MS9-P4 Structural insights into the catalytic reaction trigger and inhibition of D-3-hydroxybutyrate dehydrogenase

Hiroki Kanazawa[1,2], Md. Mominul Hoque[1,3], Masaru Tsunoda[1], Kaoru Suzuki[1], Tamotsu Yamamoto[4], Gota Kawai[5], Jiro Kondo[2], Akio Takénaka[1,5]

1. Faculty of Pharmacy, Iwaki Meisei University, Chuodai-iino, Iwaki 970-8551, Japan
2. Department of Materials and Life Sciences, Sophia University, Kioi-cho, Chiyoda-ku, Tokyo 102-8554 Japan
3. Department of Biochemistry and Molecular Biology, University of Rajshahi, Rajshahi-6205, Bangladesh
4. Asahi Kasei Pharma Corporation, Tagata-gun, Shizuoka 410-2323, Japan
5. Research Institute, Chiba Institute of Technology, Tsudanuma, Narashino, Chiba 275-0016, Japan

email: h.kanazawa@eagle.sophia.ac.jp

It's known that acetyl-CoA which carries an acetyl group as energy source for the TCA cycle is prepared through the two different pathways, glycolysis and β-fatty acid oxidation. However, excess amount of acetyl-CoA is converted to acetoacetate in liver, and further converted to D-3-hydroxybutyrate as a water-solvable form. These two ketone bodies are transported *via* bloodstream to peripheral organisms which consume lots of energy, and then acetyl-CoA is reproduced. It is D-3-hydroxybutyrate dehydrogenase which catalyze the reversible conversion. In order to clarify the structural mechanisms of the catalytic reaction with the cognate substrate D-3-hydroxybutyrate and of the reaction inhibition with inhibitors, the enzyme from *Alcaligenes faecalis* has been X-ray analyzed in the liganded states with the substrate, and with two kinds of inhibitors, malonate and methylmalonate.

Four subunits form a tetrameric enzyme. In each subunit, the substrate is trapped on the nicotineamide plane of NAD^+ bound in a central part of the enzyme. The OMIT map definitively shows that the bound ligand is D-3-hydroxybutyrate but not acetoacetate. The carboxylate group of the substrate form four hydrogen bonds with Gln94, Gln196, Lys152 and His144. The methyl group prefers to accommodate in the nearby hydrophobic pocket, so that the OH group of substrate is facilitated to form a hydrogen bond with the hydroxy group of Tyr155 (Fig. 1a). In this geometry, an active H atom attached to the C^3 atom of the substrate in the sp^3 configuration is positioned at a distance of 3.1 Å from the nicotineamide C^4 atom in a direction of the plane normal. In addition, the donor-acceptor relationship of the hydrogen bonds suggests that the Tyr155 OH group is allowed to ionize by the two donations from the Ser142 OH and the ribose OH groups. A structural comparison of the enzymes with/without ligands suggests that the domain movement to form a hydrogen bond with the substrate facilitates the active H-atom movement of the substrate as the trigger of the catalytic reaction. In the complexes with inhibitors (Fig. 1b,c), however, their principal carboxylate groups interact with the enzyme, while the interactions of other groups are changed. The crucial determinant for inhibition is that the inhibitors have no active H atom at C^3. In addition, the second determinant is the Tyr155 OH group which is perturbed by the inhibitors to donate the H atom for hydrogen-bond formation, losing the nucleophilicity.

