Crystal Structure of Human Membrane-integrated Protein Responsible for Biosynthesis of Cysteinyl Leukotrienes

The human membrane-integrated protein leukotriene C_4 synthase (LTC_4S) embedded in the nuclear membrane is the first enzyme involved in the biosynthesis of cysteinyl leukotriene (Cys-LT), and its inhibitor is expected to be a new therapeutic agent for symptoms caused by proinflammatory acute smooth muscle constriction in conditions such as anaphylaxis, allergy, and asthma. LTC_4S conjugates leukotriene (LT) A_4 and reduced glutathione (GSH) to synthesize LTC_4 (Fig. 1). LTC_4 and its metabolites, LTD_4 and LTE_4, are collectively called Cys-LTs, and Cys-LTs have been known as slow-reacting substance of anaphylaxis (SRS-A) [1,2]. The smooth muscle constriction activity of Cys-LT is one thousand-fold higher than that of histamine, a well-known inflammatory substance. LTC_4S is a potential target in the development of new therapeutic agents for anaphylaxis, allergy, and asthma, and the three-dimensional structure of LTC_4S enables us to apply modern method for drug discovery.

We elucidated the crystal structure of human LTC_4S at 3.3 Å resolution using beamline **BL44B2**, which makes human LTC_4S one of three human membrane-integrated proteins whose structures were determined crystallographically first [3]. Indeed, one of the bottlenecks is the production of a fully active human membrane-integrated protein at crystallographic quality and quantity using a heterologous expression system. We established a heterologous expression system using the fission yeast *Schizosaccharomyces pombe* to obtain a fully active LTC_4S on the milligram scale from a few liters of yeast culture.

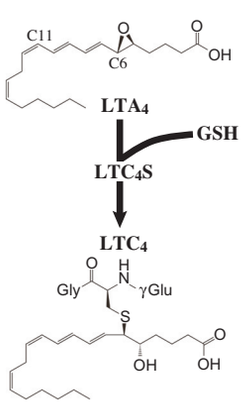

Fig. 1. Biosynthesis of Cys-LT. LTC_4S catalyzes conjugation between LTA_4 and reduced GSH to synthesize LTC_4, which is further metabolized to LTE_4 through LTD_4 by hydrolytic enzymes.

The crystal structure of LTC_4S revealed that trimeric LTC_4S in a three-fold symmetry is a biological functional unit [3]. An LTC_4S monomer is composed of five α-helices, the first four of which are transmembrane α-helices and the fifth α-helix protruding into the bulk solvent from nuclear membrane (Fig. 2(a,b)). The V-shaped cleft surrounded by five α-helices is the substrate binding site. All the five α-helices contribute to the binding of a unique U-shaped GSH to the upper side of the cleft (Fig. 2(c)). For example, the positive charge of Arg51 of α-helix II is essential to the binding of GSH [4]. The remaining space extending from GSH to the bottom of the cleft would be the binding site of LTA_4. The depth and shape of the space fits well to the molecular shape of the aliphatic chain of LTA_4 having two *cis*-double bonds at C11 and C14 (Fig. 3). Indeed, the hexyl moiety of *S*-hexyl glutathione as a weak inhibitor occupies the space in the *S*-hexyl GSH complex crystal (Fig. 2(d)). Furthermore, the aliphatic chain of dodecyl maltoside occupies the space in the GSH complex crystal. Together with these results, the active site is located between the two adjacent monomers in the trimeric LTC_4S, and the trimeric LTC_4S is the biological functional unit.

The crystal structure of LTC_4S shows that the conjugation of LTA_4 and GSH is induced by acid-base catalysis, in which Arg31 and Arg104 are the acid and base, respectively. During the course of the conjugation, LTC_4S activates the thiol group of bound GSH for the nucleophilic attack of the sixth carbon of LTA_4 and provide a hydrogen to the negatively charged oxygen derived from the epoxide group at the fifth carbon. The side chain of Arg104 as the base decreases the pKa of the thiol group of GSH as the reactive species under the physiological condition due to a hydrogen bond between Arg104 and the thiol group of GSH (Fig. 2(c)). When LTA_4 binds to the GSH-binding LTC_4S (Fig. 3), the activated thiol group would attack C6 on the opposite side against the epoxy oxygen of LTA_4, and a negative charge would grow on the epoxy oxygen concurrently with the breaking of the bond between the epoxy oxygen and C6. Finally, Arg31 as the acid provides a proton to the negatively charged oxygen at C5, resulting in LTC_4 as the conjugated product.

