THE EFFECT OF PYRIDINE NUCLEOTIDES ON THE ACTIVITY OF NADP-DEPENDENT ISOCITRATE DEHYDROGENASE FROM BRAIN CYTOSOL

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1. Introduction

In the cytoplasm of central nervous system cells the oxidation of citrate may procede via the NADP-dependent isocitrate dehydrogenase (EC 1.1.1.42 (IDH-NADP)) [1]. Atkinson [2] and Chen and Plaut [3] have suggested that the activity of IDH-NAD is regulated by adenine and pyridine nucleotides. Previously we have reported that ATP, according to its concentration, may activate or competitively inhibit IDH-NADP from brain cytosol [6]. Up to now there have been no reports about the effect of pyridine nucleotides on the activity of this enzyme. The present study deals with this problem.

2. Materials and methods

Brains from Wistar rats of about 200 g body weight were used. IDH-NADP was isolated and purified according to the procedure in [6]. Activity of the enzyme was measured spectrophotometrically by Ochoa's procedure [7]. Protein was estimated by the method of Lowry et al. [8].

3. Results

The activity of IDH-NADP from brain cytosol is increased by NADPH in concentrations below 0.125 μM. Higher concentrations inhibit the activity (fig. 1A). This inhibition is competitive with isocitrate (fig. 1B).

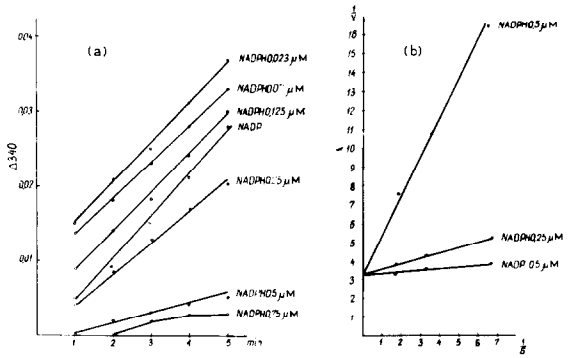

Fig. 1. Effect of NADPH on the activity of NADP-dependent isocitrate dehydrogenase. Incubation mixtures contained: 40 μmoles of Tris–HCl, pH 6.7; 18 μmoles of $MgCl_2$; 9 μg of protein; 0.5 μmole of NADP; and the indicated amounts of NADPH. (A) Contained 0.6 μmole of isocitrate. (B) contained 0.1–0.6 μmole of isocitrate. The final volume was 3.2 ml.

Similarly, NADH below 0.125 μM activates the enzyme while higher NADH concentrations inhibit it competitively (fig. 2A and B). Oxidized NAD activates IDH-NADP at all concentrations studied (fig. 3). The presence of mercaptoethanol or DTT decreases the inhibition produced by NADPH and NADH (fig. 4).

4. Discussion

Earlier studies on the effects of nucleotides on IDH-NADP activity are fragmentary and dealt with the enzyme from liver and microorganisms [4,5].

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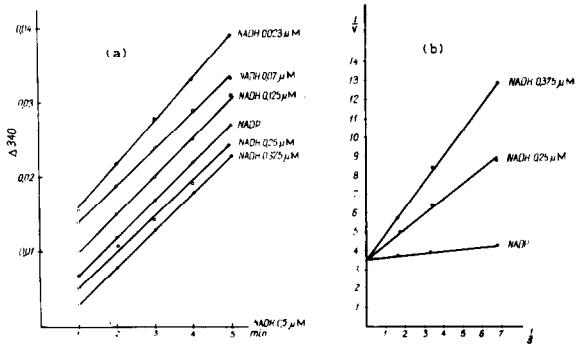

Fig. 2. Effect of NADH on the activity of NADP-dependent isocitrate dehydrogenase. Incubation mixtures contained: 40 μmoles of Tris–HCl, pH 6.7; 18 μmoles of $MgCl_2$; 9 μg of protein; 0.5 μmole of NADP; and the indicated amounts of NADH. (A) Contained 0.6 μmole of isocitrate. (B) Contained 0.1–0.6 μmole of isocitrate. The final volume was 3.2 ml.

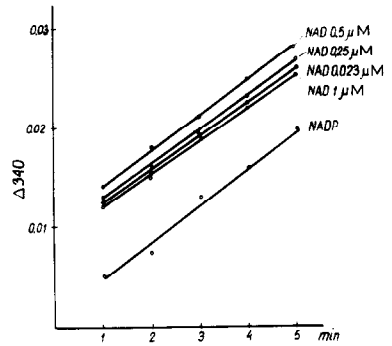

Fig. 3. Activation of NADP-dependent isocitrate dehydrogenase in the presence of NAD. Incubation mixture contained: 40 μmoles of Tris–HCl, pH 6.7; 18 μmoles of $MgCl_2$; 9 μg of protein; 0.5 μmole of NADP; 0.6 μmole of isocitrate; and the indicated amounts of NAD, in a final volume of 3.2 ml.

The present results on the inhibition of IDH-NADP by NADPH (fig. 1) are in good agreement with those of Chen and Plaut on liver IDH-NADP [3]. These authors also demonstrated an inhibition of activity of this enzyme by the reaction product which was competitive with isocitrate. Pyridine nucleotide transhydrogenase activity has been shown by Kaplan [9] to be insignificant in brain. Therefore the observed inhibitions of IDH-NADP by NADPH and NADH (figs. 1 and 2) are due directly to these compounds.

From an analysis of the results of their studies on soluble liver IDH-NADP in the presence of NADPH,

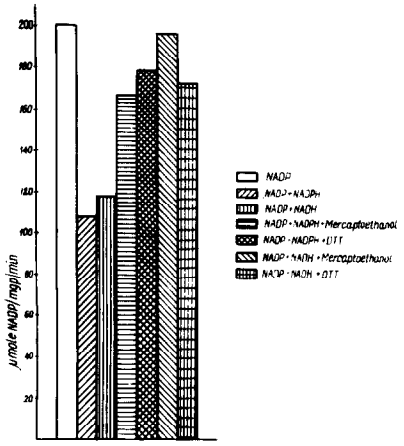

Fig. 4. Effect of mercaptoethanol and DTT on the activity of NADP-dependent isocitrate dehydrogenase in the presence of reduced pyridine nucleotides. Incubation mixture contained: 40 μmoles of Tris–HCl, pH 6.7; 18 μmoles of $MgCl_2$; 9 μg of protein; 0.5 μmole of NADP; 0.6 μmole of isocitrate; and 1 mmole of DTT, 1 mmole of mercaptoethanol, 0.375 μmole of NADPH, 0.375 μmole of NADH as indicated in the figure, in a final volume of 3.2 ml.

Illingworth and Tipton [4] suggested that two molecules of the product NADPH are involved in the reaction with a single molecule of enzyme. A similar mechanism may be expected for the brain enzyme. The fact that NADPH, NADH and also ATP [6] produce inhibitions of IDH-NADPH, which are competitive with isocitrate, suggests that all these compounds operate at the same site on the enzyme.

IDH-NADP activity is associated with the presence of active SH groups [10]. Mercaptoethanol and DTT both decrease the inhibition of IDH-NADP activity by reduced pyridine nucleotides (fig. 4) which suggests that the active centre of the enzyme is associated with active SH groups. The inhibitory effects of NADPH, NADH and ATP on IDH-NADP activity suggests that the levels of these nucleotides may have a regulatory effect on the oxidation of citrate in brain cytosol.

Acknowledgements

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