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Allosteric control of a Lactobacillus malate dehydrogenase (decarboxylating) by two glycolytic intermediate products

Recently, the regulatory properties of an inducible malate dehydrogenase (decarboxylating) (L-malate:NAD oxidoreductase (decarboxylating), EC 1.1.1.39) from *Streptococcus faecalis* strain MR were described in two reports[1,2]. The streptococcal enzyme was distinguished from other bacterial malate dehydrogenases (decarboxylating)[3–6] by the fact that its catalytic function could be regulated by two negative effectors which were intermediate products of glycolysis, namely, fructose 1, 6-diphosphate (Fru-1,6-P_2) and 3-phosphoglyceric acid (3-*P*-glycerate). A survey of other lactic acid bacteria revealed that the ability to utilize malate as an energy source for growth was not restricted to the group D streptococci. This report describes the biochemical and regulatory properties of a malate dehydrogenase (decarboxylating) from a strain of *Lactobacillus casei* capable of growing at the expense of the dicarboxylic acid. It also points up numerous similarities between the streptococcal and Lactobacillus malate dehydrogenases (decarboxylating).

L. casei strain 64H, obtained from Dr. F. Gasser, was maintained on Lactobacillus-carrying medium[7] supplemented with 0.5% diammonium L(+)-malate. This medium was used to cultivate the organism throughout the course of this work. Cells in the stationary phase of growth were harvested from unshaken cultures incubated at 30° and washed twice in 0.05 M Tris–HCl buffer (pH 8.05). Crude extracts were prepared from cells resuspended in the same buffer by disruption in a Branson sonic oscillator operating at 70 W of power for 6 min. Cell debris was removed by centrifugation at 30 000 × *g* for 20 min.

Malate dehydrogenase (decarboxylating) was partially purified by the following treatment. Streptomycin sulfate was added to extracts to a final concentration of 1.36%; they were then centrifuged as above. The supernatant fluid was heated at 60° for 5 min; denatured protein was removed by centrifugation. To the heat-treated supernatant fluid, solid $(NH_4)_2SO_4$ was added to 50% saturation. Following removal of the precipitated protein, the $(NH_4)_2SO_4$ concentration was increased to 75% of saturation. The resultant protein precipitate was collected by centrifugation, dissolved in a small volume (1–3 ml) of 0.05 M Tris–HCl buffer (pH 8.5) and stored at −20° until used. A 5–6-fold purification of the lactobacillus malate dehydrogenase (decarboxylating) was obtained by this treatment with a recovery of 87% of the original activity.

Malate dehydrogenase (decarboxylating) activity was measured by following NAD^+ reduction at 340 nm in a 1-ml reaction mixture containing: Tris–HCl buffer (pH 8.5), 35 μmoles; $MnCl_2$, 0.1 μmole; NH_4Cl, 1 μmole; NAD, 0.5 μmole; sodium L(+)-malate, 5 μmoles; and sufficient enzyme to give a rate of 0.4–0.7 absorbance units/min.

The properties of the lactobacillus malate dehydrogenase (decarboxylating) closely resembled those of the streptococcal enzyme[1]. In addition to a specific requirement for NAD^+ as cofactor and relative stability at 60°, the optimal pH of this malate dehydrogenase (decarboxylating) was between 8.0 and 8.5. A K_m of 0.42 mM was obtained for malate using 0.5 mM NAD^+ in the reaction mixture while at a concen-

tration of 5 mM malate a K_m of 0.11 mM was obtained for NAD^+; these values are similar to those obtained with the *S. faecalis* malate dehydrogenase (decarboxylating).

The similarities between the two enzymes extended beyond the general properties described above. The catalytic function of the lactobacillus malate dehydrogenase (decarboxylating) was also inhibited by Fru-1,6-P_2 and 3-*P*-glycerate. Although Lineweaver–Burk plots suggested that Fru-1,6-P_2 and 3-*P*-glycerate behaved as classical competitive inhibitors for malate within the concentration ranges tested (Fig. 1); other data indicated that this was not the case. The degree of inhibition was a sigmoidal function of the concentration of the two glycolytic intermediate products at a concentration of 5 mM malate; no significant decrease in the reaction rate could

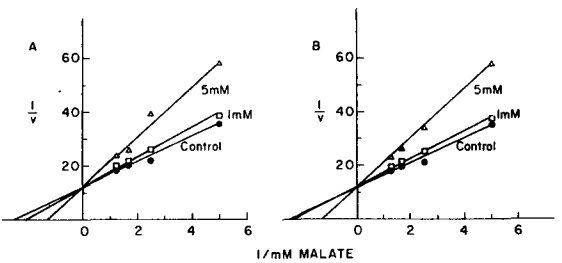

Fig. 1. Inhibition of the *L. casei* malate dehydrogenase (decarboxylating) by two intermediate products of glycolysis. A. Fru-1,6-P_2. B. 3-*P*-Glycerate.

be measured at levels below 3 mM Fru-1,6-P_2 or 6 mM 3-*P*-glycerate (Fig. 2). Sigmoidal kinetics are characteristic of an enzyme possessing multiple binding sites for a ligand which interact in a cooperative fashion[8]. The nature of the inhibition curves suggests that Fru-1,6-P_2 and 3-*P*-glycerate are negative effectors for the lactobacillus malate dehydrogenase (decarboxylating)[9]. Hill plots of 3-*P*-glycerate inhibition data yielded

