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## Inhibition of mitochondrial malate dehydrogenase by citrate

Several lines of evidence indicate the presence of two forms of malate dehydrogenase (L-malate:NAD oxidoreductase, EC 1.1.1.37), exhibiting different properties, in a single tissue: one form is apparently mitochondrial in origin, the other is found in the supernatant fraction[1-3]. The former is inhibited by high concentrations of oxaloacetate: the occurrence of this substrate inhibition is considered a typical property of the mitochondrial enzyme[2,3].

The present report shows that pig mitochondrial malate dehydrogenase, unlike the supernatant enzyme, is inhibited by citrate and fumarate. The inhibition of malate oxidation by fumarate had already been observed by STRAUB[4] using his enzyme preparation, which can be now considered as the mitochondrial one[5].

NADH, oxaloacetate and pig heart mitochondrial malate dehydrogenase (720 units/mg) were obtained from Boehringer and Soehne, and pig supernatant malate dehydrogenase (63 units/mg) from General Biochemicals. The enzyme preparations were appropriately diluted before use with 0.017 M phosphate buffer (pH 7.6), containing 2 mM $\beta$-mercaptoethanol. The enzymic reaction was studied at pH 7.6, in the direction of the reduction of oxaloacetate. The initial rate of NADH oxidation was measured at 366 m$\mu$ with an Eppendorf photometer, at room temperature.

Table I shows the effect of some compounds, tested at 3.3 mM concentration,

TABLE I

EFFECT OF DIFFERENT COMPOUNDS ON THE ACTIVITY OF MITOCHONDRIAL AND SUPERNATANT MALATE DEHYDROGENASES

The reaction mixtures contained, in a final volume of 3 ml: 0.017 M phosphate buffer (pH 7.6), 0.067 mM oxaloacetate and 0.09 mM NADH. The reaction was started by the addition of 0.03 ml of the enzyme solution (about 0.15 $\mu$g of protein in the case of the mitochondrial, and 0.75 $\mu$g of protein in the case of the supernatant one, respectively). The oxidation of NADH was followed at 366 m$\mu$, in 10-mm light path cuvettes. The results are expressed as $\Delta A \times 10^3$/min.

| *Additions* | *Malate dehydrogenase activity* | |
|---|---|---|
| | *Mitochondrial* | *Supernatant* |
| None | 49 | 43 |
| 3.3 mM citrate | 28 | 43 |
| 3.3 mM fumarate | 7 | 42 |
| 6.7 mM DL-isocitrate | 52 | 44 |
| 3.3 mM maleate | 51 | 42 |

on the reduction of oxaloacetate by mitochondrial and supernatant malate dehydrogenases. The mitochondrial enzyme was specifically inhibited by citrate and fumarate; at the concentration tested, isocitrate, maleate and other compounds not reported in Table I (succinate, *cis*-aconitate, aspartate, glutamate, pyruvate and phosphoenolpyruvate) did not inhibit the two enzymes.

The inhibition of the mitochondrial enzyme by citrate was studied in more

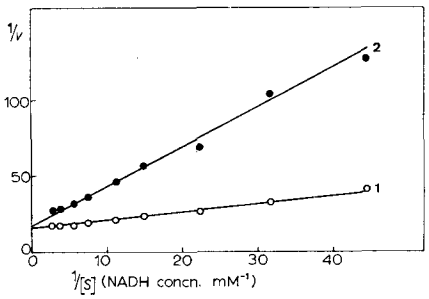


Fig. 1. Plots of reciprocal initial velocity *vs.* reciprocal NADH concentration. The reaction mixtures contained, in a final volume of 6 ml: 0.017 M phosphate buffer (pH 7.6), 0.067 mM oxaloacetate and 0.03 ml of mitochondrial malate dehydrogenase solution (0.15 $\mu$g of protein). The oxidation of NADH was followed at 366 m$\mu$, in 20-mm light path cuvettes. Initial velocities were expressed as $\Delta A$/min. Curve 1, no addition; Curve 2, 5 mM citrate.

detail. According to the results reported in Fig. 1, in the presence of a fixed concentration of oxaloacetate (0.067 mM), the inhibition by citrate was found to be competitive with NADH: 5 mM citrate enhanced the NADH concentration giving half-maximum velocity from 0.03 mM to about 0.15 mM. When the oxaloacetate concentration was varied in the range at which no substrate inhibition was observed, and in the presence of a fixed concentration of NADH (0.09 mM), then the effect of citrate was essentially on the maximum velocity (Fig. 2).

