Crystal Structure of Acyl-coa Thioesterase PaaI showing 'Half-of-the-sites Reactivity'

Many proteins undergo structural changes in response to an external stimulus, and the structural changes, in turn, allow proteins to demonstrate their specific functions. The structural changes observed in crystallography and other methods are not necessarily large in scale; therefore, only proteins that undergo obvious structural changes have been the subject of research. How to analyze the subtle and complex structural changes of proteins is still one of basic propositions in structural biology. Here, such small conformational changes in an oligomeric enzyme called acyl-CoA thioesterase PaaI have been captured by applying a novel analysis method, and the oligomerization-function relationship of this protein has been explained successfully [1].

The PaaI protein is an enzyme known to be associated with the degradation of biodegradable plastics, and functions in association as a tetrameric oligomer composed of four identical subunits. To elucidate the oligomerization-function relationship of this oligomeric protein, several crystal structures of PaaI with and without coenzyme A ligands were determined on the basis of X-ray diffraction data collected at the RIKEN Structural Biology beamlines **BL44B2** and **BL45XU**. Interestingly, only two of four intersubunit active sites of the PaaI tetramer were found to be occupied by the ligands, which is a phenomenon so-called 'half-of-the-sites reactivity' (Fig. 1). This half-of-the-sites reactivity is a seemingly wasteful phenomenon observed in oligomeric enzymes where only one-half of several active parts are used, which was so far hypothesized 35 years ago [2]. Presently determined PaaI structures provide the first direct proof of half-of-the-sites reactivity in general enzymes.

To elucidate the structural basis of half-of-the-sites reactivity, a detailed structural comparison between several liganded and unliganded PaaI structures was tried. For this purpose, a novel analysis method referred to as the 'multiple superposition method' has been developed. In the multiple superposition method, the unliganded and liganded structures are precisely compared by multiple superpositions of corresponding protein atoms, where the conformational change is decomposed into following two elements: an overall rearrangement of the subunit referred to as a rigid-body shift and an intrasubunit local deformation referred to as a local shift. Applying this method allowed us to show that a subtle rigid-body rearrangement of subunits within 2º upon binding

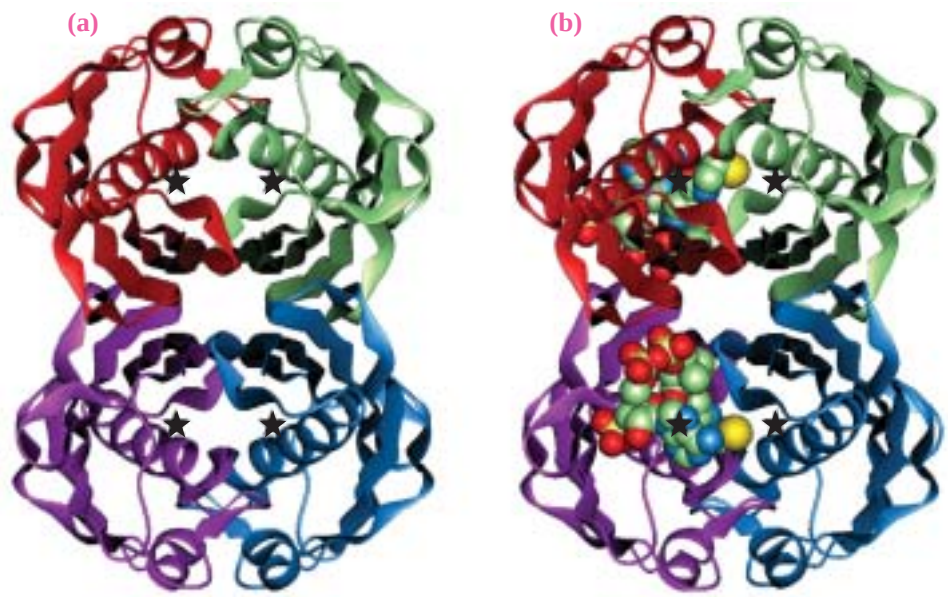

Fig. 1. Crystal structures of acyl-CoA thioesterase PaaI in **(a)** unliganded form and **(b)** coenzyme A liganded form, showing 'half-of-the-sites reactivity' of oligomeric enzyme. Four different subunits of the PaaI tetramer are distinguished by color-coding. The ligand coenzyme A molecules are depicted as space-filling models. Of four active sites indicated by asterisks, only two sites can bind ligands. Note that the structural change upon ligand binding is not obvious.

of the first two ligand molecules cooperatively induces a structural change in remaining two active sites, which may be unfavorable for further binding of ligands (Fig. 2).

