[MS5-P34] Structural studies on CBS-pyrophosphatase with adenylate ligands

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In biosynthetic reactions formed pyrophosphate re-enters the metabolic cycle after hydrolysis to orthophosphate by inorganic pyrophosphatase (PPase, EC 3.6.1.1). The enzyme exists in two non-homologous forms; integral membrane-bound and soluble, that is further divided into two non-homologous families, I and II, with distinct tertiary structures. Common family II PPases are homodimers of two-domain subunits. A quarter of family II PPases contain a large regulatory insert within catalytic domain containing pair of CBS domains and a DRTGG domain, called CBS-PPase. CBS domain pairs are present in several protein families and function as a regulatory domain by binding adenylate nucleotides. We have shown that several CBS-PPases are regulated with adenylate nucleotides. For instance a CBS-PPase from *Moorella thermoacetica* is strongly inhibited by AMP and ADP, and activated by ATP, and from *Clostridium perfringens* is inhibited by AMP and activated by a novel effector, diadenosine 5′,5-P1,P4-tetraphosphate (AP4A). This five flexible domain containing protein is challensing to stabilise for ordered crystalline form. Several ligands are know, but correct combination is difficult to find due to similarity and solubility problems. Several structures of continuously working family II PPase are solved in different orientations. We have solved X-ray structures of regulatory part of *C. perfringens* CBS-PPase with inhibitory and activatory ligands [1]. This was first structure pair where it can be seen conformational change in CBS domain pair due to opposite effectors.

[1] Tuominen, H., Salminen, A., Oksanen, E., Jämsen, J., Heikkilä, O., Lehtiö, L., Magretova, N. N., Goldman, A., Baykov, A. A. & Lahti, R. 2010: Crystal structures of the CBS and DRTGG domains of the regulatory region of *Clostridium perfringens* pyrophosphatase complexed with the inhibitor, AMP, and activator, diadenosine tetraphosphate. *J. Mol. Biol.*, **398**(3): 400-413.

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