Crystal Structure of the Membrane-bound Bifunctional Transglycosylase PBP1b from *Escherichia coli*

Ming-Ta Sung (宋明達)[1,2], Yen-Ting Lai (賴彥廷)[1], Chia-Ying Huang (黃佳瑩)[1,3], Lien-Yang Chou (卓聯洋)[1], Hao-Wei Shih (施皓維)[1,4], Wei-Chieh Cheng (鄭偉杰)[1], Chi-Huey Wong (翁啟惠)[1], and Che Ma (馬徹)[1]

[1]Genomics Research Center, Academia Sinica, Taipei, Taiwan
[2]Institutes of Biochemistry, National Yang-Ming University, Taipei, Taiwan
[3]Institutes of Microbiology and Immunology, National Yang-Ming University, Taipei, Taiwan
[4]Department of Chemistry, National Taiwan University, Taipei, Taiwan

The crystal structure of *E. coli* PBP1b in complex with moenomycin was solved at 2.16-Å resolution (Fig. 1). By using a multiwavelength anomalous dispersion (MAD) approach with crystals from selenomethionine-labeled proteins, the phase information was obtained to generate a protein electron density map. The structure was built from residues 66–800, except 2 loop regions with absent electron density (residues 249–267 and 399–406), and was refined to good quality with R_{work} and R_{free} values of 20.6% and 25.1%, respectively.

The presence of the transmembrane (TM) helix in our structure allowed us to postulate the orientation of the *E. coli* PBP1b molecule in lipid bilayers. It is commonly accepted that tryptophan and tyrosine residues have a higher frequency to be found at the lipid–water interface in membrane proteins. We examined all plausible tryptophan and tyrosine residues in the transglycosylase (TG) domain and TM helix and found a plane consisting of tryptophan and tyrosine residues that might be associated with lipids (Fig. 1).

In addition to the TM, TG, and TP (transpeptidase) domains that are commonly found in bifunctional transglycosylases, an unexpected domain was observed in our crystal structure (Fig. 1). This domain, comprising residues 109–200, folds with a 5-antiparallel-stranded β-sheet and 1 α-helix. By using Dali search, this domain was found to be structurally homologous to domains in UvrB (RMSD is 1.8 Å for 82 Cα atoms). Based on the highly similar fold, we referred to this domain as UB2H (UvrB domain 2 homolog) domain.

The overall fold of the TG domain in our structure, in complex with moenomycin, is highly similar to the 2 available transglycosylase structures (PDB ID code 2OLV and 3D3H). However, the residues involved in potential interactions with moenomycin showed similarities and differences in these transglycosylase structures [1]. The interacting residues of the TG domain around the E ring, the F ring, the phosphate group, and the carboxylate group of moenomycin are more conserved than the interacting residues with the remaining parts (Fig. 2). The conserved interacting residues in the binding pocket of transglycosylases can be considered as the most critical region to be studied in the process of antibiotic design. Our result is in agreement with the previous findings to define the minimal pharmacophore in moenomycin, in which the EF-ring phosphoglycerate portion together with either the C or the D ring forms critical interactions with proteins. The crystal structure of *E. coli* PBP1b represents a structural platform of transglycosylase, in particular for Gram-negative bacterial pathogens, for the development of antibiotics.

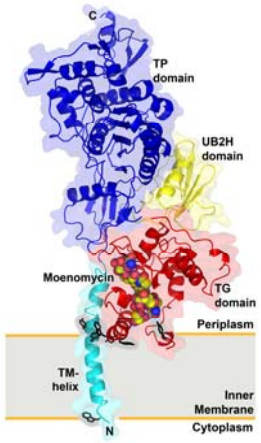

Fig. 1: The crystal structure of PBP1b is represented as a ribbon diagram. The TM, UB2H, TG, and TP domains are color coded in cyan, yellow, red, and blue, respectively. Moenomycin is represented as van der Waals spheres. Tryptophan and tyrosine residues located near the water–membrane interfaces are shown in black sticks. The proposed membrane location is indicated by a gray rectangle.

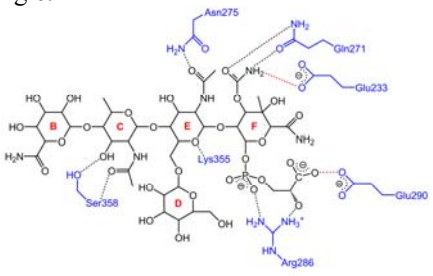

Fig. 2: The potential hydrogen-bonding interactions (distance cutoff 3.2 Å) between *E. coli* PBP1b and moenomycin are shown as dashed lines in black. The interactions between the putative active sites (E233 and E290) and moenomycin are shown as dashed lines in red.

Reference

[1] M.-T. Sung, Y.-T. Lai, C.-Y. Huang, L.-Y. Chou, H.-W. Shih, W.-C. Cheng, C.-H. Wong, and C. Ma, Proc. Natl. Acad. Sci. USA **106**, 8824 (2009).