According to RAVAL AND WOLFE[6], mitochondrial malate dehydrogenase has a compulsory substrate binding order mechanism with no kinetically discernible ternary complex; according to this mechanism, the addition of NADH to the enzyme is the preferred reaction pathway when oxaloacetate and NADH are the substrates. RAVAL AND WOLFE[7] have observed that the substrate inhibition by oxaloacetate is of mixed type with NADH, and have proposed that the inhibition is due both to the formation of an inactive binary complex between enzyme and

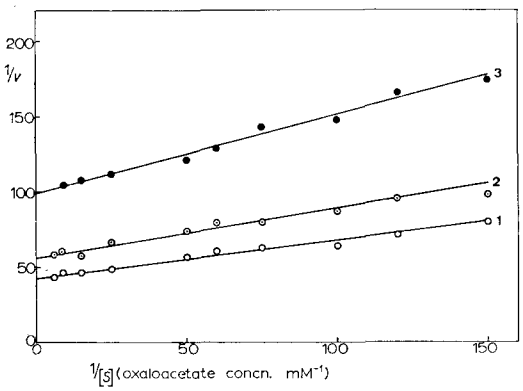


Fig. 2. Plots of reciprocal initial velocity *vs.* reciprocal oxaloacetate concentration. The reaction mixtures contained, in a final volume of 6 ml: 0.017 M phosphate buffer (pH 7.6), 0.09 mM NADH and 0.03 ml of mitochondrial malate dehydrogenase solution (0.075 $\mu$g of protein). The oxidation of NADH was followed at 366 m$\mu$, in 20-mm light path cuvettes. Initial velocities were expressed as $\Delta A$/min. Curve 1, no addition; Curve 2, 1 mM citrate; Curve 3, 5 mM citrate.

oxaloacetate and to the formation of an inactive ternary complex between enzyme, $NAD^+$ and oxaloacetate. Our results can be explained by assuming that citrate (or fumarate) is bound at the same site as oxaloacetate; thus, the binding of NADH to the enzyme would be prevented as in the case of substrate inhibition by oxaloacetate. The formation of inactive ternary complexes between enzyme, citrate and either $NAD^+$ or NADH can be ruled out because of the competitive nature of the citrate inhibition with respect to NADH.

It is tempting to speculate on a possible biological regulatory role for the peculiar inhibition of mitochondrial malate dehydrogenase by citrate and fumarate. Although the mitochondrial enzyme is essentially involved in the tricarboxylic acid cycle, both mitochondrial and supernatant forms are supposed to participate to lipogenesis and gluconeogenesis[8,9]. As far as lipogenesis is concerned, it could be suggested that the inhibition of mitochondrial malate dehydrogenase by citrate can prevent the transformation of oxaloacetate (formed from pyruvate inside the mitochondria) into malate, a reaction favoured by the equilibrium position. Oxaloacetate could then be utilized more efficiently for the synthesis of citrate, which is supposed[9] to leak out from mitochondria and to be used as the source of CoASAc for the synthesis of fatty acids. The oxaloacetate newly formed in this reaction would then produce pyruvate, *via* the supernatant malate dehydrogenase (which is not affected by citrate, according to our results) and malic enzyme (EC 1.1.1.40); by these reactions, NADH is transformed into NADPH, needed for the synthesis of fatty acids[8,9]. The inhibition of mitochondrial malate dehydrogenase by citrate could therefore be considered as another way by which citrate regulates the synthesis of fatty acids, in addition to its activating effect on CoASAc carboxylase (EC 6.4.1.2)[10].

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