Protein Biotinylation Visualized using a Complex Structure of Biotin Protein Ligase with a Substrate

We have determined the three-dimensional (3D) structure of a protein complex related to the metabolic syndrome. Organisms have an energy storage system in which excess calories are converted into adipose (fatty acid) for emergency. However, in human beings, this system could be the main reason for metabolic syndrome disorder including cardiac disorder and cerebral stroke. These diet-induced diseases and obesity can be overcome by regulating the activity of acetyl-CoA carboxylase (ACC), which is a key enzyme in fatty acid biosynthesis. It is reported that ACC-deficient mutant mice have a normal life span, a high fatty acid oxidation rate, and low amounts of fat [1]. In the living cell, ACC works only after the covalent attachment of biotin to its special lysine residue located at the biotin carboxyl carrier protein (BCCP) region. This biotinylation of BCCP is catalyzed by biotin protein ligase (BPL) in all organisms (Fig. 1). Therefore, one of the methods for

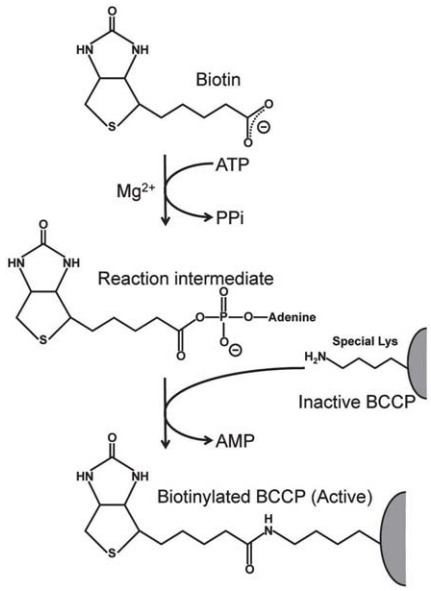

Fig. 1. Biotinylation of BCCP by BPL.

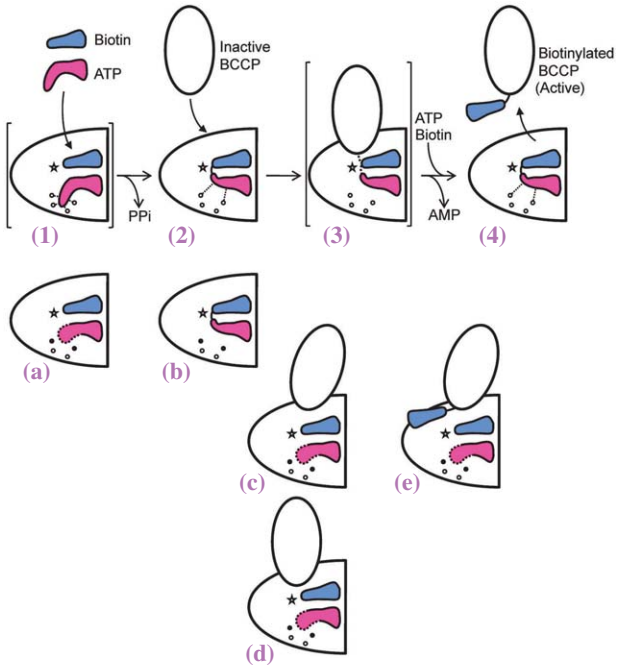

Fig. 2. Stabilization of reaction intermediates using BPL mutants. Objects (1)-(4) schematically denote the wild-type reaction procedure in which unstable states that cannot be observed by usual methods are indicated by parentheses. BPL is depicted as a half ellipsoid in which the amino acid residues to be mutated and the active site are indicated as circles and an asterisk, respectively. Covalent bonds, transition-state interactions and noncovalent interactions are indicated as thick solid lines, thick dotted lines and thin dotted lines, respectively. As derivatives from the substrates (ATP and biotin), the states (2) and (4) bind the reaction intermediate biotinyl-5'-AMP, and the state (3) binds the transition-state intermediate. Objects **(a)**-**(e)** schematically denote all the forms that were observed in the crystals of this work. The mutated residues of BPL are indicated by filled circles. The fuzzy boundary of the ATP molecule indicates a mutation-induced partial disorder due to the decrease in the number of noncovalent interactions. Presumably, in the crystals of mutant proteins, the substrate itself acted as an inactive intermediate analogue, thereby allowing the stabilization of BPL-BCCP complexes. The crystal forms **(a)** and **(b)** may correspond to the wild-type's states (1) and (2), respectively. Similarly, the forms **(c)** and **(d)** may correspond to intermediate states between (2) and (3), and the form **(e)** to a state between (3) and (4). The observation of forms **(b)** and **(e)** indicates that the mutants used retain essentially the same enzymatic function as that of the wild-type BPL.

the effective control of ACC in the human body would be the pharmaceutical inhibition of BCCP biotinylation. For the development of such medicine, scientists have to determine the 3D structure of BPL complexed with BCCP, which requires the preparation of BPL:BCCP complex crystals.

