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Purification and Properties of NAD-Dependent Malate Dehydrogenase from Mesembryanthemum crystallinum L. Exhibiting Crassulacean Acid Metabolism

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Abstract:

By DEAE-cellulose anion exchange column chromatography, three different forms of NAD-dependent malate dehydrogenase (EC 1.1.1.37) (NAD-MDH) were isolated from leaves of Mesembryanthemum crystallinum L. in the crassulacean acid metabolism mode. NAD-MDH that eluted last from an anion exchange column was purified to a specific activity of 1, 096 units (mg protein)⁻¹ using Blue Sepharose CL-6B chromatography. Citrate and adenosine 5'-triphosphate effectively inhibited the activity of NAD-MDH. The inhibition of the enzymatic activity by citrate was reversed by inorganic phosphate and the degree of reversal increased with increasing the concentration of oxaloacetate, the substrate of the reaction. The optimal pH for NAD-MDH activity was around 7.5. Citrate inhibited the enzymatic activity over a wide range of pH and caused a shift in the optimal pH. The enzymatic activity in the presence of citrate was increased by adding inorganic phosphate below pH 7.5. On the basic side, higher than pH 7.5, however, the inhibition by citrate was enhanced by adding inorganic phosphate.

Keywords:

Crassulacean acid metabolism, Enzyme purification, Mesembryanthemum crystallinum L., NAD-dependent malate dehydrogenase (EC 1.1.1.37)

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