Report BM-30, Proposal 30.01.764.

Initial and essential data collections were done at BM30-A (crystal screening, initial diffraction data of the native and heavy atom soaked crystals). High-resolution data sets were obtained on ID23-1.

Abstract of the submitted paper (Accepted in BMC Structural Biology):

Background

The import of solutes into the bacterial cytoplasm involves several types of membrane transporters, which may be driven by ATP hydrolysis (ABC transporters) or by an ion or H^+ electrochemical membrane potential, as in the tripartite ATP-independent periplasmic system (TRAP). In both the ABC and TRAP systems, a specific periplasmic protein from the ESR family (Extracytoplasmic Solute Receptors) is often involved for recruiting the solute and presenting it to the membrane complex. In *Rhodobacter sphaeroides*, TakP (previously named SmoM) is an ESR from a TRAP transporter and binds α-keto acids *in vitro*.

Results

We describe the high-resolution crystal structures of TakP in its unliganded form and as a complex with sodium-pyruvate. The results show a limited "Venus flytrap" conformational change induced by substrate binding. In the liganded structure, a cation (most probably a sodium ion) is present and plays a key role in the association of the pyruvate to the protein. The structure of the binding pocket gives a rationale for the relative affinities of various ligands that were tested from a fluorescence assay. The protein appears to be dimeric in solution and in the crystals, with a helix-swapping structure largely participating in the dimer formation. A 30 Å-long water channel buried at the dimer interface connects the two ligand binding cavities of the dimer.

Conclusions

The concerted recruitment by TakP of the substrate group with a cation could represent a first step in the coupled transport of both partners, providing the driving force for solute import. Furthermore, the unexpected dimeric structure of TakP suggests a molecular mechanism of solute uptake by the dimeric ESR via the connecting channel at the dimeric interface.

Data collection statistics:

Table 2 - Summary of crystal parameters, data collection and refinement statistics.

Data collection

		Unbound Selenomethionine ($P2_1$)			Pyruvate ($C2$)
Wavelength, Å		0.97960	0.97940	0.97565	0.97565
Resolution range, Å		68-1.7	68-1.8	68-2.0	48-1.4
Cell parameters:	a	104.61			118.02
	b	63.94			78.06
	c	127.95			95.14
	β	106.64			124.98
No. of measured reflections		581707	506662	374656	500384
No. of unique reflections		175148	149602	109442	137318
R_{sym} [a,b]		8.7 (46.2)	8.0 (25.3)	7.4 (18.3)	7.7 (49.1)
I/σI [b]		12.3 (2.2)	13.1 (3.6)	13.5 (5.3)	15 (3.1)
Completeness, % [b]		98.5 (95.5)	99.5 (98.2)	99.6 (98.6)	99 (97.9)
Sites (n)		40			
Refinement					
Resolution range, Å		68-1.7			48-1.4
R_{cryst} / R_{free}		17.9 / 20.5			17.3 / 18.4
No. of non hydrogen atoms:					
Total		11298			5795
Protein		1054			5304
Glycerol		24			
Pyruvate		-			12
Ion		-			2
Water		731			477
Average B-factors, Å² :					
Main-chain		16.1			9.6
Side-chain		18.2			11.3
Glycerol		35.8			-
Pyruvate		-			9.2
Ion		-			4.4
Water		24.8			19.6
RMSD bonds, Å		0.012			0.007
RMSD angles, °		1.30			1.14
PDB code		2HZK			2HZL

[a] $R_{sym}=\Sigma_h\Sigma_i|I_i(\mathbf{h}) - \langle I(\mathbf{h})\rangle / \Sigma_h\Sigma_i I_i(\mathbf{h})$

[b] Number in parenthesis refer to the last 0.1Å shell