silico analysis shows that the localization of the putative sugar-binding site is close to the dimeric interfaces. This supports the proposal of a new mechanism of transcriptional regulation that is in complete agreement with functional studies recently reported on a protein belonging to the same family.

Keywords: SorC; sorbitol operon regulator; X-ray crystallography; transcriptional regulation

FA1-MS04-P04

Crystallographic Analysis of the PAS Domain of RsbP, a Stress-Response Phosphatase in *Bacillus Subtilis.*

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The bacterium *Bacillus subtilis* exploits a signaling cascade to respond to deleterious effects of stresses or sudden changes in the environment. Through the cascade, stress information converges on and is transmitted to the general stress response transcription factor σ^B, leading to the expression of more than 200 general stress genes to exclude stresses or to repair the damage. The activity of σ^B in forming the complete transcriptional complex is governed by the signaling cascade with two distinct branches, which are composed of a set of signal transduction proteins termed *r*egulators of *s*igma *B* (Rsb). One branch is specific for energy stresses, such as carbon, phosphate, or oxygen limitation, and the other is specific for environmental stresses, such as acid, ethanol, heat, or salt shock. Each branch terminates with two serine phosphatase RsbP and RsbU. Both of them dephosphorylate the common downstream factor, RsbV. Depending on the phosphorylation state of RsbV, RsbW regulates σ^B activity *via* a partner-switching mechanism. RsbP is an essential protein to sense energy limitation in conjunction with the α/β hydrolase RsbQ that is co-expressed with RsbP from the *rsbQP* operon. The amino acid sequence of RsbP indicates that it is comprised of three domains: Per-Arnt-Sim (PAS) domain, coiled-coil domain and PPM/PP2C Ser/Thr phosphatase domain. Since PAS domains are structural modules that can be found in proteins in all of life and are involved in many signaling proteins where they are used as a signal sensor domain, RsbP-PAS is expected to sense energy stress information. We have crystallized the recombinant RsbP-PAS using 40% PEG400 as a precipitant. The crystals belong to space group $P2_1$, with unit cell parameters of *a*=55.2, *b*=71.7, *c*=60.2 Å, β=92.1°. The X-ray crystal structure of RsbP-PAS was determined by the SeMet SAD phasing at a resolution of 1.7 Å. RsbP-PAS maintains a typical PAS fold with 5 α-helices and 5 β-strands, and forms a homodimer through mainly hydrophobic interactions between the two monomers. Two small and highly hydrophobic cavities are located in the protein core region, which closely match the ligand/co-factor binding sites found in other PAS domains. These results suggest that RsbP senses some small hydrophobic compounds transmitted from RsbQ in the protein core as stress signals. Slight structural rearrangements around the cavities are predicted upon ligand binding, which may initiate a switch in regulating RsbP's phosphatase activity.

Keywords: signal transduction; structure-function relationships; macromolecular crystallography

FA1-MS04-P05

Structure of a Secondary Vitamin D_3 Binding Site of Milk β-Lactoglobulin for Vitamin D Transport.

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β-lactoglobulin (LG), one of the most investigated proteins, is a major bovine milk protein with a predominantly β structure. The structural function of the only α-helix with three turns at the C-terminus is unknown. Vitamin D_3 binds to the central calyx formed by the β-strands. Whether there are two vitamin D binding-sites in each LG molecule has been a subject of controversy. Here, we report a second vitamin D_3 binding site identified by synchrotron X-ray diffraction (at 2.4 Å resolution) and fluorescence spectral analyses. In the central calyx binding mode, the aliphatic tail of vitamin D_3 clearly inserts into the binding cavity, where the 3-OH group of vitamin D_3 binds externally through interacts with the carbonyl of Lys60 forming a hydrogen bond. The second binding site, however, is near the surface at the C-terminus (residues 136-149) containing part of an α-helix and a β-strand I. A remarkable feature of the second exosite is that it combines an amphipathic α-helix providing non-polar residues (Phe136, Ala139, and Leu140) and a β-strand providing a non-polar (Ile147) and a buried polar residue (Arg148). They are linked by a hydrophobic γ-turn loop (Ala142, Leu143, Pro144, and Met145). Thus, the binding pocket furnishes strong hydrophobic force to stabilize vitamin D_3 binding. Using site-directed mutagenesis, we showed that the γ-turn loop play a crucial role in the binding. Using mice as an animal model, we demonstrated that LG is a major fraction of milk proteins responsible for uptake of vitamin D. Most interestingly, dosing mice with LG supplemented with vitamin D_3 revealed that native LG containing two binding sites gave a saturated concentration of plasma 25-hydroxyvitamin D at a dose ratio 2:1 (D_3:LG), whereas heated LG containing one exosite (lacking a central calyx) gave a ratio 1:1. This finding provides a new insight into the interactions between vitamin D_3 and LG, in which the exosite may provide another route for the transport of vitamin D_3 in vitamin D_3 fortified dairy products. LG has a functional advantage in the transport of vitamin D, indicating that supplementing vitamin D in milk effectively enhances its uptake.

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Keywords: vitamin D; β-Lactoglobulin; transport