Substrate specificity of D-2-hydroxyisocaproate dehydrogenase from *Lactobacillus casei*

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The D-hydroxyisocaproate dehydrogenase (D-HicDH) is a homodimer enzyme which consists of 333 amino acids per subunit and has a molecular weight of 2 x 37 kDa. It catalyses the NAD dependent reversible, stereospecific interconversion between aliphatic branched and unbranched 2-ketocarboxylic acids and D-2-hydroxycarboxylic acids. Because of its broad substrate specificity it is possibly used in stereospecific amino acid production and hence of high biotechnological interest.

Wild type protein D-HicDH was produced recombinantly in *E.coli* and crystallized as a ternary complex in the presence of NAD^+ and the 2-ketoisocaproate substrate with ammonium sulphate as the precipitating agent. Four different crystal forms of D-hydroxyisocaproate dehydrogenase have been found [1] and one of them, a hexagonal crystal, has been characterized by X-ray diffraction so far [2]. This structure has been solved to 1.9 Å resolution [3].

We collected two complete datasets of two different D-HicDH crystal forms at the EMBL BW7B beamline under cryo-cooling conditions using 25 % glycerol as cryoprotectant. One crystal belongs to the hexagonal spacegroup $P6_322$ with cell parameters a = b = 131.33 Å, c = 125.55 Å and diffracted to 1.85 Å resolution. The second crystal belongs to the cubic spacegroup $P4_332$ with cell parameters a = b = c = 149.33 Å and diffracted to 2.0 Å resolution. Both crystals contain one D-HicDH subunit in the asymmetric unit with the Matthews parameter V_m of 4.23 $Å^3 Da^{-1}$ (hexagonal crystal) and 3.76 $Å^3 Da^{-1}$ (cubic crystal), corresponding to a solvent content of 70.7 % (hexagonal crystal) and 67.0 % (cubic crystal).

The three C-terminal amino acids missing in the previously reported D-HicDH structure [3] can be detected in either of these structures. The hexagonal crystal contains 2-ketocaproate and the cubic one phenylpyruvate bound to the active site of the enzyme. Currently we are crystallizing D-HicDH mutants with altered substrate specificity. Together with the ternary complex structures of wild-type D-HicDH structures of these mutants will refine our understanding of the substrate specificity of D-HicDH.

References

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