The crystallization of BPL:BCCP complexes is difficult because the complexes are inherently unstable as they are essentially intermediates (Fig. 2). Previously, we determined the crystal structure of archaeal BPL [2]. On the basis of the 3D structural information of BPL, eight mutants were designed to stabilize the intermediate complexes. By using these BPL mutants, the crystallization screening of BPL:BCCP complexes was performed and was successful for two mutants that could potentially form complexes but would only react slowly with BCCP. We have determined the crystal structures of BPL:BCCP complexes for the first time in the world using the synchrotron radiation at beamline **BL26B1** (Fig. 3). In this work, a huge number of combinations of mutants, ligands and crystallization conditions were carried out, and about 30 relevant crystal structures were determined to elucidate the BCCP biotinylation reaction. In one of the crystal forms, surprisingly, two different states of biotinylation were observed in a single crystal: one of the complexes was in the unbiotinylated state before the reaction and the other complex showed the biotinylated state after the reaction. On the basis of these various crystal structures, we could successfully discuss the activation mechanism of ACC [3].

The 3D structural information presented here will be useful for the development of drugs for metabolic syndrome. For instance, large structural changes were observed at the C-terminal domain during the reaction (Fig. 3). Therefore, a certain ligand to arrest the structural change in the C-terminal domain can be used to control the BCCP biotinylation effectively. As another perspective from this work, our approach using mutant proteins may be applied to the structural determination of other recalcitrant but important target proteins that are difficult to crystallize owing to their poor crystallizability or inherent instability.

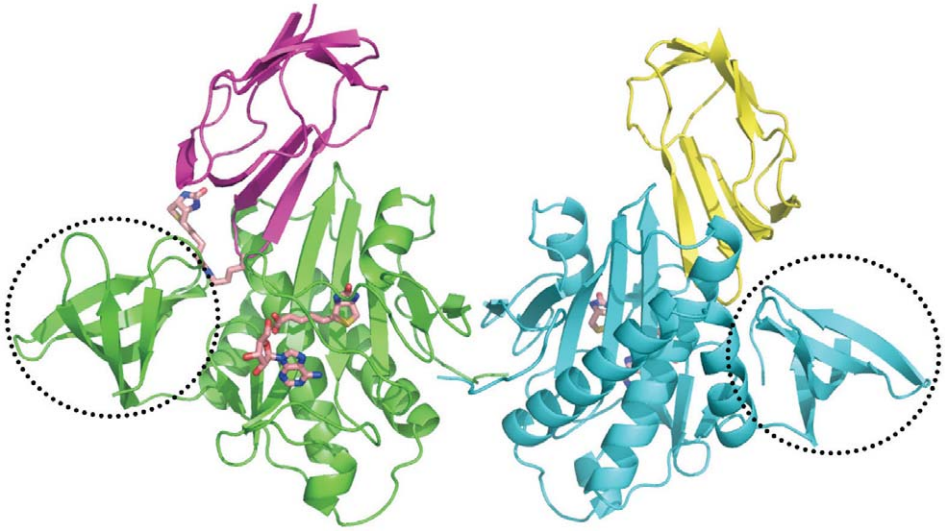

Fig. 3. Crystal structure of BPL:BCCP complex. BPL is a dimeric protein whose subunits are depicted as green and blue ribbon models. In the present crystal form of the BPL:BCCP complex, each BPL subunit binds a BCCP molecule (magenta or yellow model) to form a 2:2 complex. Two different states of the BCCP biotinylation reaction are observed in the same crystal: the right-side complex (blue and yellow models) and the left-side complex (green and magenta models) correspond to the states before and after the biotinylation, respectively. Dotted circles indicate the BPL's C-terminal domains that show large structural changes during the reaction, thereby suggesting a potential drug design target.

Naoki Kunishima* and Bagautdin Bagautdinov

SPring-8/RIKEN

*E-mail: kunisima@spring8.or.jp

References

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