CONTROL OF NADP$^+$-SPECIFIC ISOCITRATE DEHYDROGENASE FROM *ACINETOBACTER* BY NUCLEOTIDES

H.-P. KLEBER and H. AURICH

Section of Biological Sciences, Dept. of Biochemistry, Karl Marx University, Leipzig, G.D.R.

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1. Introduction

In mammalian tissues and yeasts NAD$^+$- and NADP$^+$-specific forms of isocitrate dehydrogenase are described. The most important activators of NAD$^+$-linked isocitrate dehydrogenase are AMP and ADP, while ATP has been found to be inhibitory [1]. However, *Acinetobacter* and most other bacteria contain only the NADP$^+$-specific form of the enzyme [2].

A study of the NADP$^+$-linked isocitrate dehydrogenase (threo-D_s-isocitrate: NADP$^+$ oxidoreductase (decarboxylating); EC 1.1.1.42) of *Acinetobacter* [3–5] and *E. coli* [6] has revealed the existence of multiple forms of the enzyme in these organisms. In *Ac. lwoffi* [7] only the higher mol. wt. isoenzyme was found to be stimulated by AMP and ADP, while ATP exerted essentially no effect.

In this communication we report the stimulatory effects of AMP and ADP on the higher molecular weight form of NADP$^+$-linked isocitrate dehydrogenase (mol. wt around 360 000) from *Ac. calcoaceticus* and the inhibition of this enzyme by nucleoside triphosphates.

2. Materials and methods

Ac. calcoaceticus was cultured aerobically at 30°C in a minimal medium [8] containing 2% sodium acetate as sole carbon source. The cells were harvested at the end of exponential growth phase, washed, suspended in buffer of composition 50 mM Tris–HCl (pH 7.5), 10 mM $MgCl_2$, 1 mM EDTA and disrupted by ultrasonication. The homogenate was centrifuged at 20 000 *g* for 1 h at 4°C. The supernatant obtained was fractionated with ammonium sulphate. The material precipitating between 40% and 65% saturation was dissolved in a small volume of buffer (see above). The resulting solution was applied to a Sephadex G-200 column (2.5 × 80 cm) equilibrated with 50 mM Tris–HCl/1 mM EDTA buffer, pH 7.7. Fractions of 4 ml were collected. The active peak fractions of the higher molecular weight form of the enzyme were pooled and concentrated [4,5]. The specific activity of the enzyme used for all the experiments reported here was 9.2 μmoles of NADPH formed/min/mg protein.

The activity of the isocitrate dehydrogenase 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 7.5, 2 mM DL-isocitrate, 1.5 mM NADP$^+$, and 0.5 mM $MnCl_2$. The reaction was initiated by the addition of enzyme. Protein was determined by the method of Lowry et al. [9].

3. Results and discussion

As previously described [10], only the higher molecular weight form of isocitrate dehydrogenase was stimulated by AMP and ADP. The effect of 1 mM AMP on enzyme activity was examined at 1 mM NADP$^+$ and various isocitrate concentrations (fig.1A) or 2 mM DL-isocitrate and various NADP$^+$ concentrations (fig.1B). The figure shows that AMP influences V_{max} and to a smaller extent the apparent K_M values for isocitrate and NADP$^+$. In *Ac. lwoffi* [7] the stimulation by AMP and ADP is primarily an effect exerted on V_{max}.

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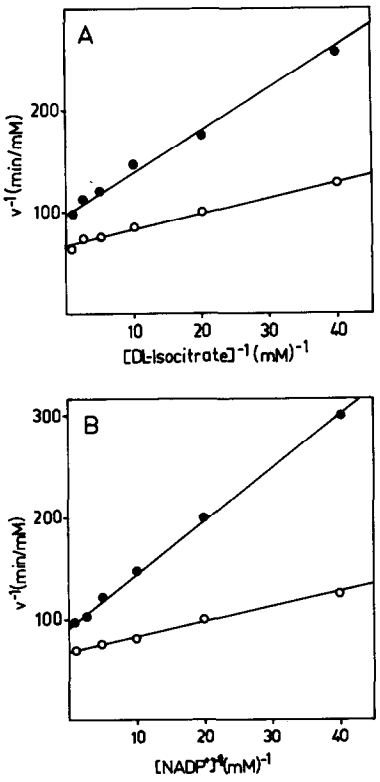

Fig.1. Effect of AMP on isocitrate dehydrogenase of *Ac. calcoaceticus*. Assays were performed in 0.1 M Tris–HCl, pH 7.5, and 0.5 mM $MnCl_2$. The results are plotted in the form of double-reciprocal plots of enzyme activity (mM/min) against the concentration of DL-isocitrate (A, in the presence of 1 mM $NADP^+$) or $NADP^+$ (B, in the presence of 2 mM DL-isocitrate), in each case in the absence of effector (•) or in the presence of 1 mM AMP (○).

