Crystal Structure of the NADP-dependent 3-hydroxyisobutyrate Dehydrogenase

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3-Hydroxyisobutyrate, a central metabolite in the valine catabolic pathway, is reversibly oxidized to methylmalonate semialdehyde by a specific NAD/NADP-dependent dehydrogenase. To gain insight into the function of this enzyme at atomic level, we have determined the first crystal structures of 3-hydroxyisobutyrate dehydrogenase from *Thermus thermophilus* HB8: holo enzyme, 3-hydroxyisobutyrate complex, and sulfate ion complex. The crystal structures reveal a unique tetramer consisting of four identical protomers. The protomer folds into two distinct domains with open/closed interdomain conformations. The cofactor NADP(H) and the substrate 3-hydroxyisobutyrate are bound at the cleft between the two domains of the closed protomer. The observed tetramer structure might be important for the catalytic function through forming the active site involving two adjacent subunits. A kinetics study confirms that this enzyme has strict substrate specificity for 3-hydroxyisobutyrate and serine, but it cannot distinguish the chirality of the substrates. This enzyme prefers the physiological cofactor NADP rather than NAD. We propose a reaction mechanism based on the structures of cofactor/substrate bound at the cleft; Lys^{165} is the probable catalytic residue of the enzyme.

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