ESRF	Experiment title: The Use of ESRF to Study Structural Intermediates of the Reaction Catalysed by Porphobilinogen Deaminase	Experiment number: LS 284
Beamline: ID9-BL3	Date of Experiment: from: 26-Nov-95 07:OOh to: 28-Nov-95 07:OOh	Date of Report: 1 July 1996
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Report:

In view of experiment LS 284, a total of 169 mg of the Gln^{59} mutant of porphobilinogen deaminase (Gln^{59}-PBGD) was purified to homogeneity from 108 g (wet weight) of E. coli TG 1 recO/pPA448 cells [1]. This variant of PBGD was chosen because kinetic experiments had previously shown that the enzyme-substrate complexes ES_2, followed by ES_3, are accumulated during the pre-steady-state period of the reaction [2]. Five weeks before beam time, this material as well as some previously purified batches of wild-type PBGD were used to set up crystallisation experiments for the production of high-quality crystals. These experiments were carried out as described previously [3] at the outstation of the European Molecular Biology Laboratory (EMBL) in Grenoble, to avoid the need for transportation of crystals. An abundance of high-quality crystals of the above-mentioned variants of PBGD were available on time.

During data collection, the crystals were immobilised in a flow cell of 1.0 mm i. d. (P. D. Carr, unpublished work) and exposed to a continuous flow (0.02 ml/min) of a solution of substrate (1 mM) by means of a motor-driven syringe pump. Laue patterns were recorded before reaction initiation and then repeatedly in an approximately geometrical series of time points, i. e. after 7", 30", 1', 2', 4', 8', 16', 32', 1 h and 2 h into the reaction. This was done with a total of 6 crystals. The exposure time for each CCD Laue pattern was

around 1.5 ms. The detector readout time was 8 s. The data were processed with the Daresbury Laue Suite [4] [5]. At each time point then the Laue images from the different crystals could be combined to make a data set. These generally have a completeness of 70% to 2.3 Å resolution. In addition in separate experiments, a large number of Laue exposures from a single crystal were practical to make data sets at 25' ± 7' and 2 h ± 10' on BL3 yielding data sets of 85% completeness. Also a wild-type crystal yielded 47 Laue images giving 148'779 Laue processed intensities (singles and deconvoluted multiples) and these reduced to 13'949 unique reflections with *R*merge of 10.2% and 90% completeness. In a final case a crystal from a time sequence experiment was disconnected from the flow cell substrate supply at 2 h and monochromatic data to 2.0 Å collected on BL19 on CCD (with A. W. Thompson) at 12 h ± 30'. As well as the data reduction details, which document the high-quality Laue data recorded on BL3, it has been possible to start comparing all of these time points in the data acquisition pathway. At present this is basically in terms of the diffraction intensity differences. Reflection intensity differences greater than 7 σ occur at 2 h into the reaction. The data sets at these time points are now the subject of protein structure refinement but also indicate the need for a fuller exploration of the crystal reaction between l/2 h and longer. A particularly intriguing feature of the chemical reaction is that although none of the ES_i offer a spectroscopic signal the product, hydroxymethylbilane, is unstable and undergoes non-enzymic cyclisation to colourless uroporphyrinogen I. The latter is readily oxidised to pink/red uroporphyrin I, the colour of which was noticeable in the crystal at 2 h and very marked at 12 h. This indicates that the reaction has gone to full completion without major rearrangement of the protein structure domains or break-up of the crystal. This enzyme offers considerable scope for exploration of the time-course of the reaction in the crystal and on a vitally important molecule in chemistry and biology.

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