Although the biological significance of half-of-the-sites reactivity has been unknown to date, the present work successfully suggests it for the first time. The molecular size of acyl-CoA, which is the substrate of PaaI, is relatively large as that of a biological ligand. The tetrameric PaaI molecule effectively accommodates the large ligand utilizing its asymmetric rigid-body rearrangement of subunits, thereby resulting in the half-of-the-sites reactivity. Therefore, the effective recognition of acyl-CoA at the first two active sites might be of advantage for PaaI even if the demerit of losing activity at remaining two is taken into account.

On the other hand, the activity regulation of this type of oligomeric enzyme through the binding of another ligand as a modulator at remaining sites may also be a pivotal role of half-of-the-sites reactivity. Human thioesterase III (hTEIII) is known as a homologue of PaaI. It was reported that hTEIII is activated by the infection of HIV that causes AIDS [3]. Therefore, the elucidation of the hTEIII activation mechanism may be useful for the structure-based drug design of AIDS therapy. Although the crystal structure of hTEIII is still unknown, half-of-the-sites reactivity can be suggested as a principle for activity regulation. In conclusion, the biological role of half-of-the-sites reactivity presented here may be one of the fundamental catalytic strategies of oligomeric enzymes.

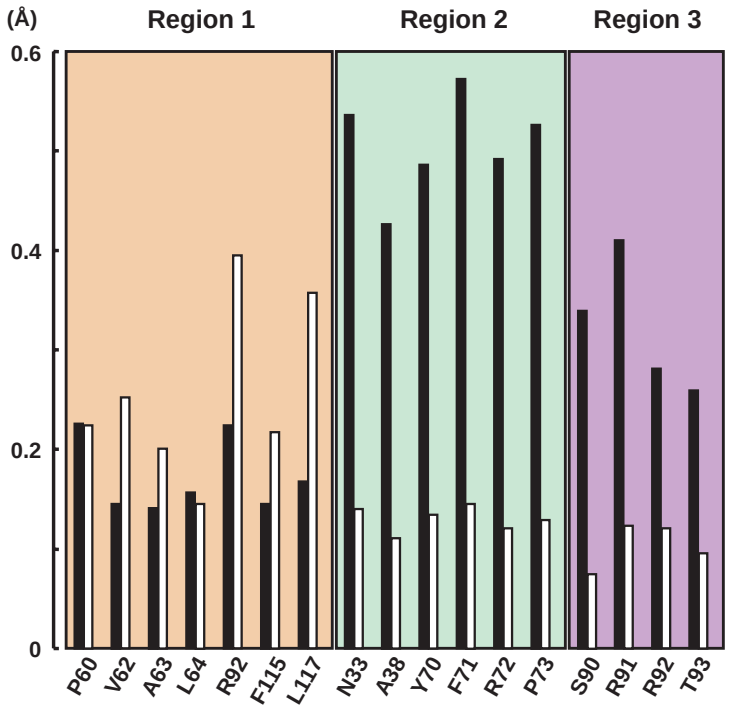

Fig. 2. Subtle structural changes of ligand recognition atoms in PaaI captured by the multiple superposition method. Rigid-body shifts of recognition atoms upon ligand binding are calculated and averaged by residue. In the PaaI tetramer, the ligand coenzyme A molecule is recognized by regions 1, 2 and 3, which are distinguished by different colors. The rigid-body shifts at occupied and vacant active sites are separately shown as closed and open bars, respectively. Different and complementary patterns of rigid-body structural changes are observed between the occupied and vacant sites, indicating a cooperativity upon ligand binding.

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References

[1] *N. Kunishima, Y. Asada, Ma. Sugahara, J. Ishijima, Y. Nodake, Mitsu. Sugahara, M. Miyano, S. Kuramitsu, S. Yokoyama and Michi. Sugahara: J. Mol. Biol.* ***352*** *(2005) 212.*
[2] A. Levitzki *et al.*: Biochemistry **10** (1971) 3371.
[3] H. Watanabe *et al.*: Biochem. Biophys. Res. Commun. **238** (1997) 234.