Quaternary-Structure Change of Fatty Acid β-Oxidation Multienzyme Complex as Revealed by Small-Angle X-ray Scattering Analysis

Fatty acid is a major molecule for biological energy storage. In energy extraction, the molecule is conjugated with coenzyme A (CoA) to form acyl-CoA, which is successively decomposed by β-oxidation (Fig. 1). This metabolic pathway is a cycle of reactions catalyzed by acyl-CoA dehydrogenase (ACD), enoyl-CoA hydratase (ECH), 3-hydroxyacyl-CoA dehydrogenase (HACD), and 3-ketoacyl-CoA thiolase (KACT). Since many organisms possess these enzymes, their reactions are essential to maintain fundamental biological activity. Interestingly, the molecular architecture of β-oxidation enzymes significantly varies among organisms, suggesting that each enzyme is highly optimized for its functional role. In eukaryotic mitochondrion, a multienzyme complex, called the fatty acid β-oxidation multienzyme (FOM) complex, processes acyl-CoA derivatives with a long acyl group. The complex, catalyzing three consecutive reactions by ECH, HACD and KACT, is composed of two polypeptide chains, namely, α and β. The α-subunit exhibits ECH and HACD activities whereas the KACT active site is located in the β-subunit. A homologous multienzyme complex is also found in some prokaryotes, such as *Escherichia coli* and *Pseudomonas fragi*. The remarkable similarity in the amino acid sequence between the mitochondrial and bacterial FOM complexes suggests that they operate using a common catalytic mechanism with a similar protein architecture.

We determined the three-dimensional structures of the FOM complex from *P. fragi* in five crystal forms, among which three forms display distinct quaternary structures [1,2]. These structures revealed that the local environments of the six active sites in the $\alpha_2\beta_2$ heterotetramer are significantly different among the three forms. Thus, quaternary-structure conversion presumably occurs in the consecutive three reactions. However, the crystal packing inevitably restricts all of the structures. To explore the structural event during the chain reaction, the conformation of the complex should be investigated in a solution. Small-angle X-ray scattering (SAXS) with synchrotron radiation is suitable for probing the overall structure of large protein complexes in a solution. Therefore, we analyzed the ligand-induced conformational change of the FOM complex from *P. fragi* by SAXS [2].

The bacterial FOM complex overproduced in *E. coli* was purified to homogeneity. The X-ray scattering intensity of the protein solution was measured with synchrotron radiation at beamline **BL40B2**. We acquired the SAXS data in three different solution conditions: the ligand-free state, the states in the presence of nicotineamideadenine dinucleotide (NAD^+; cofactor for HACD), and of 3-hydroxyhexadecanoyl-CoA (HAC; substrate for HACD).

The conventional SAXS analysis, including the Guinier analysis, indicated that these ligands could induce significant conformational change. However, all of the experimental scattering profiles remarkably deviate from those calculated with known crystal structures. Rigid-body optimization of the structure to the experimental scattering data revealed that the rearrangement of the seven domains in the complex could reasonably account for the discrepancy (Fig. 2). As a result, the structural state for each condition could be estimated (Fig. 3). In the ligand-free state, the FOM complex

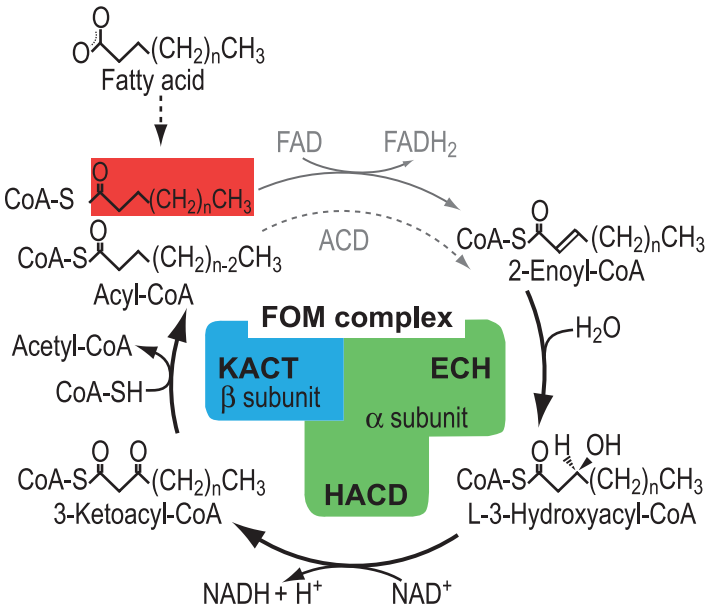

Fig. 1. Fatty acid β-oxidation cycle. The reactions catalyzed by the FOM complex are highlighted. The red box in the acyl-CoA molecule at the beginning of the cycle indicates the acyl group, which comes from the fatty acid molecule. Every cycle of the four reactions shortens the acyl group by two carbon units, which are released as acetyl-CoA.

