



<b>Experiment title:</b> Physical Estimation of Triplet Phases for Solving Macromolecular Structures. 1. Exploratory Experiments	<b>Experiment number:</b> MI-166	
<b>Beamline:</b> SNBL BMO1	<b>Date of Experiment:</b> from: 01.03.97 07:00 to:05.03.97 07:00	<b>Date of Report:</b> 28.08.97
<b>Shifts:</b> 9	<b>Local contact(s):</b> K. Knudsen	<i>Received at ESRF :</i> <b>3 SEP. 1997</b>

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## Report:

The first 24 hrs. of this beamtime was in one-bunch mode, hence unsuited for work with weakly scattering samples. This time was spent on alignment of the diffractometer, tests of polarization conditions and of software, and initial tests of *Bellidiflorin* crystals.

*Bellidiflorin* is a natural product of unknown structure, isolated from various species of lichens. It contains C, H, O and Fe, and crystallizes in space group  $P2_12_12_1$ . A model of the chelate centre based largely on an EXAFS study has been published [1]. The unit-cell volume  $V \sim 19.300 \text{ \AA}^3$  is comparable to that of a small protein, and *B.* is therefore a possible candidate for physical phase estimation (PPE). In the period until 04.03, 17 hrs., eighteen different crystals of the compound were examined. None of them were of adequately small mosaicity to allow PPE. Three to four crystals were suitable for measurement of integrated intensities for the purpose of structure determination, and have been saved. Cell parameters determined from one of them are:  $a = 37,55 \text{ \AA}$ ,  $b = 24,45 \text{ \AA}$ ,  $c = 21,05 \text{ \AA}$ ,  $\alpha = \beta = \gamma = 90^\circ$ .

*Guinea-fowl lysozyme*: The structure of this glycosidase was recently determined [2]. It crystallizes in the hexagonal space group  $P6_122$ , with  $V \sim 425.000 \text{ \AA}^3$ . The crystals had in general small mosaicity, but all specimens that were examined consisted of more than one crystal block. The crystal used for the phase measurements was a twin, full-width at half-maximum (FWHM) values for the larger individual from  $\omega$  rocking curves were in the range  $0,004 - 0,010^\circ$ . Two intensity profiles showing splitting of different magnitude are reproduced in Fig. 1. Three-beam interference profiles were collected by repeated Y-scans for 28 pairs of triplets -H/L/H-L and H/-L/-H+L, corresponding to phases  $+\Phi_3$  and  $-\Phi_3$ . From these profiles

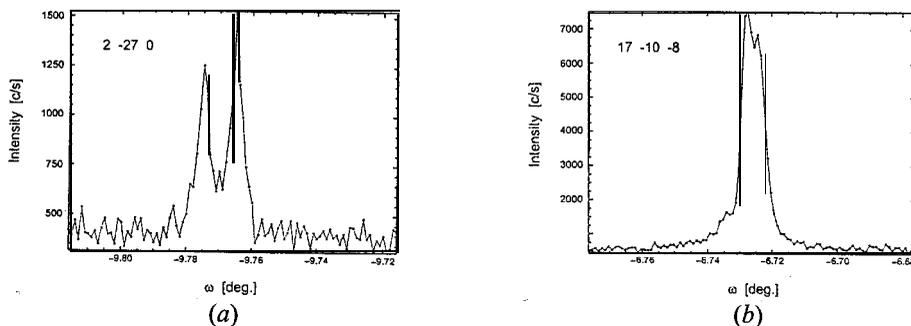


Fig. 1. Intensity profiles from  $\omega$  scans of the reflections 2 -27 0 and 17 -10 -8,  $\lambda = 1,000 \text{ \AA}$

20 triplet phases could be estimated. We ascribe the reduction in number both to problems due to twinning and to the inability, at the time, to calculate optimized wavelengths to minimize the interference from other strong and closely adjacent reflections. Three examples of experimental profile pairs are shown in Fig. 2; the upper profile in each pair corresponds to the triplet

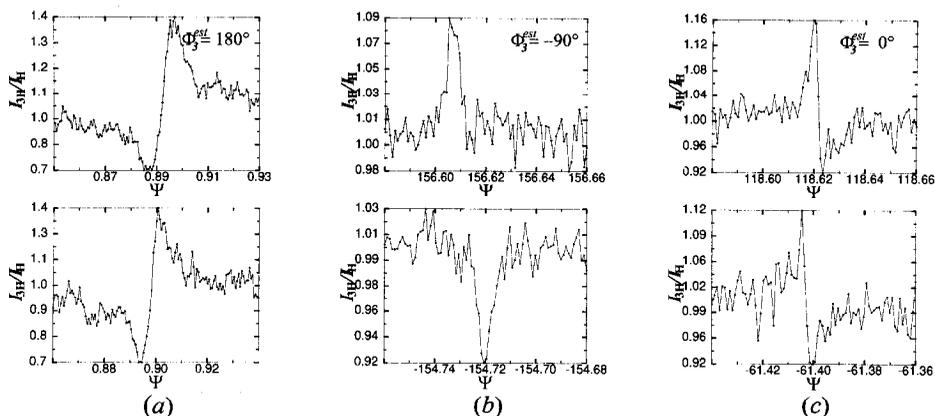


Fig. 2.  $\Psi$ -scan profiles of three interference maxima; the estimated triplet phase,  $\Phi_3^{\text{est}}$ , is given in the upper profile of each pair. (a) Triplet 2 2 1 / -1 -1 0 / -1 -1 -1,  $\Phi_3^{\text{calc}} = 180^\circ$ . (b) Triplet 3 1 3 / -1 -1 0 / -2 0 -3,  $\Phi_3^{\text{calc}} = -87^\circ$ . (c) Triplet 11 4 9 / 2 -1 0 / -13 -3 -9,  $\Phi_3^{\text{calc}} = -22^\circ$ .

-H/L/H-L. The average difference,  $|\Delta\Phi_3|$ , between the estimated triplet phases and those calculated from the crystallographic refinement is  $21,5^\circ$ . Previous PPE on protein crystals includes tetragonal lysozyme,  $V \sim 237.200 \text{ \AA}^3$  [3]. In the present study we have demonstrated the feasibility of PPE with a different crystal form having a larger unit cell. The crystals of hexagonal lysozyme were very resistant to X-rays, there were hardly any changes in the intensity profiles of the test reflections after about 20 hrs. of exposure. Part of the work on guinea-fowl lysozyme was done in the subsequent SNBL Experiment 01-02-65.

The Y-scans were made on a 6-circle Huber diffractometer (University of Karlsruhe) located on the Swiss-Norwegian Beamline, ESRF.

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- [3] Weckert, E., Schwegle, W. & Hümmel, K. (1993) *Proc. Roy. Soc. Lond. A* **442**, 33-46.