[MS5-P02] Structure change for substrate recognition in conjugated polyketone reductase

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Abbreviations: D-PL, D-Pantoyl lactone; KPL, ketopantoyl lactone; CPR, conjugated polyketone reductase; TIM, triose-phosphate isomerase. D-PL is an important chiral intermediate for the chemical synthesis of D-pantothenic acid (vitamin B5), which is a component of coenzyme A in vivo, and therefore an indispensable ingredient in the daily diet.[1,2] The conventional synthetic process for D-PL requires optical resolution of racemic PL. On the other hand, the enzymatic reduction of KPL by CPR-C2 from Candida parapsilosis IFO 0708 was reported for the first time to cause the stereospecific synthesis of D-PL in excellently high yields,[3-5] and hence is expected to be an efficient alternative process for producing D-PL. This enzyme was identified as an NADPH-dependent KPL reductase that belongs to the aldo-keto reductases superfamily.[6] To elucidate the structural basis of the substrate specificity, we determined the crystal structures of the apo CPR-C2 and CPR-C2. NADPH complex at 1.70 and 1.80 Å resolutions, respectively. CPR-C2 adopted a triose-phosphate isomerase (TIM) barrel fold at the core of the structure. Binding with the cofactor NADPH induced conformational changes in which Thr27 and Lys28 moved 15 and 5.0 Å, respectively. These residues form hydrogen bonds with the adenosine 2'-phosphate group of NADPH. Based on the comparison of the CPR-C2.NADPH structure with 3-α-hydroxysteroid dehydrogenase and mutation analyses, we constructed substrate-binding models with KPL, which provided insight into the substrate specificity by the cofactor-induced structure. The results will be useful for the rational design of CPR-C2 mutants targeted for use in the industrial manufacture of KPL.

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