Structural Versatility of a Bacterial Aconitase as Revealed by Small-Angle X-Ray Scattering Analysis

Nowadays, to catalogue comprehensive three-dimensional folding patterns of proteins has an air of authenticity. On the other hand, the structural information of macromolecular complexes is still limited. Their structural analysis is an important task in modern structural biology, because many biological functions the require formation of complexes. In particular, macromolecular complexes maintained by weak protein-protein interactions are of considerable interest, because their versatile nature plays a vital role in dynamic biological processes. For instance, a multienzyme complex could exhibit an allosteric effect among the active sites. Temporal assembly of these enzymes yields a timely metabolic regulation, responding to different conditions. Although conventional biochemical studies focused on the catalytic properties of purified enzymes under diluted conditions, little is known about their proprieties under physiological conditions, where macromolecules are densely crowded. The intracellular environment could promote macromolecular association by weak interactions. Recent proteomic analyses results revealed several supramacromolecular complexes *in vivo*. However, their structural analyses are generally difficult, owing to their low stability *in vitro*.

Aconitase B (AcnB) from *Escherichia coli* is an iron-sulfur protein, catalyzing a reversible isomerization of citrate into isocitrate (Fig. 1). A previous study indicated that the protein could assume not only a monomer but also a homodimer in the cell [1]. The catalytic property apparently varied, depending on the oligomerization state. Consideration of the potential consequence tempted us to explore a linkage with a metabolon, that is, a multienzyme cluster catalyzing the consecutive reactions in the Krebs cycle [2]. The pairwise interactions between the component enzymes were too weak to detect *in vitro*. However, experimental data *in vivo* implied a possibility of a direct interaction between AcnB and isocitrate dehydrogenase (ICDH). ICDH catalyzes the next reaction of AcnB in the Krebs cycle (Fig. 1). Thus, the hypothetical interaction could affect the consecutive reactions.

We investigated the versatile architecture of AcnB and the interference in the reaction with ICDH [3]. Small-angle X-ray scattering (SAXS) is advantageous for analyzing protein structures with fragile interactions. The experimental data provide structural information of macromolecules in solution without any specific modifications. Although the data are of limited resolution, they could be interpreted using known atomic structures of the component proteins. Because direct binding of AcnB to ICDH was undetectable *in vitro*, a fusion protein of the two proteins was analyzed. The linker with 27 amino acid residues (about 80 Å), introduced by a genetic manipulation, connected the N-terminus of AcnB to the C-terminus of ICDH. The increased local concentration for each protein region promotes the weak intermolecular interaction. The SAXS data of *E. coli* AcnB, ICDH, and the fusion protein (AcnB-ICDH) were obtained with the synchrotron radiation at beamline **BL40B2**.

The conventional SAXS analysis indicated that all the three proteins formed homodimers in solution. These observations were consistent with the results of the chemical crosslinking [3]. The structural state of each protein was estimated, using the experimental SAXS data and the known three-dimensional structure (Fig. 2). The crystal structure of homodimeric ICDH reasonably explained the corresponding scattering profile. In contrast, only the monomeric structure was known for AcnB (PDB 1L5J). To account for the experimental SAXS data of AcnB, a three-dimensional model of the homodimeric AcnB was constructed by rigid-body modeling, using the atomic coordinates of the AcnB monomer. No structural state of the homodimeric AcnB-ICDH could be explained using

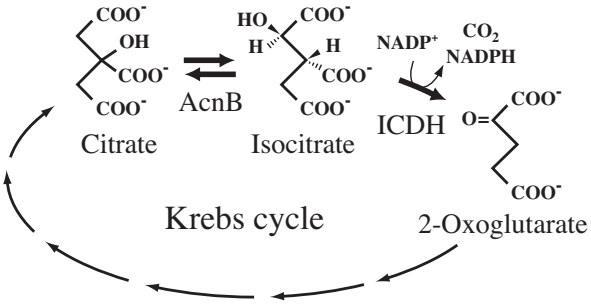

Fig. 1. Catalytic reactions of AcnB and ICDH.

any combination of the AcnB and ICDH homodimers, even though the linker was sufficiently long to form a hypothetical "heterodimer-of-homodimers." Instead, a rigid-body modeling with the SAXS data indicated that the fusion protein maintained only the homodimeric architecture of ICDH but not that of AcnB. Only one of the two monomeric AcnB regions associated with the homodimeric ICDH region.

