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A feasibility study of experimental triplet-phase determination in small proteins

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Abstract. It is shown for the first time that the measurement of triplet phases of three-beam cases where strong structure factors are involved is possible in small protein structures, for example myoglobin. The exploitation of the triplet phase from the interference profile is not affected as long as the structure factors of unavoidable overlapping three-beam cases are small.

Introduction. Recently, it has been shown that triplet phase invariants $\Phi = \phi(-\mathbf{h}) + \phi(\mathbf{g}) + \phi(\mathbf{h} - \mathbf{g})$ (ϕ's represent phase angles of structure factors; **h**, **g** are reciprocal-lattice vectors) can be experimentally determined by means of three-beam interference experiments with an accuracy of about 45° (Hümmer, Weckert & Bondza, 1990). The method has been applied to several organic non-centrosymmetric small-molecule structures. With synchrotron radiation, it is possible to select for each three-beam case an appropriate wavelength (in our case between 0.7 and 3.0 Å), so that no adjacent multiple-reflection positions occur within a ψ-angular range of $\pm 0.1°$ with respect to the three-beam case of interest. Thus, the influence of any adjacent multiple-beam reflection on the main one is negligible. For a given unit cell the total number of possible three-beam cases decreases with increasing wavelength, therefore larger cells require longer wavelengths to avoid overlapping of three-beam interference profiles. As experiments encounter difficulties for various reasons for wavelengths longer than 2.8 or 3.0 Å, non-overlapping three-beam cases are difficult to find for unit cells larger than about 10 000 $Å^3$. In preliminary experiments using small-molecule structures we investigated the way in which ψ-scan profiles of three-beam cases with strong structure factors are influenced by overlapping three-beam reflections with weak structure factors. The structure-factor moduli of the main three-beam cases

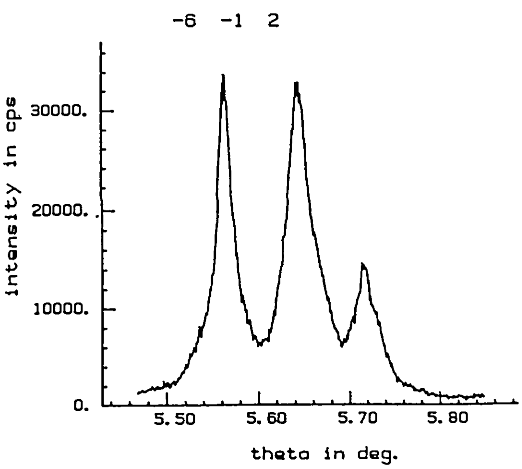

Fig. 1. ω scan of the reflection $\bar{6}\bar{1}2$, $\lambda = 1.906$ Å.

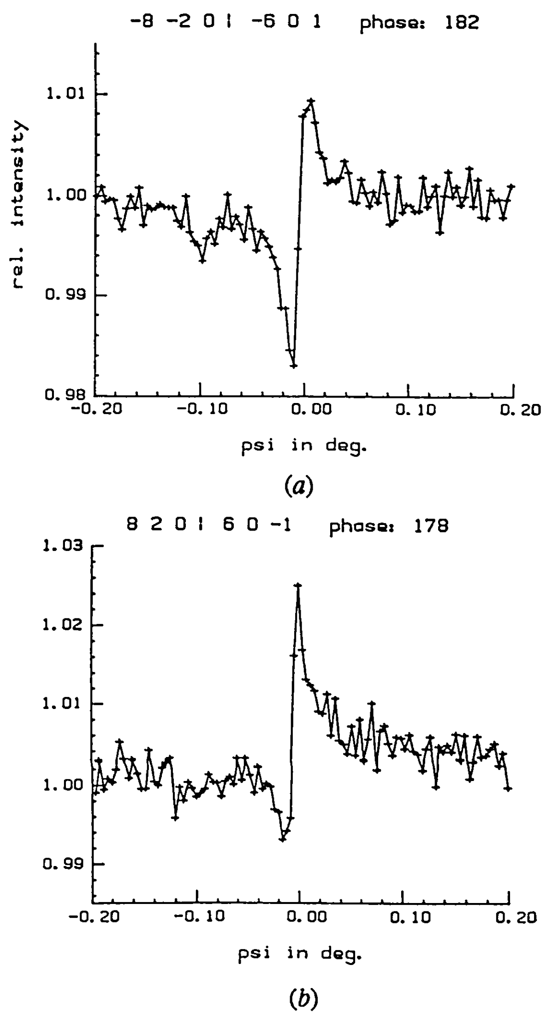

Fig. 2. (*a*), (*b*) ψ-scan profiles of a 180° triplet phase; $|F(\bar{8}\bar{2}0)| = 578$, $|F(\bar{6}01)| = 1052$, $|F(\bar{2}\bar{2}\bar{1})| = 817$, $\lambda = 1.5405$ Å.