The crystal structure of the human membrane-

integrated protein LTC_4S reveals unique features for the strict substrate specificity and the conjugation catalyzed by two arginine residues in an acid-base-catalytic manner. We hope that this work contributes to the advancement of the pathobiology of inflammatory diseases and to the development of new therapeutics.

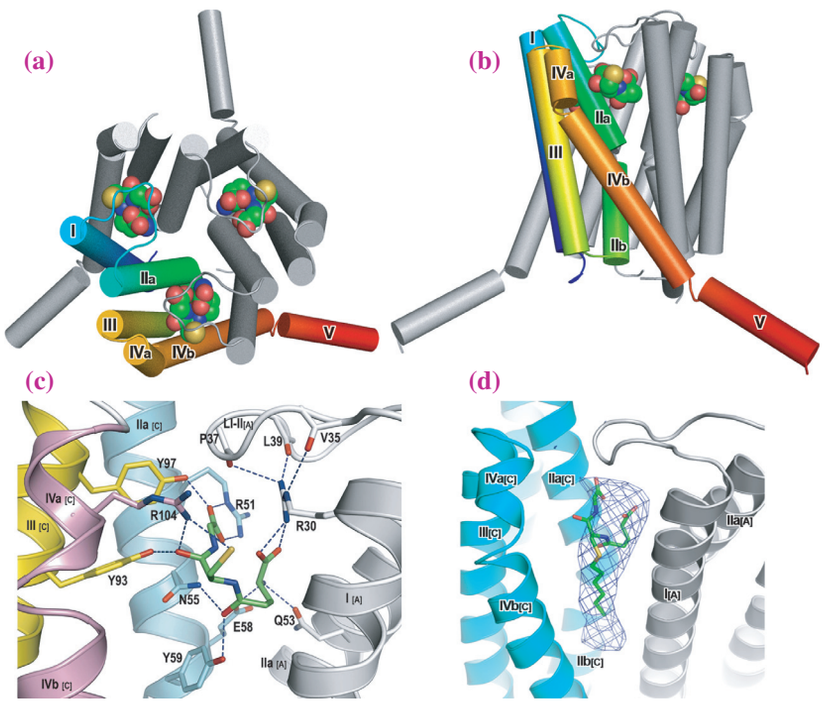

Fig. 2. Crystal structure of LTC_4S. **(a,b)** Schematic representations of trimeric LTC_4S. α-Helices from a monomer are tube models in rainbow color, and the bound GSH is in space-filling model. In all panels, the sequential number on each α-helix is the order of the α-helix from the N-terminus. The small letter following the sequential number means that the α-helix is subdivided. A small capital in square brackets corresponds to the monomer, which contains the α-helix. **(c)** Close-up view of the bound GSH. Dashed lines are polar interactions participating in GSH binding. **(d)** Electron density of inhibitor *S*-hexyl GSH at 5 Å resolution.

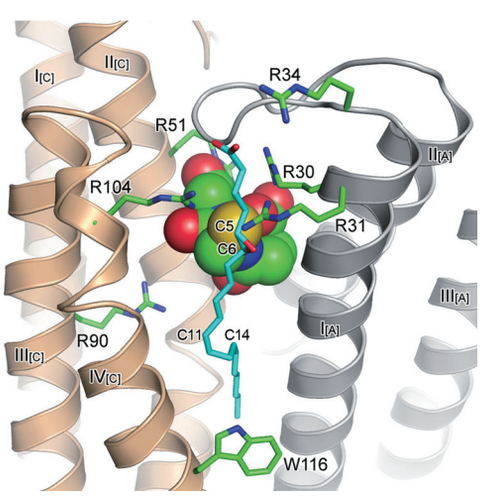

Fig. 3. LTA_4 binding model. The stick model with cyan carbon is the modeled LTA_4, and the CPK model is the bound GSH in which sulfur, carbon, nitrogen, and oxygen are in yellow, green, blue, and red, respectively.

Hideo Ago* and Masashi Miyano

SPring-8 / RIKEN

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

References

[1] S.-E. Dahlén *et al.*: Nature **288** (1980) 484.
[2] K.F. Austen: Prostaglandins Other Lipid Mediat. **83** (2007) 182.
[3] *H. Ago, Y. Kanaoka, D. Irikura, B.K. Lam, T. Shimamura, K.F. Austen and M. Miyano: Nature **448** (2007) 609.*
[4] B.K. Lam *et al.*: J. Biol. Chem. **272** (1997) 13923.