THE RELATIONSHIP OF SOLUBLE AND MITOCHONDRIAL ISOCITRATE DEHYDROGENASES IN METABOLIC REGULATION

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It has been shown that the soluble $NADP^+$-specific isocitrate dehydrogenases from both prokaryotic and eukaryotic cells are inhibited by oxalacetate and glyoxylate in a concerted manner. We have now compared the mitochondrial and soluble isocitrate dehydrogenases from N. crassa and S. cerevisiae with respect to their inhibition by these compounds. The NAD^+-linked mitochondrial enzymes were not inhibited, whereas the $NADP^+$-linked enzymes were. The significance of this inhibition is discussed with respect to the regulatory role of these two enzymes.

The regulatory role for the mitochondrial NAD^+-specific isocitrate dehydrogenase (IDH) has been established. The activity of this enzyme, isolated from various sources, fluctuates with the concentration of nucleotides (see reviews 1, 2), citrate (3), and perhaps anions as well (4). Additional evidence for the control of the NAD^+-specific IDH by citrate and AMP in intact yeast mitochondria has been presented by Bernofsky and Utter (5).

Despite the importance of this enzyme in intramitochondrial carbohydrate metabolism, the function of the extramitochondrial $NADP^+$-specific IDH remains unclear. Its presence in the cytosol appears to be universal (6), and it has been suggested that its role is to produce NADH via a transhydrogenase (7) and to generate NADPH for reductive biosynthesis of fatty acids.

We first obtained evidence that the soluble $NADP^+$-specific IDH enzymes might have a regulatory function by the demonstration that this enzyme

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from Salmonella typhimurium was inhibited by purine nucleoside triphosphates (8). Further investigation established that this enzyme in the protozoan Crithidia fasciculata was inhibited by nucleoside triphosphates at a site distinct from the substrate loci (9). This latter observation that the soluble $NADP^+$-IDH was subject to heterotropic modification at an allosteric site indicated that this enzyme might be of importance in the regulation of carbohydrate metabolism in the cytosol.

The finding of Shiio and Ozaki (10) that the $NADP^+$-IDH from Brevibacterium flavum and several other organisms could be inhibited by oxalacetate and glyoxylate gave additional support to a possible regulatory function for this enzyme. It was subsequently shown by Marr and Weber (11) that the $NADP^+$-IDH from C. fasciculata was also subject to concerted inhibition by these two organic acids. A regulatory function for this enzyme involving an allosteric locus for a nucleotide and a non-allosteric locus for oxalacetate and glyoxylate was proposed (11, 12).

In an attempt to determine whether this response to these compounds was specific for the soluble enzyme, the intramitochondrial NAD^+-linked IDH enzymes from Neurospora crassa and Saccharomyces cerevisiae were examined. These organisms were chosen since the prokaryotic cells we studied previously lacked an NAD^+-linked IDH, and the preparations of C. fasciculata were not purified free of malic dehydrogenase, which interfered with the assay when oxalacetate was present.

MATERIALS AND METHODS

The enzymes from N. crassa and S. cerevisiae were purified according to the methods of Sanwal et al. (13) and Hathaway and Atkinson (14), respectively. All chemicals were purchased from the Sigma Chemical Company. Oxalacetate was prepared immediately prior to each experiment. Protein

was determined by the method of Lowry et al. (15) or Warburg and Christian (16).

The reduction of NAD^+ and $NADP^+$ was measured at 340 nm using a Gilford recording spectrophotometer. Reactions were carried out at 25°.

RESULTS AND DISCUSSION

Figures 1 and 2 illustrate the effects of oxalacetate plus glyoxylate on the $NADP^+$- and NAD^+-linked enzymes from N. crassa and S. cerevisiae. The $NADP^+$ enzymes were inhibited although neither NAD^+ enzyme was significantly affected. The apparent slight inhibition of this latter enzyme, as seen in Figure 2, was due to some contamination by the malic dehydrogenase. Comparison of the shapes of the curves for the $NADP^+$ enzymes suggests two different mechanisms for the concerted inhibition. In the concentrations used, neither oxalacetate nor glyoxylate alone inhibited the enzymes.