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were chosen to fulfil the following condition: $2 \le Q_{hg} \le 6$ with $Q_{hg} = F'(\mathbf{g})F'(\mathbf{h}-\mathbf{g})/F'(\mathbf{h})^2$ [F' are structure-factor moduli corrected for polarization (Hümmer, Weckert & Bondza, 1990)]. It was found that in spite of this overlap of weak reflections the triplet phase of the 'main' three-beam case can be exploited provided that $Q_{hg}(\text{weak}) \le 0.1Q_{hg}(\text{main})$. Thus, one can be confident that experimental phase determination is also possible for large structures where overlapping of multiple reflections is unavoidable. In this paper we report the first experimental determination of triplet phases in sperm-whale myoglobin.

Experimental. A special ψ-circle diffractometer, installed at beamline C of storage ring DORIS II at HASYLAB, Hamburg, was used. The experimental procedure is described in recent papers (Hümmer, Weckert & Bondza, 1989, 1990). Crystals of myoglobin (typical dimensions ~300 µm) were kept with some mother liquid in glass capillaries. The quality of a typical crystal used for experimental phase determination can be seen from Fig. 1, where an ω scan of the $\bar{6}\bar{1}2$ reflection is shown. The beam divergence for this experiment was about 0.01°. Three crystal blocks can be seen, the best one with a FWHM of about 0.023°. The angular difference between the blocks (about 0.1°) is large enough to enable us to use only one of these blocks for the three-beam interference experiments. We chose three-beam cases among the strongest reflections possible and selected wavelengths such that Q_{hg} for weak overlapping reflections within a ψ-angular range of ±0.1° was smaller than 10% of Q_{hg} of the main three-beam case under investigation.

Results. Measured ψ-scan profiles for 'strong' three-beam cases with different triplet phases are shown in Figs. 2–4. The profiles refer to so-called 'in-out' ψ scans reading from left to right. ψ = 0° marks the exact three-beam position. The intensity scale gives the relative change of the integrated two-beam intensity. In each figure the indices of the primary **h** reflection and the secondary **g** reflection as well as the triplet phase of the 'main' three-beam case are given. In each case the two centrosymmetrically related three-beam cases **0/h/g** and **0/–h/–g** are com-

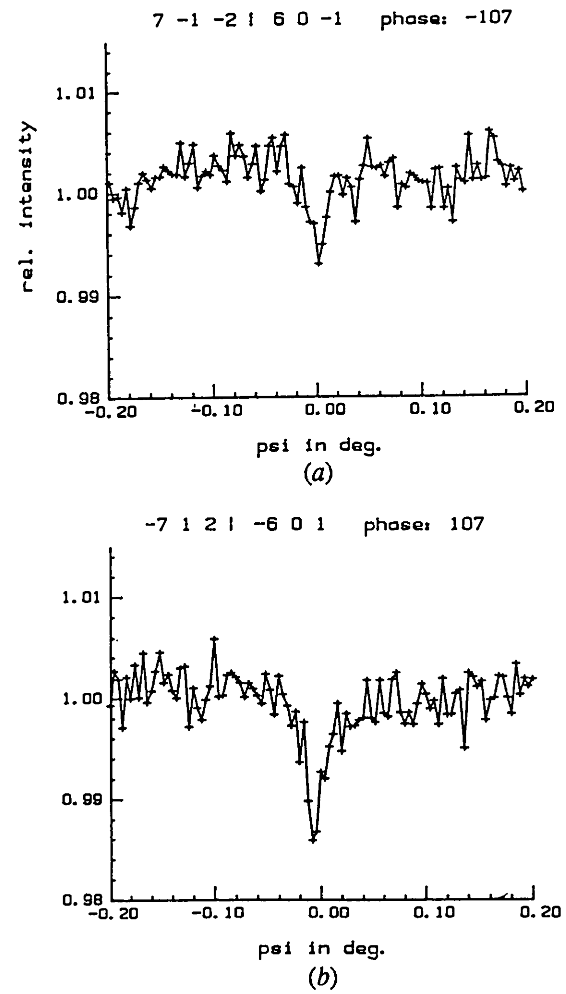

Fig. 3. (a), (b) ψ-scan profiles of a ±107° triplet phase with *Aufhellung*; $|F(\bar{7}12)|$ = 501, $|F(\bar{6}01)|$ = 1052, $|F(\bar{1}11)|$ = 1097, λ = 1.961 Å.

