ESRF	**Experiment title:** Probing protein-ligand interactions using the periplasmic oligopeptide binding protein, OppA	**Experiment number:** LS1383
Beamline: ID02B	**Date of experiment:** From: 12/05/99 to: 15/05/99	**Date of report:** 28/02/00
Shifts: BAG	**Local contact(s):** Julien Lescar	*Received at ESRF:*

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Report:

OppA is a periplasmic binding protein involved in the uptake of extracellular peptides by bacteria such as *E. coli*. It is the largest binding protein known, having 517 amino acids and a molecular weight of 58.8kDa. The structure of the protein [1] revealed that, in common with other periplasmic binding proteins, OppA engulfs its ligand between two domains joined by a flexible hinge.

Oppa binds peptides between two and five residues in length with little regard to sequence. Tight binding (Kd ~10^{-6}M) is achieved by the protein utilising the hydrogen bonding potential of the peptide backbone and strategically placed charged groups to bind the N and C termini of the peptide. The ligand side-chains are accommodated in large hydrated cavities in the protein. The overall conformation of the protein remains essentially unchanged on binding different ligands [2].

Current studies are focussed on exploring the effects of pH and temperature on ligand binding to OppA. Two different crystal forms (I and II) of unliganded OppA were grown at pH 7.5 (previously all former crystals had been grown at pH 5.5) to determine whether a change in pH would alter the three-dimensional structure of OppA and, therefore, affect

peptide binding. A full data set was collected on both forms at the ESRF on station ID2B (λ=0.92Å). Full data collection statistics on both forms are given in table 1 below.

Table 1. Data collection statistics

	Form I	**Form II**
Number of images	150[a]	180
Oscillation range	0.5°	1.0°
Space group	$P3_221$	$P2_1$
Resolution	1.65Å	2.3Å
Rmerge	7.2% (25.7%)[b]	8.1% (28.9%)[b]
Completeness	95.1%	99.6%

[a]A low and high resolution data set was collected, [b]R_{merge} in the highest resolution bin

Crystals of form I diffracted to 1.65Å and belong to the trigonal space group $P3_221$ with cell dimensions a=b=80.9, c=164.6Å, $\alpha=\beta=90.0°$ and $\gamma=120.0°$. Structure solution by molecular replacement using the entire previously determined OppA model proved unsuccessful. However, molecular replacement studies using individual domains of OppA as separate models were successful and the structure was solved in this way. Rigid body refinement was carried out on the domains followed by model building of the missing residues into the electron density maps. The structure was subsequently refined to a current R_{free} of 27.6% and R factor of 21.7%. The new structure of OppA was superposed onto the original model and was found to have a large conformational change in the overall three-dimensional structure. Domains 1 and 3 have opened relative to each other, via the hinge region, leaving the binding site fully exposed to the external environment. The form II crystals diffracted to 2.3Å and belong to the monoclinic space group $P2_1$ with cell dimensions a=70.5Å, b=120.3Å, c=77.3Å, $\alpha=90.0°$, $\beta=112.8°$ and $\gamma=90.0°$. Structure solution by molecular replacement using the refined model of the form I crystals was straightforward, indicating that both crystals had the same overall structure.

Future work will involve trials to co-crystallise a ligand into this new OppA crystal form to see how ligands will bind into the exposed binding site.

References

[1] J.R.H. Tame, G.N. Murshudov, E.J. Dodson, T.K. Neil, G.G. Dodson, C.F. Higgins and A.K. Wilkinson, Science, 264, 1578-1781 (1994)

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[3] S.H. Sleigh, P.R. Seavers, A.J. Wilkinson, J.E. Ladbury and J.R.H. Tame, J. Mol. Biol., 291, 393-415 (1999)

[4] T.G. Davies, R.E. Hubbard and J.R.H. Tame, Protein Science, 8, 1432-1444 (1999).