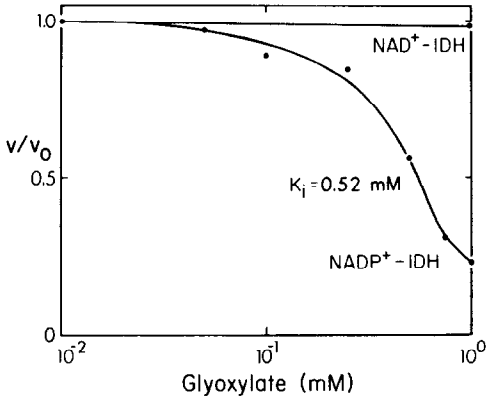

Fig. 1. Effect of oxalacetate and glyoxylate on the NAD^+ and $NADP^+$-IDH from N. crassa. Reaction mixtures for the $NADP^+$-IDH contained 500 μmoles of Tris-HCl buffer, pH 7.6, 0.2 μmole of $MgCl_2$, 0.3 μmole of $NADP^+$, and 2.9 mg of enzyme protein. Reaction mixtures for the NAD^+-IDH contained 400 μmoles of Tris-HCl buffer, pH 7.6, 10 μmoles of $MgSO_4$, 9 μmoles of NAD^+, 5 μmoles of AMP, and 0.18 mg of enzyme protein. Both enzymes were assayed in the presence of 10^{-4} M oxalacetate and the concentration of glyoxylate was varied as indicated on the abscissa. The total volume was 3 ml. Reactions were started by the addition of 1.5 μmoles of D-isocitrate.

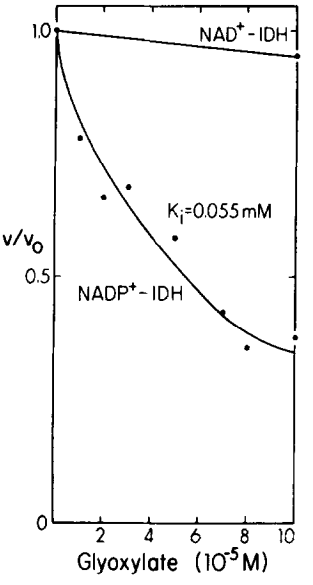

Fig. 2. Effect of oxalacetate and glyoxylate on the NAD^+ and $NADP^+$-IDH from S. cerevisiae. Reaction mixtures for the $NADP^+$-IDH contained 100 μmoles of Tris-HCl buffer, pH 7, 10 μmoles of $MgCl_2$, 1.5 μmoles of $NADP^+$, and 0.38 mg of enzyme protein. Both enzymes were assayed in the presence of 10^{-4} M oxalacetate and the concentration of glyoxylate was varied as indicated on the abscissa. Reaction mixtures for the NAD^+-IDH were identical except 1 μmole of NAD^+, 0.5 μmole of AMP, and 0.07 mg of enzyme protein were used. The total volume was 3 ml. Reactions were started by the addition of 1.5 μmoles of D-isocitrate.

Concerted inhibition of the $NADP^+$-linked enzyme has been demonstrated in several genera of bacteria, fungi, protozoa, and the mammalian pig heart system (10, 11). In a preliminary survey of other organisms, it was found that the soluble $NADP^+$-linked IDH enzymes from Mycobacterium phlei and S. typhimurium were also inhibited in a concerted manner by oxalacetate and glyoxylate. However, only two systems in which the NAD^+- and $NADP^+$-linked enzymes from the same organisms could be contrasted have thus far been examined.

The difference between the NAD^+- and $NADP^+$-IDH described here is perhaps of significance in the regulation of cell metabolism. However, be-

cause of the limited number of organisms examined thus far, it is not yet possible to define the extent of the phenomenon. We should like to postulate that this type of feedback inhibition of the soluble $NADP^+$-IDH influences the concentration of citrate in the cytoplasm and thereby alters other metabolic sequences. Because of the position of the $NADP^+$-IDH in that portion of the tricarboxylic acid cycle which is extramitochondrial, and the direction of the aconitase equilibrium, this IDH appears to be ideally suited to control the citrate concentration in the cytosol. Since the aconitase equilibrium is in the direction of citrate production (17), a highly active IDH is required to alter the equilibrium and permit the oxidation of citrate to α-ketoglutarate. The finding that the $K_m^{isocitrate}$ for the soluble $NADP^+$-IDH enzymes is usually in the micromolar concentration range and the $K_m^{aconitate}$ is generally about ten-fold higher (18) is in agreement with this hypothesis. The inhibition of the IDH by oxalacetate and glyoxylate would allow citrate to accumulate. Because of the multiplicity of effects of citrate on cytoplasmic metabolism, it would be difficult to ascribe any particular effect to this increase in citrate concentration. However, because of the apparent specificity of this inhibition for the soluble $NADP^+$-linked IDH, it is suggested that this enzyme, probably in conjunction with the citrate cleaving enzyme, is a focal point in the control of cytoplasmic carbohydrate metabolism.

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