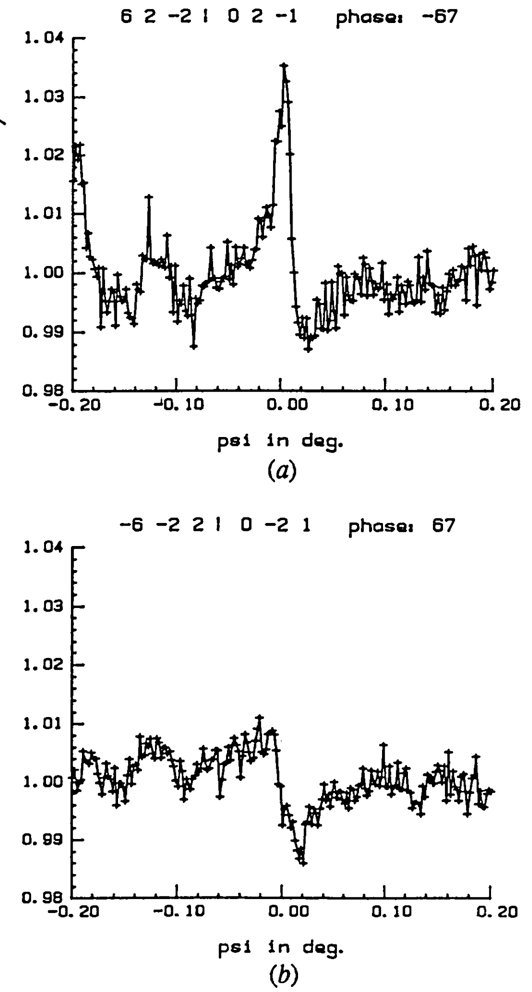

Fig. 4. (a), (b) ψ-scan profiles of a ±67° triplet phase; $|F(\bar{6}\bar{2}2)|$ = 538, $|F(0\bar{2}1)|$ = 1280, $|F(\bar{6}01)|$ = 1052, λ = 1.9349 Å.

pared in order to evaluate phase-independent *Umweganregung* or *Aufhellung* effects (Weckert & Hümmer, 1990; Hümmer, Weckert & Bondza, 1990). As can be seen, the triplet phase of the 'main' three-beam case can be deduced from the measured ψ-scan profiles within the experimental error of about 45°. Figs. 2(*a*) and (*b*) show typical profiles for a triplet phase near 180° where almost no phase-independent effects are involved. In Figs. 3(*a*) and (*b*), the ψ-scan profiles of a triplet phase near $\mp 90°$ with *Aufhellung* are shown. Here the constructive interfering term of the −107° triplet phase is overcompensated by phase-independent *Aufhellung* (Hümmer, Weckert & Bondza, 1990; Chang, Huang, Tang & Lee, 1989). From the different extent of the constructively interfering part of the profiles shown in Figs. 4(*a*) and (*b*), one would deduce a triplet phase near ±45°. This agrees within experimental error with the calculated phase of ±67°. The phases were calculated from known atomic coordinates (Hartmann, Steigemann, Reuscher & Parak, 1987).

Discussion. It is shown that experimental phase determination is also possible for large structures, where overlapping of several multiple reflections is unavoidable provided that the structure factors involved in the underlying multiple reflections fulfil the condition $Q_{hg}(\text{weak}) \leq 0.1Q_{hg}(\text{main})$. In that case the perturbation of the ψ-scan profile of the main three-beam reflection is weak and it can be exploited for phase determination. For the wavelengths used, there were about 150 to 300 three-beam cases within a ψ-angular range of ±0.1° with respect to the three-beam position of the main three-beam case. The relatively high fluctuations of the 'two-beam' intensity outside the actual ψ-scan profile may be due to these underlying multiple reflections.

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