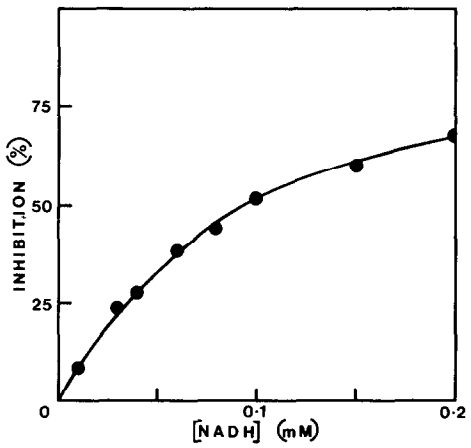

Fig. 1. Inhibition of *Acinetobacter* α-KGDH by NADH. Assays were carried out under standard conditions with 5 mM α-ketoglutarate.

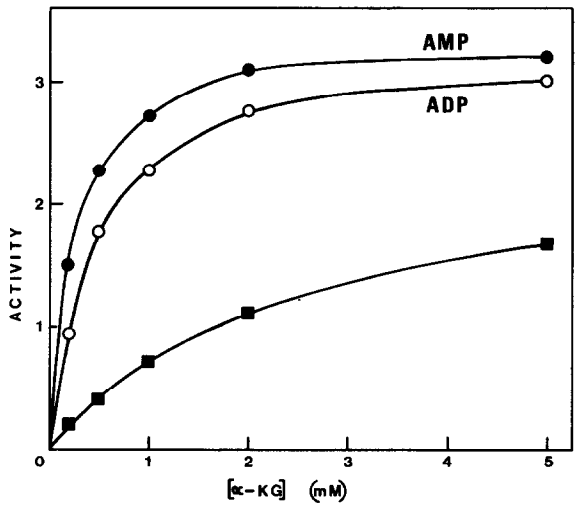

Fig. 2. Dependence of *Acinetobacter* α-KGDH on α-ketoglutarate concentration in the absence and presence of nucleotides. (■—■—■), no added nucleotide; (●—●—●), with 0.2 mM AMP; (○—○—○), with 0.2 mM ADP. Activities are in arbitrary units.

present in the reaction mixture prior to the introduction of enzyme.

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

3. Results and discussion

The effect of NADH on enzyme activity was first examined. It was found that NADH is a powerful inhibitor of the enzyme complex, 50% inhibition being produced by 0.1 mM NADH. Fig. 1 shows the dependence of inhibition on NADH concentration.

The effect of AMP on this inhibition was then examined. At a concentration of 0.2 mM, AMP completely overcame the inhibition produced by 0.1 mM NADH but, unexpectedly, the activity measured in the presence of both NADH and AMP was considerably greater than that measured for enzyme alone in the absence of any effectors. These results were confirmed by an entirely independent assay procedure [3] in which the acylation of coenzyme A was followed polarographically. Thus AMP was capable not only of overcoming the inhibition by NADH but also of stimulating the basal level of enzyme activity. This was confirmed by various assays of α-KGDH activity in the absence and presence of AMP which showed that AMP stimulated activity, the more so at lower α-ketoglutarate concentrations. The effects of AMP, ADP and ATP on the kinetic behaviour of α-KGDH were investigated.

Fig. 2 shows the dependence of α-KGDH activity on α-ketoglutarate concentration and the effects of AMP and ADP on this dependence. Both of these nucleotides produce considerable stimulation of activity accompanied by a marked lowering of the apparent K_m for α-ketoglutarate. Double reciprocal plots of these data indicate a shift of the K_m from 2.5 mM in the absence of added nucleotide to 0.27 mM in the presence of 0.2 mM AMP and 0.43 mM in the presence of 0.2 mM ADP. ATP produced no stimulation but, on the contrary, a small degree of inhibition.

The relative effects of AMP/ADP/ATP as a function of nucleotide concentration were examined at a low concentration (0.5 mM) of α-ketoglutarate, and the results are presented in fig. 3. AMP is seen to be the most effective activator of the enzyme, achieving a 450% increase in activity at 0.2 mM concentration. ADP is somewhat less powerful an activator, while ATP is exclusively inhibitory over the concentration range examined.

The dependences of enzyme activity on the concentrations of NAD and CoA were also examined in the absence and presence of the adenine nucleotides. There appeared to be little change in the K_m values for these other substrates and certainly nothing comparable with the large change in the K_m for α-ketoglutarate.

Our comparative studies of the regulation of another tricarboxylic acid cycle enzyme, citrate synthase, in diverse organisms [1] has revealed a marked distinction between the enzymes from aerobic and facultatively anaerobic Gram-negative bacteria. The citrate synthases from strict aerobes, such as *Acinetobacter,* are inhibited by NADH and this inhibition is overcome by AMP or ADP. On the other hand, the facultative organisms, such as *E. coli,* show NADH inhibition of citrate synthase but no adenylate reactivation.

