EUROPEAN SYNCHROTRON RADIATION FACILITY
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ESRF

ESRF	**Experiment title:** Structure of Hamster Aphrodisin	**Experiment number:** BAG-LS1508
Beamline:	**Date of experiment:** from: 16 to: 18.9.99	**Date of report:** 29.2.2000
Shifts:	**Local contact(s):**	*Received at ESRF:*

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Report:

Data collection and processing

A Se-MAD experiment was performed on the ID14-4 beamline at ESRF. The X-ray fluorescence and transmission from the crystal were measured as functions of the incident X-ray energy in the vicinity of the Se-K edge. The wavelenghts used for the data collection were chosen based on the absorption edge of the Selenium, 12677 ev (h= 0.978), 12657 ev (h= 0.9795) corresponding to the minimum f' and maximum f'', respectively. A third, remote energy was selected at 13296 ev (h= 0.9324) where the effect of the absorption were negligible. MAD data were collected from a single crystal in an arbitroty setting using a Quantum-CCD detector, after optimizing the oscillation range using STRATEGY (Ravelli et al, 1997). The data obtained at the remote energy were indexed and intergrated using DENZO (Otwinowsky, 1993). The near edge data set were scaled to the remote energy data set using SCALA (CCP4, 1994), and structure factor amplitudes were calculated using TRUNCATE (CCP4, 1994). The dataprocessing are given in table 1.

Phasing, model building and refinement

The sites of ten of the expected twelve selenium atom per asymetric unit were located, refined and phased using the program SOLVE. Initial Solve phases were improved by density modification with DM using a fourth data set at 1.5Å resolution. After the solvent flattening, the electron density maps obtained from the MAD phases were readily interpretable and were used to construct the main chain of one molecule of the tetramer. The Cα main chain of one subunit was built using part of the main secondary structure of the Porcine OBP (Spinelli et al, 1998).The model was preliminary refined wtih simulated annealing and torsion

angle refinement using the program CNS version 0.9 (Brunger, personal communication). In the meantime , an additional dataset was collected at high resolution on ID14 EH4 (ESRF, Grenoble). This dataset extend to a resolution of 1.6Å. Cycles of refinement with REFMAC were alternated with manual re-fitting into Sigmaa-weighted (Read, 1986) electron density maps with the graphic program TURBO-FRODO (Roussel and Cambillau, 1991). The final model has a R and Rfree factors of 19.5 and 23.5% respectively with an uninterruped backbone but no density for 13 residues pertaining to the his-tag which were systematically cut in the four molecules and also 2, 3 or 4 residues of N-terminal domain were not defined. The geometry of the model is good, according to the PROCHECK criteria (Laskowski et al., 1993) with 90.8% residues in the most favourable area and 9.2% in the additional allowed region.

Table 1 : Final statistics of the MAD data collection.

Dataset	_. (Å)	Resolution (Å)	R_{sym} (%)	R_{anom} (%)	I/_. (I)	Completeness (%)	Anomalous completeness (%)	Redondancy
f max	0.9780	2.795	3.6 (6.3)	5.0 (6.5)	12.4	98.1	81.4	2.5
f min	0.9795	2.795	3.3 (5.6)	3.3 (4.6)	15.0	98.3	83.3	2.6
Remote	0.9324	2.795	4.1 (5.5)	4.2 (5.3)	10.6	97.5	79.5	2.5
Native	0.9330	1.60	9.9 (4.1)	-	4.0	89.2	-	2.4

Values of R_{sym} and R_{anom} in parentheses are for the outer resolution shell.

$R_{sym} = \_ | (I+) - (I-)| / \_(I)$

$= \_i\_h |I_{ih}^{+} - (I^{+})_h | + | I_{ih}^{-} - (I^{-})h | / \_i\_h I_{ih}^{+,-}$

Resolution (Å)	15-1.63
Total number of reflections	88968
R_{work}/R^{a}_{free}	19.4/23.4
Mean B factors (Å^3)	
Main-chain	26.00
Side-chain	29.02
Solvent	49.06
rms B factors (Å^3)	
Main-chain	1.661
Side-chain	2.691
rms deviations (Å)	
Bond angles (°)	1.42
Bond lenght (Å)	0.011

[a]Number of reflections : 2202