The enzymatic assay revealed the variable catalytic property of AcnB, depending on the oligomerization state [3]. To perform the consecutive reaction from citrate to 2-oxoglutarate, AcnB-ICDH exhibited a slower catalysis than that observed in the mixture of the AcnB and ICDH proteins in an equimolar ratio. Considering the catalysis of each enzyme, monomerization of AcnB would attenuate the overall performance of the consecutive reaction, either by the decelerated hydration of the intermediate product in AcnB, or by the partial inhibition of ICDH activity. In addition, we observed that homodimerization of AcnB generated a substrate-dependent negative cooperativity between the two active sites [4]. This allosteric effect selectively promoted the isomerization from isocitrate, but not from citrate, at the intracellular substrate concentration. The dissociable homodimerization of AcnB, together with ICDH, may be involved in the complex control of the Krebs cycle.

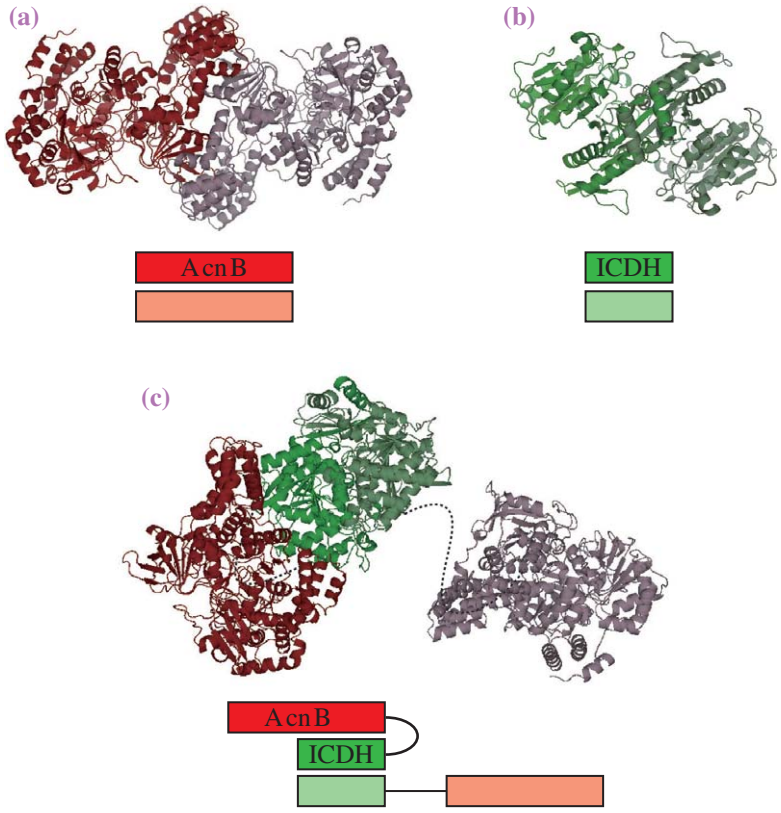

Fig. 2. Structural models of **(a)** AcnB, **(b)** ICDH, and **(c)** AcnB-ICDH in solution, which could account for the experimental SAXS profiles. The structure in **(b)** is the same as that observed in the crystal (PDB 3ICD). The models in **(a)** and **(c)** were constructed by a rigid-body modeling method [3], using the experimental SAXS profiles and atomic coordinates of PDB 1L5J (AcnB) and 3ICD (ICDH).

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