Although isocitrate dehydrogenase from *Ac. calcoaceticus* obeys classical Michaelis–Menten kinetics, it is inhibited by nucleoside triphosphates. ATP served as a model inhibitor in the following experiments. As indicated in fig.2A, inhibition by ATP with respect to DL-isocitrate was of a competitive type and the K_i obtained by replotting the slopes against various concentrations of ATP (inset) was determined to be 1×10^{-3} M. Since the inhibition over the entire range of ATP concentration was not linear, only the lowest ATP concentrations were used to determine the K_i. A replot of the reciprocal velocity against inhibitor concentration (fig.2B) confirmed that the inhibition was nonlinear. Extrapolation of these lines gives a K_i of about 1×10^{-3} M, in agreement with that obtained in fig.2A.

As shown in fig.3A, ATP acted as a competitive inhibitor with respect to $NADP^+$. The K_i obtained by replotting the slopes of the curves against various concentrations of ATP was 5.5×10^{-4} M. The inhibition was linear over the entire range of ATP concentration. Fig.3B shows the data graphed according to the method of Dixon [11]. The K_i was determined to be 5.1×10^{-4} M. The secondary replot of the slopes of these curves against concentration of $NADP^+$ yields a line which passes through the origin, confirming the competitive nature of the inhibition.

As shown in table 1, isocitrate dehydrogenase of *Ac. calcoaceticus* is also inhibited by other nucleoside

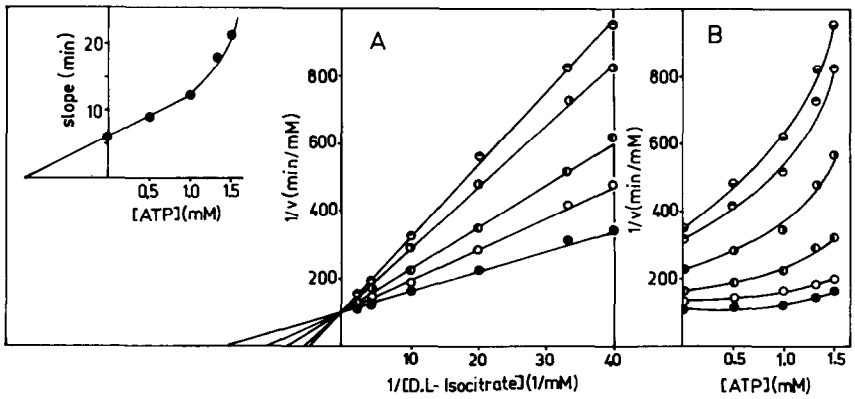

Fig.2. Inhibition of isocitrate dehydrogenase of *Ac. calcoaceticus* by ATP with DL-isocitrate as the variable substrate. The reaction mixtures contained (in μmoles) in a final volume of 2 ml: Tris–HCl buffer, pH 7.5, 200; $NADP^+$, 3; $MnCl_2$, 1. The concentrations of ATP used were: A, 0.5 (○); 1.0 (◐); 1.3 (◑); 1.5 (◒) mM (● = without ATP). The concentrations of DL-isocitrate used were: B, 0.5 (●); 0.25 (○); 0.1 (◐); 0.05 (◑); 0.03 (◒); 0.025 (◓) mM.

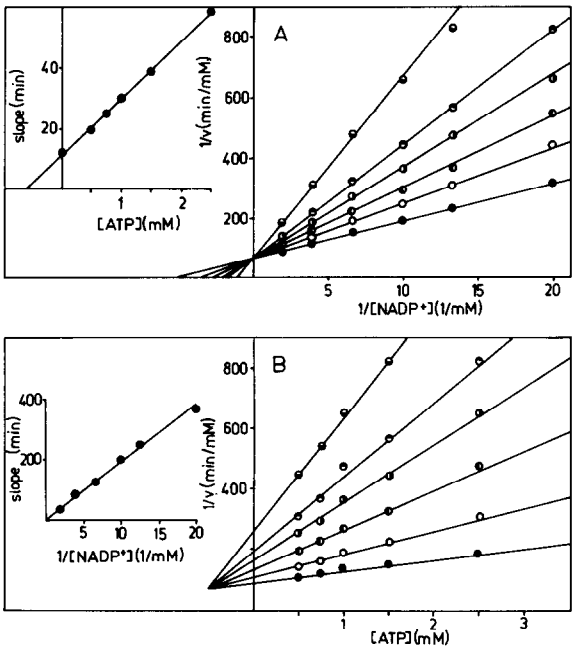

Fig.3. Inhibition of isocitrate dehydrogenase of *Ac. calcoaceticus* by ATP with $NADP^+$ as the variable substrate. The reaction mixtures contained (in μmoles) in a final volume of 2 ml: Tris–HCl buffer, pH 7.5, 200; DL-isocitrate, 2; $MnCl_2$, 1. The concentrations of ATP used were: A, 0.5 (○); 0.75 (◐); 1.0 (◑); 1.5 (◒); 2.5 (●) mM (● = without ATP). The concentrations of $NADP^+$ used were: B, 0.5 (●); 0.25 (○); 0.15 (◐); 0.1 (◑); 0.075 (◒); 0.05 (◒) mM.

