Crystal Structure of the Biotin Protein Ligase from *Pyrococcus horikoshii* OT3: Insights into the Mechanism of Biotin Activation

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Biotin protein ligase (BPL) catalyses synthesis of an activated form of biotin, biotinyl-5'-AMP, from substrates biotin and ATP, and followed biotinylation of the biotin carboxyl carrier protein subunit of acetyl-CoA carboxylase. The crystal structures of BPL from *Pyrococcus horikoshii* OT3 (*Ph*BPL) and its complexes with biotin, ATP, ADP and biotinyl-5'-AMP have been determined at 1.6, 2.0, 1.6 and 1.45Å resolution, respectively. Analysis of location of the activated intermediate and conformational rearrangements in the *Ph*BPL complexes allows us to propose structural guidelines for the biotin activation.

The structures reveal a dimer as the functional unit and each subunit contains two domains, a larger N-terminal catalytic and a smaller C-terminal domains. Dimer configuration of *Ph*BPL (enzyme) is different that of from BPL from *E.coli*, *Ec*BirA (enzyme-repressor): in *Ph*BPL, the tight dimer through N-termini shows no change upon ligand binding; in *Ec*BirA, the dimerization through the central regions of catalytic domain is controlled by the ligand binding. In crystals cocrystallized with biotin and ATP, electron density corresponding to a biotinyl-5'-AMP was observed due to the self-catalysis between substrates. An induced-fit ordering of the active site loop in the complexes makes the catalytic field suitable for the first step of BPL reaction. In *Ph*BPL, both biotin and ATP are fixed in spatially adjacent active site pockets in orientation allowing the reaction. In the bottom of the pockets, there are conserved residues like Gly45, Gly47, Gly127, Gly129 and Trp53 providing required space and orientation for substrates, as well as the conserved positively charged residues Arg48, Arg51, Arg233 and Lys111 located near to the reaction ends of substrates, which may facilitate the reaction.

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