s1.m8.p12 **Ion channels in crystals of gramicidin D complex with RbCl. Atomic resolution low-temperature synchrotron X-ray data.** M.L. Glówka, A. Olczak, J. Bojarska, M. Szczesio[a], W. L. Duax, B. M. Burkhart, W. A. Pangborn, D. A. Langs, N. Li[b], Z. Wawrzak[c], *[a]Technical University, Lódz, Poland, [b]Hauptman-Woodward Medical Research Institute Inc.Buffalo, NY, USA, [c]DND-CAT Synchrotron Research Center, Argonne, Il, USA. E-mail: marekglo@p.lodz.pl*

Keywords: Gramicidin; Double-stranded channel; Atomic resolution

Gramicidin D (gD) is a naturally occurring ionophoric antibiotic produced by *Bacillus brevis*. It is active against Gram-positive species due to channel formation in their membranes. The channels are specific for monovalent cations such as Tl+, NH_4+ and alkali metals. Passage of cations through channels is vital for numerous cell functions.

Formation of helical gramicidin channels is due to the alternate D-L configuration of gramicidin pentadecapeptide. As a result, all side chains are on the same side of β-ribbons formed by pairs of antiparallel peptides. Side chains repulsion causes the ribbons to roll up into narrow cylinders.

Three types of gramicidin channels have been observed experimentally, left and right handed antiparallel double stranded β-helices (DSbH) and single-stranded head-to-head dimers. In all X-ray studies of complexed and uncomplexed gramicidin crystal structures only DSβH forms have been observed. The distribution of cations and water in the channels provides insight into the possible mechanism of cation passage. The best resolution of the available gramicidin structures until now was only 1.4 Å.

Our data are based on a crystal of rubidium chloride complex measured at 100K with synchrotron radiation. The structure was refined with anisotropic temperature factors for all non-H atoms and with many partial occupancies. The resolution is 1.14 Å and the final R of 15% was achieved. There are 7 distinct rubidium binding sites in each of the two crystallographically independent right handed antiparallel $DS\beta H_R$ dimeric unit. Occupations factors of Rb cations are between 0.11 and 0.35 and the average ion contents are 1.59 and 1.23, respectively in the two channels.

The most interesting findings are as follow:

[1] Though each channel is chemically symmetrical, the distributions of rubidium binding sites in the two independent channels are not.
[2] The water to cation ratio in the channel interior is four or five to one.
[3] Cations are "coordinated" by delocalized p electrons of four to six carbonyl groups, that together with peptides backbone chains form the gramicidin channel walls.
[4] Five or six waters separate Rb cations during their passage through the channel.
[5] The passage of the cations through the channel is three-step process and the jump length is about 8 Å.

s1.m8.p13 **Crystal Structures of CTP Synthase reveal ATP, UTP, and Glutamine Binding Sites.** Masaru Goto,[1,2] Rie Omi,[1,2] Ikuko Miyahara,[2,3] and Ken Hirotsu[2,3], *[1]Grad. Sch. of Sci., Osaka Univ., Toyonaka, Osaka, Japan, [2]Grad. Sch. of Sci., Osaka City Univ., Osaka, Japan, [3]RIKEN Harima Inst., Sayo-gun, Hyogo, Japan. E-mail: goto@sci.osaka-cu.ac.jp*

Keywords: Crystal Structure; Conformational Change; Catalytic Triad

CTP synthase (CTPs) is a member of the class I glutamine-dependent aminotransferases which are characterized by the presence of "triad" Cys-His-Glu in the glutaminase domain. CTPs catalyzes the last step of the CTP biosynthesis, where nascent ammonia generated at the glutaminase site reacts with the ATP-phosphorylated UTP to produce CTP at the synthase domain. Since CTPs is essential for RNA, DNA and phospholipid biosynthesis, many biochemical and kinetic studies have been performed. In the absence of ATP and UTP, CTPs from *Escherichia coli* shows a very small activity for glutamine hydrolysis and is in an equilibrium state between a monomer, a dimer, and a tetramer depending on the protein concentration. In the presence of ATP and UTP, the enzyme changes its overall conformation and is folded into an active homotetramer irrespective of the protein concentration. The addition of an allosteric effector GTP induces a further conformational change to stimulate the glutamine hydrolysis. Therefore, CTPs possesses individual recognition sites for glutamine, UTP, ATP, and GTP. In the presence of ATP and UTP or ATP, UTP, and GTP, the rate of glutamine hydrolysis is identical to that of the CTP synthesis indicating that glutamine hydrolysis is coupled to the synthase reaction in a 1:1 ratio. In order to elucidate the mechanism of the regulation and catalysis of the CTPs, we have determined the crystal structures of native CTPs in the unliganded form and its complexes, CTPs with $3SO_4^{2-}$, and CTPs with glutamine [1]. The CTPs is folded into a homotetramer in a crystal with one subunit in the crystallographic asymmetric unit. The subunit is composed of a synthase domain and a glutaminase domain. Synthase domains of CTPs interact with one another to form a tetrameric CTPs with a crystallographic 222 symmetry and a cross-shaped structure. The glutaminase domain is located at each of the four corners, and has a mean main-chain B-factor higher than that of the synthase domain. Based on the binding mode of sulfate anions to the synthase site, ATP and UTP are modeled into CTPs with a geometry favorable for the reaction to occur. Glutamine bound to the glutaminase domain is situated next to a "pentad" of Glu-His-Cys-His-Glu as a potential catalyst and a water molecule. The synthase active site exposed to the solvent region and the GTP binding motifs divided into glutaminase and synthase domains provide the possible conformational change with the formation of the GTP binding site upon the binding of ATP and UTP.

[1] M. Goto, R. Omi, J. Hoseki, N. Nakagawa, I. Miyahara, K. Hirotsu, *Acta Crystallogr*. D**59**, 551-553 (2003)