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Crystallographic analysis of malate dehydrogenase from thermophilic bacterium *Thermus flavus* and the mutant EX7

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Introduction

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

On the basis of the crystal structure of the NAD(H)-dependent cytoplasmic malate dehydrogenase and its alignment with NADP(H)-dependent counterparts, the loop region between β-strand B and α-helix C in the nucleotide-binding fold was predicted as a principal determinant for the coenzyme specificity. In mutant EX7 of NAD(H)-dependent MDH from *T. flavus*, seven amino acids in positions 41-47 were replaced by the corresponding residues in the NADP(H)-dependent MDH from chloroplasts. In the mutant, coenzyme specificity was altered from NAD(H) to NADP(H)[4]. For better understanding of the coenzyme specificity in MDH, we conducted crystallographic analysis of EX7-NADP(H), EX7-NAD(H) and MDH-NADP(H) complex.

Materials and Methods

Crystals of MDH were grown at 20°C by vapor diffusion in 20~25% PEG 4000, 0.1 M Tris-HCl pH8.0. Crystals of apo-EX7 were grown at 20°C by vapor duffusion in 20~25% PEG 4000, 0.1 M Tris-HCl pH8.0~8.5 and 1 mM DTT. The crystals were soaked into a buffer containing 1 mM each coenzyme.

Results

Analysis using a data set of EX7-NADPH revealed the presence of NADPH at the nucleotide-binding site forming the Rossmann fold of MDH. The exchange of loop region resulted in the formation of hydrogen bonds between the 2'-phosphate group of adenine ring of NADPH and hydroxyl groups of Ser42 and Ser45 of EX7 mutant, playing the crucial role of these residues in the coenzyme specificity. In crytstal structure of chloroplast MDH (NADP-dependent MDH) from *Flaveria bitentis*, electron density of adenine of NADPH was observed. However, in the EX7-NADPH complex, electron density of adenine ring of NADPH was not observed,suggesting that adenine of NADPH is mobile in the . Adenine ribose of NADPH is stabilized by hydrogen bonds to main chain nitrogen atomis of Gly10, Gly13 and Ala88 in addition to Ser42 and Ser45. Hydroxyl side chain and main chain oxygen of Ser42 form hydrogen bond with Arg44, Ser45 and Phe46, indicating that Ser42 serves to stabilize the structure of the loop region. These hydrogen bonds between EX7 and NADPH, in the replaced loop assumes the high affinity of EX7 to NADPH.

Crystal structure of EX7-NADH was also determined. In the map, electron density of nicotinamide, nicotinamide ribose and pyrophosphate was observed, but electron density of adenine and adenine ribose was not observed. These observation indicates that EX7 is bound to NADH mainly through nicotinamide, nicotinamide ribose and pyrophosphate. In contrast to the EX7-NADH complex, EX7-NAPDH complex forms many hydrogen bonds through 2'-phosphate and adenine ribose. Thus, the high specificity of EX7 to NADPH rather than NADH was rationally explained.

References

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