

Crystal structure analysis of malate dehydrogenase

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Malate dehydrogenase (MDH) is the enzyme that catalyzes the last step of the reaction in the tricarboxylic acid cycle and catalyzes the dehydrogenation of malate to produce oxaloacetic acid. MDH has been purified from *Thermus flavus* AT62, and its enzymatic properties have been analyzed[1],[2]. MDH is a dimeric enzyme contains two identical subunits. The gene encoding MDH has been cloned from *Thermus flavus* AT-62 [3].

MDH is an NAD-dependent enzyme. However, the enzyme can catalyze the reaction using NADP as a coenzyme, though the catalytic efficiency is considerably low. We found that K_m value of MDH for oxaloacetic acid markedly increased (200 times), when NADP was used as a coenzyme of MDH [4]. This suggests that NADP binding to MDH induces conformational change around substrate binding site. In order to elucidate the molecular basis of the coenzyme-dependent change in substrate specificity of the enzymatic reaction, we tried to determine the structure of MDH/NADP/substrate complexes. Crystals of MDH were grown at 20°C by vapor diffusion in 18~22% PEG 6000, 0.2 M NaCl, 0.1 M Tris-

HCl (pH7.5) and 5 mM NADPH. We obtained several crystals of MDH by using a buffer containing NADP and oxaloacetic acid. The obtained crystal was analyzed using BL18B at PF. The space group for the crystal was P2₁2₁2₁. The unit cell parameters for the crystal was a=70.954 Å, b=79.129 Å, c=136.584 Å. The structure analysis revealed that MDH did not contain NADP nor oxaloacetic acid in the molecule. The structure of apo-MDH was nearly identical to that of NAD-binding form of the enzyme.

References

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