The results presented here show that in *Acinetobacter* the regulatory sensitivities of citrate synthase and α-KGDH are similar. For each enzyme NADH

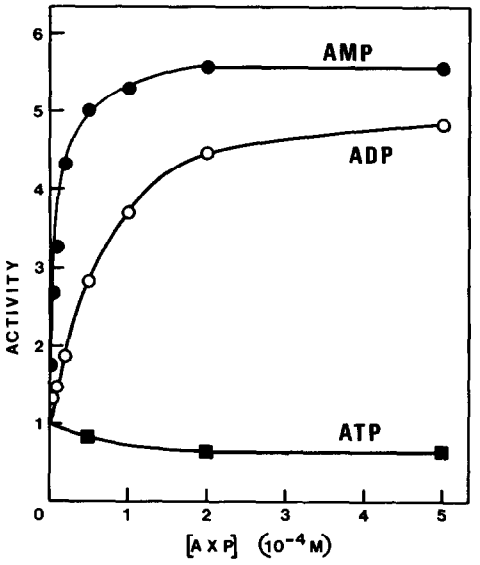

Fig. 3. Effects of adenine nucleotides on *Acinetobacter* α-KGDH activity. Assays were carried out under standard conditions with 0.5 mM α-ketoglutarate (●—●—●), AMP; (○—○—○), ADP; (■—■—■), ATP. Activities are in arbitrary units.

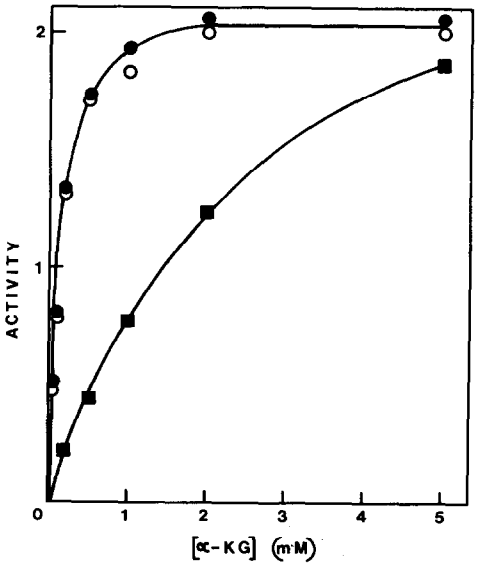

Fig. 4. Comparison of *E. coli* and *Acinetobacter* α-KGDH activity dependences on α-ketoglutarate concentration. (●—●—●), *E. coli* enzyme; (○—○—○), *E. coli* enzyme plus 0.2 mM AMP; (■—■—■), *Acinetobacter* enzyme. Activities are in arbitrary units.

acts as a negative effector and AMP/ADP exert positive effector action. It was therefore of interest to examine the sensitivity of *E. coli* α-KGDH to these metabolites. A preparation of α-KGDH was obtained from acetate-grown *E. coli* K12 by the same procedure as described above for the *Acinetobacter* enzyme. In agreement with a previous observation [4] the *E. coli* enzyme was also found to be strongly inhibited by NADH. However, AMP and ADP were unable to reverse this inhibition, nor did they produce any effect on the activity of the enzyme alone (fig. 4). Thus in *E. coli,* too, the regulation of α-KGDH is similar to that of citrate synthase; in each case NADH is inhibitory and the adenine nucleotides are without effect.

It is also interesting to compare the dependences of α-KGDH activities from the two organisms on α-ketoglutarate concentration (fig. 4). The K_m for α-ketoglutarate of the *E. coli* enzyme is very much lower than that of the *Acinetobacter* enzyme but is similar to the value obtained for the latter enzyme in the presence of AMP (fig. 2).

Further investigation of the AMP/ADP stimulation of *Acinetobacter* α-KGDH may well show this effect to be of the allosteric type. It is however noteworthy that no sigmoidicity has been observed in the rate dependences on substrates, activators or inhibitor.

Nucleotide activation of cauliflower α-KGDH was recently described [5] but the present communication is the first report of such activation of a bacterial α-KGDH. The physiological significance of the regulation of *Acinetobacter* α-KGDH may be related to the regulation of the tricarboxylic acid cycle as a whole. NADH is an end-product both of the cycle and of the α-KGDH complex, and its inhibition of both citrate synthase and α-KGDH may represent a feedback control mechanism. Stimulation by the 'low-energy' signals AMP and ADP may be part of the organism's devices for regulating energy metabolism by channelling α-ketoglutarate round the cycle and away from biosynthetic reactions.

Acknowledgements

I thank Miss Janet K. Wildman for skilled technical assistance.

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