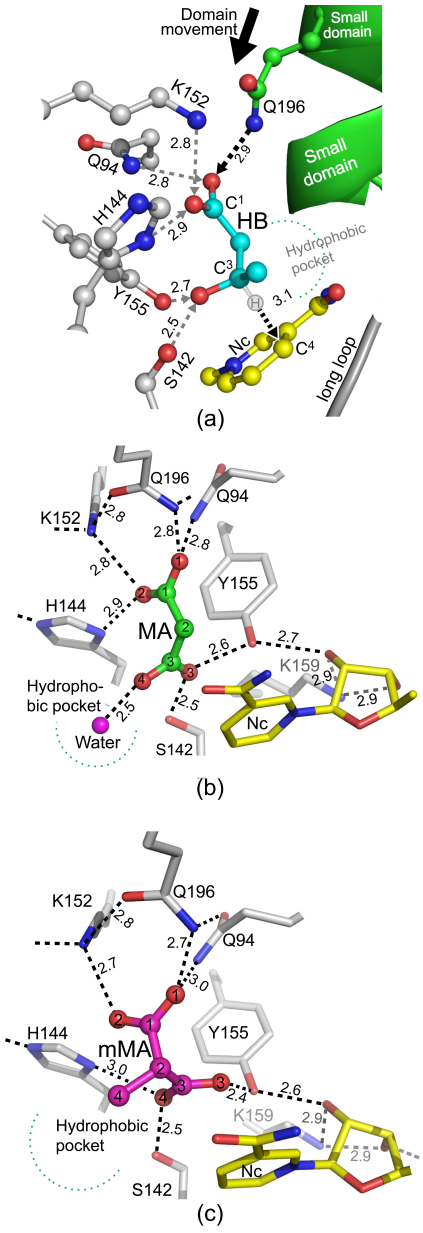

Figure 1. Interaction geometries of substrate/inhibitor binding sites found in substrate-complex (a), malonate-complex (b) and methylmalonate-complex (c). Broken lines indicate possible hydrogen bonds. Dotted half circles show the hydrophobic pocket surrounded by His144, Ala186, Trp187 and Trp257.

Keywords: Reaction mechanism, D-3-Hydroxybutyrate dehydrogenase, Substrate, Inhibitor

MS9-P5 Structures of the photosensory core module of bacteriophytochrome Agp1 from two crystal forms reveal plasticity of the Pr state

Norbert Krauß[1,2], Soshichiro Nagano[2,3], Patrick Scheerer[4], Kristina Zubow[2], Norbert Michael[5], Katsuhiko Inomata[6], Tilman Lamparter[1]

1. Karlsruher Institut für Technologie, Botanisches Institut (Germany)
2. Queen Mary University of London, School of Biological and Chemical Sciences (United Kingdom)
3. Justus-Liebig-Universität Giessen, Institut für Pflanzenphysiologie (Germany)
4. Charité - Universitätsmedizin Berlin, Institut für Medizinische Physik und Biophysik (Germany)
5. Technische Universität Berlin, Institut für Chemie (Germany)
6. Kanazawa University, Division of Material Science (Japan)

email: norbert.krauss@kit.edu

Agp1 is a canonical biliverdin-binding bacteriophytochrome from the soil bacterium *Agrobacterium fabrum* that acts as a light-regulated histidine kinase and uses the red-light-absorbing Pr form as the dark adapted state [1]. At the level of the tertiary structure, the structural changes that occur during photoconversion from Pr to the far-red-absorbing Pfr form are characterised by a transition in the so-called tongue region of the PHY domain from an antiparallel β-sheet in Pr to a partially α-helical structure in Pfr and rearrangements within the long α-helix that connects the GAF and the PHY domain. Based on those crystal structures of photosensory core modules (PCMs) where the protein subunits are arranged as parallel dimers, suggestions have been made as to how changes in the quaternary structure of the PCM associated with photoconversion lead to modulation of the activity of the histidine kinase output module [2, 3, 4]. Here we present crystal structures of the PCM of Agp1 at 2.70 Å resolution and of a surface-engineered mutant at 1.85 Å resolution in Pr. Whereas in the mutant structure the dimer subunits are found to be in anti-parallel orientation, the wild-type structure contains parallel dimers, which structurally differ significantly from the parallel dimers of the PCMs of other phytochromes in Pr. Comparison of the two Agp1-PCM structures reveals relative orientations between the PAS-GAF bidomain and the PHY domains to be different, due to movement about two hinges in the GAF-PHY connecting α-helix and in the tongue region that indicates pronounced structural flexibility which may give rise to a dynamic Pr state even at the level of the quaternary structure. The high resolution of the mutant structure enabled us to detect a sterically strained conformation of the chromophore at pyrrole ring A and its side chain that we attribute to the tight interaction with Pro461 of the PRxSF motif in the tongue region and the restricted conformational degrees of freedom which are due to the covalent linkage between biliverdin (BV) and Cys20. Based on this observation and on additional biochemical data we discuss the potential reasons for the crucial role which the tongue region plays in Pr-to-Pfr photoconversion.

References:

[1] T. Lamparter et al., *Proc. Natl. Acad. Sci. U. S. A.* 99, 11628-11633 (2002)

[2] H. Takala et al., *Nature* 509, 245-248 (2014)