ESRF	**Experiment title:** The Use of ESRF ID9 to Study Structural Intermediates of the Reaction Catalysed by Hydroxymethylbilane Synthase (HMBS) ~~Mutants R155L and K59Q~~ a)	**Experiment number:** LS 774
Beamline: ID09-BL3	**Date of Experiment:** from: 24-Jun-97 07:00h to: 30-Jun-97 07:00h	**Date of Report:** 28 February 1998
Shifts: 18	**Local contact(s):** M Wulff, R Leberman	*Received at ESRF :* - 4 MAR. 1998

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Report:

Our proposal for LS774 had originally been put on a reserve list for beamline ID09 (decision of the Review Committees, dated 11 June 97), but beam time became available on a very short notice (1 week, email from M Wulff, dated 17 June 97), much earlier than October as requested by us originally. Since our proposal envisaged the use of a flow cell for the HMBS mutants R155L and K59Q, crystals of these enzymes would have had to be grown at the EMBL Outstation in Grenoble well before the experiment (HMBS crystals need ca. 4 weeks to grow). In view of the crystals that were available in Basle at the time of beam time allocation (these were crystals of wild-type HMBS only), a plan for the optimal use of beam time, under the given circumstances, was negotiated with M Wulff. It was agreed that ID09 be used in monochromatic mode to collect data ***a)*** from crystals of wild-type HMBS, reduced, active form, to the best possible resolution (this was anticipated to be around 1.7 A), since this kind of data had previously been collected from crystals of an oxidised, inactive form of HMBS only (1.76 Å resolution) [l], and ***b)*** from similar crystals, after soaking in substrate (porphobilinogen, PBG) for ca. 2 h or c) an analogue thereof (aza-PBG).

According to this plan, LS774 used the machine in 16-bunch mode (4 nm emittance, 1% coupling, intensities 45-90 mA). Data were collected at λ = 0.8611 A and 100 K (Oxford Cryostream) using a Thompson image intensifier (220 mm diameter) coupled to a CCD camera (1242 x 1152 pixels, digitised to 16 bits, minimum sensitive surface 145 mm) which was set at a close distance

a) Amended owing to the short notice (1 week) and much earlier than October as requested by us originally.

(125 mm, for high resolution) and further away (250 mm, to gather data hidden behind ice rings). Data sets were measured from 8 samples, in all comprising a total of 645 CCD images.

Of these data sets, five were found to be of good quality and these were processed using *DEN20* and *SCALEPACK* by J Habash and J R Helliwell at the University of Manchester. The data were of high intrinsic quality for 3 of the samples (of types *a), b),* and c) (soaking times 2.5 and 3.5 h, respectively)), extending to 1.65 Å resolution. Two others were only processable to lower resolution, *i.e.* 2.6 Å (type *b))* and 3 Å (type c)). The data from wild-type HMBS then served as the reference for the other 4 sets. The results of the processing showed clearly that ID09 is capable of providing diffraction data of very high-quality consistency in monochromatic mode.

In order to provide phases at high resolution, the native structure was refined to 1.65 Å resolution by P Faulder in Manchester. Previously we had structures of this form refined at 2.4 Å for room temperature data and at 2.7 Å for 100-K data [2]. The new 1.65-A cryo structure contributes substantially to the database of known HMBS structures [1,2], since *i)* it defines the molecule to the best resolution obtained so far, and *ii)* this is achieved for the reduced, active, form of HMBS. It was found that the well-known mobile, hitherto invisible, loop between residues 48 and 57 shows signs of being clearer, especially in the 48- and 49-residue region near the active site.

The other four data sets were scaled against the cryo 1.65-A native data, and along with calculated phases from that model, difference Fourier maps were calculated. These were, disappointingly, flat, *i.* e. they showed no evidence of new electron density appearing in the active-site region. This was in accord with the low mean fractional differences for the high-resolution type *b)* and type c) (see above) data. However, the corresponding low-resolution data sets showed very high mean fractional differences. Thus the flatness of these two difference maps could also be due to a strong lack of isomorphism between the soaked and the native crystals. Molecular replacement (rigid-body) calculations followed by detailed molecular model refinement of the latter two structures will now be done.

The conclusion of these soaking experiments is that the wild-type 2.5-h freezing approach based on previously measured K59Q time-resolved Laue titration curves [3,4] was not appropriate. Hence this confirms the need, detailed in the original beam-time request, that the flash-freeze experiment at 2 h into the reaction should be done on the K59Q mutant rather than the wild-type protein. The soaking experiments with aza-PBG strongly suggest that a co-crystallization approach, rather than soaking of crystals might then have, as we know now, a better chance of success.

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References

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