Comparison between Crystal Structures of cMDH in Apo and NAD/NADH Binding Form

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Cytosolic malate dehydrogenase (cMDH) is generally known as a key enzyme in several metabolic pathways. An additional biological function associated with nucleic acid-conducting channel has been identified very recently [1]. Furthermore, the finding that anticancer natural products inhibit cMDH has raised the possibility of this multi-functional protein as a druggable target [2]. Since human cMDH alone is structurally unknown, we investigated the crystal structure of the apo form to be compared with the NAD/NADH complex.

We crystallized three types of cMDH (NAD-binding, NADH-binding and the apo forms), and collected the diffraction data at high resolution by Pharmaceutical Industry Beamline (BL32B2) in the SPring-8. As a result of structural determination, significant structural differences were observed in the NAD/NADH-binding site, especially in the entrance region including a long loop. NAD and NADH interacted with the loop to be stabilized although the loop in the apo form was included in the packing interaction of the crystal. These findings suggest that the loop moves flexibly to capture the coenzymes. On the other hand, the flexibility of the inner region seemed to be low. Finally, the possibility to use the structure information on drug design will be discussed.

[1] Basil H., *et. al*., *Proc. Natl. Acad. Sci.,* 2002, **99**, 1707. [2] Knockaert M., *et. al., J. Biol. Chem*., 2002, **277**, 25493.

Keywords: dehydrogenase, crystal structures, drug design