triphosphates. In order to determine which nucleoside triphosphates inhibit, a further investigation was carried out by sequential addition of these compounds. Table 1 shows the predicted and observed values for the reaction rate in the presence of nucleoside triphosphates. The observed inhibition pattern was of a cumulative type similar to that described by Woolfolk and Stadtman [12] and Marr and Weber [13,14].

These results and the marked inhibition noted if the enzyme is exposed to ATP before the addition of Mn^{++} to the reaction mixture [15], indicates that ATP has a direct inhibitory effect on the enzyme which is quite distinct from Mn^{++} complexing.

Summarizing we can state that similar effects of AMP or ADP on the $NADP^+$-linked isocitrate dehydrogenase of bacteria have been reported previously for the enzyme of *Ac. lwoffi* [7,16], but the additional effect of ATP inhibition is now only known for the enzyme of *Ac. calcoaceticus* and probably of *Hydrogenomonas* [17]. The regulatory behaviour of this enzyme is therefore really comparable with that of NAD^+-linked isocitrate dehydrogenases of eucaryotes, previously supposed by Weitzman and co-workers [16]. The activation of the higher molecular weight form of isocitrate dehydrogenase by glyoxylate and pyruvate and the inhibition of the enzyme by oxalacetate [15] on the one hand and the adenylate

Table 1
Cumulative inhibition of isocitrate dehydrogenase by nucleotides

Nucleotide (1 mM)	Observed	% Inhibition Cumulative predicted	Additive predicted
ATP	17		
GTP	36		
CTP	17		
UTP	15		
ITP	24		
ATP + GTP	48	47	53
ATP + GTP + CTP	55	56	70
ATP + GTP + CTP + UTP	60	62	85
ATP + GTP + CTP + UTP + ITP	70	71	109

The reaction mixtures contained (in μmoles) in a final volume of 2 ml: Tris–HCl buffer, pH 7.5, 200; DL-isocitrate, 4; $NADP^+$, 3; $MnCl_2$, 1; and the indicated nucleotide. The reaction was started by the addition of the enzyme.

control on the other, may reflect its key position in the balance between biosynthesis and energy production within the citric acid cycle as has been discussed by Weitzman and his co-workers [16].

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References

[1] Goebell, H. and Klingenberg, M. (1964) Biochem. Z. 340, 441.
[2] Ragland, T. E., Kawaski, T. and Lowenstein, J. M. (1966) J. Bacteriol. 91, 236.
[3] Self, C. H. and Weitzman, P. D. J. (1970) Nature 225, 644.
[4] Self, C. H. and Weitzman, P. D. J. (1972) Biochem. J. 130, 211.
[5] Kleber, H.-P., Diezel, W. and Aurich, H. (1974) Rev. roum. Biochim. 11, 177.
[6] Reeves, H. C., Brehmeyer, B. A. and Ajl, S. J. (1968) Science 162, 359.
[7] Parker, M. G. and Weitzman, P. D. J. (1970) FEBS Lett. 7, 324.
[8] Kleber, H.-P. and Aurich, H. (1973) Z. Allg. Mikrobiol. 13, 473.
[9] Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 193, 265.
[10] Kleber, H.-P. and Aurich, H. (1973) 9th Internat. Congr. Biochem., Stockholm, Abstr. 8b 36.
[11] Dixon, M. (1953) Biochem. J. 55, 170.
[12] Woolfolk, C. A. and Stadtman, E. R. (1967) Arch. Biochem. Biophys. 118, 736.
[13] Marr, J. J. and Weber, M. M. (1968) J. Biol. Chem. 243, 4973.
[14] Marr, J. J. and Weber, M. M. (1969) J. Biol. Chem. 244, 2503.
[15] Kleber, H.-P. (1975) Z. Allg. Mikrobiol. 15, 431.
[16] Self, C. H., Parker, M. G. and Weitzman, P. D. J. (1973) Biochem. J. 132, 215.
[17] Glaeser, H. and Schlegel, H. G. (1972) Arch. Mikrobiol. 86, 327.