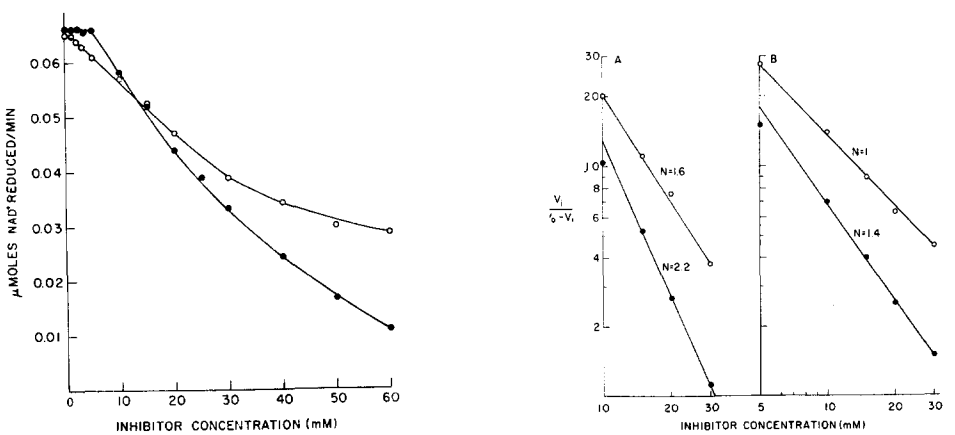

Fig. 2. Nature of the inhibition produced by Fru-1,6-P_2 (○—○) and 3-*P*-glycerate (●—●) at saturating concentrations of substrate (5 mM).

Fig. 3. Determination of the interaction strength of the inhibitors with a crude cell-free extract (●) and the partially purified malate dehydrogenase (decarboxylating) (○) from *L. casei* 64H. A. 3-*P*-Glycerate. B. Fru-1,6-P_2.

cooperativity coefficients (n values) of 2.1 and 1.7 with a crude extract and the purified malate dehydrogenase (decarboxylating), respectively (Fig. 3A). Little or no cooperativity could be demonstrated for Fru-1,6-P_2 under the same conditions (Fig. 3B).

In contrast to the streptococcal malate dehydrogenase (decarboxylating), the cooperativity coefficient of the inhibitors remained essentially unchanged between pH 7.5 and 9.5. It is of interest to note that the degree of cooperativity for Fru-1,6-P_2 was greater than that observed for 3-P-glycerate with the streptococcal enzyme, while the converse was observed to be the case for Lactobacillus malate dehydrogenase (decarboxylating). At present, it is not known whether the differences in cooperativity coefficients reflect differences in the intracellular levels of Fru-1,6-P_2 and 3-P-glycerate in the respective organisms.

Other subtle differences between the two malate dehydrogenases (decarboxylating) were noted. Both enzymes were slowly denatured at 60°. However, the addition of 1 mM $MnCl_2$ to the incubation mixture only slightly increased the stability of the lactobacillus enzyme towards heat. The rate of thermal inactivation of the streptococcal enzyme was not altered when saturating levels of malate were included in the reaction mixture. In contrast, the lactobacillus enzyme was labilized in the presence of 5 mM malate. Finally, the presence of Fru-1,6-P_2 or 3-P-glycerate in the reaction mixture during heat inactivation of the streptococcal enzyme markedly reduced the rate of denaturation; no such effect was observed with the *L. casei* enzyme.

It is entirely possible that the parallels observed between the two malate dehydrogenases (decarboxylating) represent nothing more than a series of trivial coincidences. On the other hand, the striking similarities in catalytic function and regulatory properties may reflect regions of homology in the respective proteins. An immunological study is presently underway to learn whether the streptococcal and lactobacillus malate dehydrogenases (decarboxylating) share common antigenic determinants.

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1 J. LONDON AND E. Y. MEYER, *Biochim. Biophys. Acta*, 178 (1969) 205.
2 J. LONDON AND E. Y. MEYER, *J. Bacteriol.*, 98 (1969) 705.
3 L. A. JACOBSON, R. C. BARTHOLOMAUS AND I. C. GUNSALUS, *Biochem. Biophys. Res. Commun.*, 24 (1966) 955.
4 H. KATSUKI, K. TAKEO, K. KAMEDA AND S. TANAKA, *Biochem. Biophys. Res. Commun.*, 27 (1967) 331.
5 K. TAKEO, T. MURAI, J. NAGAI AND H. KATSUKI, *Biochem. Biophys. Res. Commun.*, 29 (1967) 717.
6 B. D. SANWAL, J. A. WRIGHT AND R. SMANDO, *Biochem. Biophys. Res. Commun.*, 31 (1968) 623.
7 M. ROGOSA, *Type Strains of Lactobacillus Species*, American Type Culture Collection, 1968, p. 16.
8 J. WYMAN, *Cold Spring Harbor Symp. Quant. Biol.*, 28 (1963) 88.
9 J. C. PATTE, P. TRUFFA-BACHI AND G. N. COHEN, *Biochim. Biophys. Acta*, 128 (1966) 426.

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