C-5-07

Inhibitor Screening of Capsular Polysaccharide Synthesizing Enzyme CapF from *Staphylococcus aureus*

Koichiro Nakano[1] kk126434@mgs.k.u-tokyo.ac.jp
Takamitsu Miyafusa[1] t-miyafusa@aist.go.jp
Satoru Nagatoishi[2] nagatoishi@bioeng.t.u-tokyo.ac.jp
Jose Manuel Martinez Caaveiro[2] jose@bioeng.t.u-tokyo.ac.jp
Kouhei Tsumoto[1,2,3] tsumoto@bioeng.t.u-tokyo.ac.jp

[1] Department of Medical Genome Sciences, Graduate School of Frontier Sciences, The University of Tokyo, 4-6-1 Shirokanedai, Minato-ku, Tokyo 108-8639, Japan
[2] Department of Bioengineering, Graduate School of Engineering, The University of Tokyo, 4-6-1 Shirokanedai, Minato-ku, Tokyo 108-8639, Japan
[3] Medical Proteomics Laboratory, The Institute of Medical Science, The University of Tokyo, 4-6-1 Shirokanedai, Minato-ku, Tokyo 108-8639, Japan

Keywords: FBDD, Enzyme inhibition, *Staphylococcus aureus*, SPR, ITC

The enzyme CapF is essential for synthesizing capsular polysaccharide of *Staphylococcus aureus*, and reduces its substrate by oxidizing NADPH [1,2]. Interestingly, the affinity of CapF for NADPH is approximately 100-hold higher than that for $NADP^+$. In addition, it has been reported that NAPDH binding to Ps3αHSD [3] or SDRvv [4] form helical structures, that have a similar function of the enzyme activity in CapF. Therfore, we had a hypothesis that the recognition ability of CapF is caused by its loop structure of CapF near the NADPH-binding site of one. Here, we performed a small-compoud screening to search a inhibitor for synthesizing capsular polysaccharide by regulating the significant recognition ability of CapF based on the hypothesis.

To find the specific binder to CapF, a hit screening based on a Fragment library (FBDD) is carried out using surface plasmon resonance and enzyme activity assay. We obtained some compounds in each screening assay. These compounds were characterized by the some assays such as does-dependent binding assays and calorimetric analyses.

[1] Miyafusa, T., Caaveiro, J.M., Tanaka, Y. and Tsumoto, K., Crystal structure of the enzyme CapF of *Staphylococcus aureus* reveals a unique architecture composed of two functional domains, *Biochemical Journal*, 443:671-680, 2012.

[2] Miyafusa, T., Caaveiro, J.M., Tanaka, Y., Tanner, M.E. and Tsumoto, K., Crystal structure of the capsular polysaccharide synthesizing protein CapE of *Staphylococcus aureus*, *Bioscience Reports*, 33:463-474, 2013.

[3] Buysschaert, G., Verstraete, K., Savvides, S.N. and Vergauwen, B., Structural and biochemical characterization of an atypical short-chain dehydrogenase/reductase reveals an unusual cofactor preference, *The FEBS Journal*, 280:1358-1370, 2013.

[4] Nakamura, S., Oda, M., Kataoka, S., Ueda, S., Uchiyama, S., Yoshida, T., Kobayashi, Y. and Ohkubo, T., Apo- and holo-structures of 3alpha-hydroxysteroid dehydrogenase from Pseudomonas sp. B-0831. Loop-helix transition induced by coenzyme binding. *The Journal of Medicinal Chemistry*, 281:31876-31884, 2006.