



	Experiment title: MAD, determination of <i>E. coli</i> KDPG Aldolase	Experiment number: LS-1683
Beamline:	Date of experiment: from: August to:	Date of report: 24/08/00
Shifts:	Local contact(s): Gordon Leonard	<i>Received at ESRF:</i>

Names and affiliations of applicants (* indicates experimentalists):

James H. Naismith

Louise Buchanan

David Sanders

All St. Andrews

Report:

Data were collected at ESRF Grenoble on the tuneable beamline ID14-4. An EXAFS scan was used to confirm the presence of the seleniums in the crystal and locate the edge. Three data sets were thus collected. One set of data was collected on the selenium edge at $\lambda = 0.9794\text{\AA}$, one at the point of inflection on the selenium edge, $\lambda = 0.9798\text{\AA}$, and a third on the high-energy side of the selenium edge, $\lambda = 0.9393\text{\AA}$. Data were collected as 108 images with an exposure time of 5 s, 1° non-overlapping images, with a crystal to detector distance of 250mm. Data were integrated with *DENZO* and scaled with *SCALEPACK* to 2.5\AA , treating Bijovet pairs independently. The crystal was $P2_12_12_1$ with cell constants of $a = 55.06\text{\AA}$, $b = 84.32\text{\AA}$, $c = 134.32\text{\AA}$, $\alpha = \beta = \gamma = 90^\circ$. Summary data for the three wavelengths is shown in table1. Complete statistical analysis of the data for all three wavelengths is shown in table3.

Selenium Positional Identification

The program *SOLVE* was used to identify the locations of the selenium's in the KDPG aldolase and calculate the phases. It identified 10 selenium sites, shown in table2 The Z score for the sites found is 70.94, with a figure of merit value of 0.54. At this stage it was still unclear if the protein existed as a dimer or trimer. As there are 4 methionines in the sequence and 10 sites found it was deduced that the protein was trimeric, with 2 of the selenium's being disordered and thus not located by *SOLVE*. The sites were not identified via the

NCS symmetry matrix, calculated using *NCSFIND*; it found only a 2-fold symmetry axis not the true 3-fold. Thus the relationship was examined in *O* and the 3-fold axis located. The centre of the 3-fold was determined in three-dimensional space and this point was used as the centre point from which a mask of 40Å was calculated. The mask was used for the modification of the initial *SOLVE* map using the NCS features of DM. The resulting map was readily interpretable.

	$\lambda = 0.9794$	$\lambda = 0.9798$	$\lambda = 0.9393$
Unique Reflections	22040	18160	27901
I/σ	21.9	21.4	23.0
Average Redundancy	2.0	2.1	1.98
Completeness (%)	92.7	93.7	88.2
Anom Complete (%)	84.4	84.3	87.4
R_{merge} (%)	5.4	6.3	5.5
refined f'/f''	-9.24/5.54	-8.85/2.71	-2.25/3.35

Table1 – Summary of data from all three wavelengths

Site	x	y	z	Occupancy	B	Height / σ
1	0.58	0.168	0.204	0.641	24.15	18.8
2	0.150	0.362	0.170	0.658	19.51	15.4
3	0.222	0.118	0.088	0.955	39.04	16.6
4	0.280	0.161	0.054	0.8207	29.94	15.4
5	0.479	0.521	0.102	0.869	58.73	10.5
6	0.047	0.092	0.199	0.801	42.99	16.2
7	0.201	0.324	0.130	0.714	27.07	14.4
8	0.973	0.665	0.128	0.451	39.00	9.1
9	0.717	0.235	0.004	0.359	23.83	8.6
10	0.410	0.882	0.061	0.571	36.60	8.7

Table2 – Results from SOLVE ran using the ID14-4 data