assumes a flexible architecture, in which the position of the HACD region relative to the remaining ECH/KACT regions fluctuates in a rigid-body manner. The binding of two NAD^+ molecules induces the two-fold-symmetric structure whereas HAC occupies one of the two HACD active sites in the asymmetric heterotetramer. This contention is consistent with the previous crystallographic view that the precedent/following ECH/KACT reactions occur in the symmetric/asymmetric forms, respectively [1]. The ligand-induced conformational change is involved in mechanism of the chain reactions coupled with substrate channeling, which is important for controlling the flux of lipophilic compounds in cellular metabolism [3].

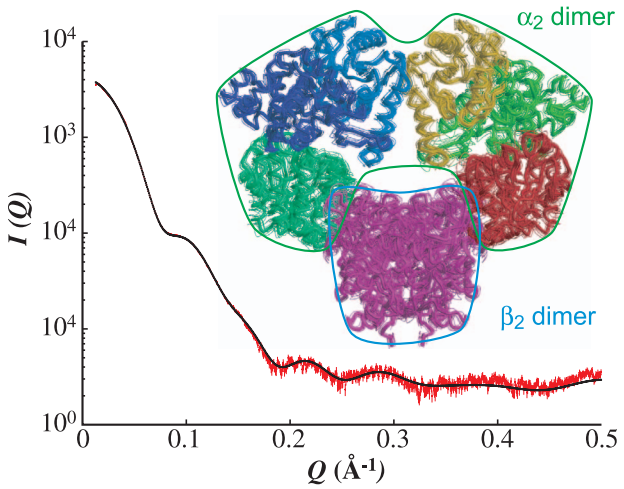

Fig. 2. Rigid-body optimization of atomic structure with experimental scattering data. Only the results for the NAD^+-bound state is shown. The graph represents the experimental scattering intensity with error bars (red). The black line indicates the scattering calculated with the mean structure (thick-tubing model) among the ten optimized structures (thin-tubing model). The structural models are colored to distinguish the seven rigid-body segments for the optimization. The α- and β-subunits are encircled with green and blue lines, respectively.

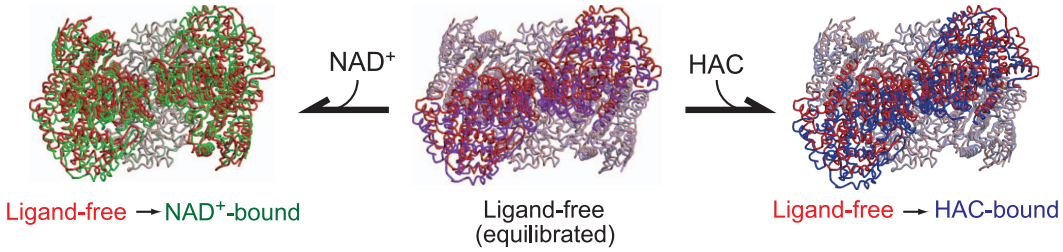

Fig. 3. Ligand-induced conformational change of FOM complex. Each structure is viewed from the top of the molecule drawn in Fig. 2. The HACD region is highlighted with the bright colors. The structures in the NAD^+-bound (green, left) and HAC-bound (blue, right) states were respectively superimposed onto the ligand-free structure (red). We presume that the ligand-free state (middle) is in a structural equilibrium, as represented by the two identical asymmetric models (red and magenta) related by the two-fold axis of the β_2 dimer.

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References

[1] M. Ishikawa *et al.*: EMBO J. **23** (2004) 2745.
[2] *D. Tsuchiya, N. Shimizu, M. Ishikawa, Y. Suzuki and K. Morikawa: Structure* ***14*** *(2006) 237.*
[3] D. Tsuchiya *et al.*: "Functional and Structural Biology on the Lipo-network" (Transworld Research Network, India, 2006), 117.