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**Crystal Structures of the Biotin Protein Ligase and Biotin Carboxyl Carrier Protein from *Pyrococcus horikoshii* OT3: Stages of Biotin Activation and Biotinylation.** Bagautdin Bagautdinov, Naoki Kunishima, Advanced Protein Crystallography Research Group, RIKEN SPring-8 Center, Harima Institute, 1-1-1 Kouto, Hyogo 679-5148, Japan.

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 (BCCP) subunit of acetyl-CoA carboxylase. Here we present the crystal structures of BPL and BCCP from *Pyrococcus horikoshii* OT3. BPL structures liganded with biotin, ATP, ADP, ADP:biotin, biotinyl-5'-AMP, biotinyl-5'-AMP:Mg<sup>2+</sup>(Mn<sup>2+</sup>) as well as of the mutated K111G (K111A) BPL protein in apo- and in complexes with biotinyl-5'-AMP have been analyzed. The structures reveal the tight dimer through N-termini as the functional unit (Bagautdinov *et al. J. Mol. Biol.* **353**, 322). The catalytic requirement of the BPL active site is positioning of reactants and neutralization of their negative charges by K111, R48, R51, R233. At mutation of K111 to G(A) BPL produces biotinyl-5'-AMP, possibly due to the neutralization of the reactant ends by three positively charged arginines. Presence of the divalent metals Mg<sup>2+</sup>(Mn<sup>2+</sup>) at reaction causes trapping of leaving  $\beta$ -,  $\gamma$ -phosphates in the active site cavity. The biotinyl domain BCCP structure characterized by higher mobility of the local residues -M111-K112-M113-, where K112 is candidate for biotinylation by BPL. The details of the BPL-BCCP interactions involved at biotinylation will be discussed.