Crystal structure of the ATP-binding cassette of the glucose ABC transport system from *Sulfolobus solfataricus*

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The hyperthermophilic Archaeon *Sulfolobus Solfataricus* has been shown to possess a typical bacterial binding-protein dependent ABC transport system dedicated to glucose uptake. The capture of glucose is ensured by GlcS, a glucose-binding protein anchored in the membrane. After binding, the glucose molecule is docked at the entrance of the translocation pore formed by two sugar permeases (GlcT and GlcU). Through an ATP-driven mechanism, the ATP-binding cassette (GlcV) provides the energy required for the translocation of the glucose molecule along the channel. We aim at describing the structure-function relationships of GlcV as an ATP-hydrolysing subunit and at identifying the structural elements and events involved in generating the driving-force provided by GlcV.

Wild-type GlcV could be over-produced in *E. coli* and purified in large amounts by means of ion exchange, hydrophobic interactions and dye-ligand affinity chromatography. After initial crystallisation experiments and rounds of optimisation using the hanging drop method, two crystal forms have been characterised, both belonging to the space group $P2_12_12_1$ (Form I: a = 46.01 Å, b = 48.25 Å, c = 183.04 Å and Form II: a = 47.0 Å, b = 146.64 Å, c = 178.5 Å). From self-rotation and self-patterson calculations, it was deduced that the two crystal forms are related, with Form II having three molecules in the asymmetric unit (instead of 1 for Form I) arranged along the 2-fold screw axis in the b direction.

As demonstrated recently, iodine atoms can be a powerful source of phase information and may be used as anomalous scatterers for SIRAS and SAS methods [1]. Iodine derivatized crystals of GlcV were obtained by cross-seeding from a native crystal replacing the sodium chloride present in the crystallisation buffer by sodium iodine (0.5 M). A dataset was collected at BW7A (EMBL, DESY) using a wavelength of 1.7 Å in order to record a significant anomalous scattering (f' = -1.0 ē; f'' = 8.2 ē).

Table: Crystallographic data statistic of the Iodine derivative (Form I)

Data	**λ(Å)**	**Resolution (Å)**	**Rmerge (%)**	**Completeness (%)**	**Redundancy**	**I/σ(I)**
Iodine	1.7	37 - 2.1 (2.0 - 2.1)	10 (27)	98.7 (93.8)	5 to 6	14.6 (5.7)

From the GlcV-iodine data, a partial substructure of five iodine atom positions could be solved by direct methods using SnB [2]. Using SHARP [3], seven additional sites were located in the residual anomalous difference Fourier map and the iodine atom parameters were refined using only the anomalous data. After a density modification procedure using SOLOMON [4], the phases were combined with data at 1.65 Å collected on the same type of crystal (ID14-2, ESRF, Grenoble). ARP/wARP [5] was then used to extend and improve the phases (warp mode: slow protocol for 6 free atom models + averaging). Using the warpNtrace mode, the main chain of GlcV could be automatically built (336 residues, 4 fragments).

Currently the refinement of GlcV structure is being completed (current: Rfactor = 22.5 % and Rfree = 26.5 %). GlcV is built-up from two domains with the N-terminal domain (200 aa) forming the ATPase unit. The active site pocket, where ATP is hydrolysed, is defined by the typical motifs showed to be involved in the ATP-binding (Walker A motif, ...). The overall structure is very similar to Malk from *Thermococcus litoralis* [6]. The C-terminal domain, involved in regulation of the transport system, is made from a very small β-barrel and of a β-sheet. We are currently investigating complexes mimicking various steps in the catalytic cycle of this ATPase.

References

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