

s1.m7.p19 **Crystal structure of a γ -butyrolactone autoregulator receptor protein in *Streptomyces coelicolor* A3(2).** Ryo Natsume,^a Sueharu Horinouchi^b and Toshiya Senda^c, ^aJapan Biological Information Research Center (JBIRC), Japan biological informatics consortium, 2-41-6 Aomi, Koto-ku, Tokyo 135-0064, Japan, ^bDepartment of Biotechnology, Graduate School of Agriculture and Life Sciences, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan, and ^cBiological Information Research Center (BIRC), National Institute of Advanced Industrial Science and Technology (AIST), 2-41-6 Aomi, Koto-ku, Tokyo 135-0061, Japan. E-mail: rnatsume@jbirc.aist.go.jp

Keywords: Autoregulator; γ -butyrolactones; Receptor

The γ -butyrolactone-type autoregulator/receptor systems in the Gram-positive bacterial genus *Streptomyces* regulate morphological differentiation or antibiotic production, or both. The autoregulator receptors act as DNA-binding proteins, and on binding their cognate ligands (γ -butyrolactones) they are released from the DNA, thus serving as repressors. CprB in *Streptomyces coelicolor* A3(2), a homologue of the A-factor-receptor protein, ArpA, in *Streptomyces griseus*, was crystallized [1] and its crystal structure was determined at 2.4 Å resolution by MAD method [2]. The overall structure of CprB shows that the γ -butyrolactone receptors belong to the TetR family. CprB forms homo-dimer linked by an inter-subunit S-S bridge. Each subunit is composed of two domains, a DNA-binding domain and a regulatory domain. The DNA-binding domain contains a typical helix-turn-helix motif, whose residues are well conserved among the related proteins. The regulatory domain contains a hydrophobic cavity. The cavity seems to be the ligand-binding pocket, as the cavity is large enough to accommodate an entire molecule of typical γ -butyrolactones and the side chain of the conserved Trp residue, which is essential for ligand-binding, lines the inner surface of the cavity. On the basis of the crystal structure of CprB and on the analogy of the characteristics of ligand-TetR binding, the binding of γ -butyrolactones to the regulatory domain of the receptors is supposed to induce the relocation of the DNA-binding domain through conformational changes of residues located between the ligand-binding site and the DNA-binding domain, which would result in the dissociation of the receptors from their target DNA.

- [1] Natsume, R., Takeshita, R., Sugiyama, M., Senda, T. & Horinouchi, S. (2003). Crystallization of CprB, an autoregulator-receptor protein in *Streptomyces coelicolor* A3(2). *Acta Crystallogr. D*, **59**, 2313-2315
- [2] Natsume, R., Ohnishi, Y., Senda, T. & Horinouchi, S. (2004). Crystal structure of a γ -butyrolactone autoregulator receptor protein in *Streptomyces coelicolor* A3(2). *J. Mol. Biol.*, **336**, 409-419

s1.m7.p20 **Crystallisation and preliminary X-ray analysis of Glycolipid Transfer Protein.** Yvonne Nymalm, Gun West, Heidi Kidron, Tomi Airene, Peter Mattjus and Tiina Salminen, Åbo Akademi University, Department of Biochemistry and Pharmacy, Turku, Finland. E-mail: ynymalm@abo.fi

Keywords: Lipid transfer; Glycolipid; Crystallization

Glycolipid transfer protein (GLTP) is a soluble protein that transfers glycolipids between membranes [1]. In contrast to non-specific lipid transfer proteins it transfers only certain glycolipids. The exact mechanism of GLTP for extracting lipids from the membrane is not known, but it is presumed that GLTP causes a local disturbance in the structure of the bilayer and this facilitates the lipid removal [2].

GLTP consists of 209 amino acids, with a mass of 22 kDa and a basic isoelectric point. The amino acid sequence is conserved between species, which indicates that GLTP has an important role. Still, GLTP has no sequence similarity to other lipid transfer proteins and few other sequence homologues have been found. Two such homologues are the fungal protein HET-C2 and ACD11 from *Arabidopsis thaliana* [3,4]. Both proteins show an *in vitro* capacity to transfer lipids [5,6].

Bovine GLTP has been expressed and purified as a His-tag fusion protein. A crystal grown at +4°C in 0.1 M Sodium acetate, 0.1 M MES pH 6.6 and 30 % w/v PEG 8000, with the hanging drop vapor diffusion method, was analyzed at the X13 beamline at EMBL/DESY (Hamburg, Germany). The crystal was cryoprotected with 30 % glycerol. The crystal diffracted to 1.6 Å, the space group was P21 and the unit cell dimensions were a=55.4 b=34.9 c=58.2 a=g=90 b=116.2. Crystallization of selenomethionine and heavy atom derivatives of GLTP is now in progress.

- [1] Mattjus, P., Kline, A. Pike, H.M., Molotovskiy, J.G., and Brown, R.E. (2002) *Biochemistry* **41**, 266-273
- [2] Sasaki, T. (1990) *Experientia* **46**, 611-616
- [3] Saupe, S., Descamps, C., Turcq, B. and Begueret, J. (1994) *PNAS* **91**, 5927-5931
- [4] Wuetrich, K. L., Bovet, L., Hunziker, P.E., Donnison, I. S., and Hörtensteiner, S. (2000) *Plant J.* **21**, 189-198
- [5] Mattjus, P., Turcq, B., Pike, H.M., Molotovskiy, J.G., and Brown, R.E. (2002) *Biochemistry* **42**, 535-542
- [6] Broedersen, P., Petersen, M., Pike, H.M., Olszak, B., Skov, S., Odum, N., Jorgensen, L.B., Brown, R.E., and Mundy, J. (2002) *Genes Dev.* **16**, 490-502