

26. A HISTORICAL PERSPECTIVE

The temperature factors shown in Table 26.1.3.2 most deserve comment. All those obtained for the significant sites (of which there are no more than two for any derivative) are comparable with the overall value for the protein crystals themselves. The very large values obtained for other sites show that these sites are of little importance at high angles and may not represent real sites of heavy-atom attachment. The minor site of MHTS is clearly the SO_3^- group of this molecule (see Section 26.1.3.1).

26.1.3.9. Calculation of phase values

Blow (1958), in his determination of the phase angles of the noncentrosymmetric [100] zone of horse haemoglobin, and, later, Cullis *et al.* (1961, 1962), in their determination of the three-dimensional structure of horse haemoglobin, used anomalous-scattering data to supplement the information available from the isomorphous-replacement differences. In each of these studies, phase determination had been carried out by constructing probability curves from the multiple-isomorphous-replacement data and, when the most probable phase angle had been deduced, the anomalous-scattering data were examined. For many reflections, they allowed a choice to be made between two apparently equally probable values of phase angle given by the isomorphous data. This procedure was clearly rather arbitrary and subjective, and a method of combining anomalous scattering with isomorphous replacement in a more rigorous way was described by Blow & Rossmann (1961). In their method, which was subsequently employed for the low-resolution work on lysozyme, use was made of the fact that the mirror image of the Argand diagram for a $\bar{h}\bar{k}\bar{l}$ reflection is similar to the Argand diagram for the hkl reflection, but for the reversal of the sense of the imaginary part of the heavy-atom contribution. The data for the $\bar{h}\bar{k}\bar{l}$ reflections may therefore be treated as though they came from a separate isomorphous compound, with parameters identical to those of the original compound, but with the opposite sign for the imaginary component of the atomic scattering factor.

In the low-resolution lysozyme phase determination (Section 26.1.2.7), intensities of the Friedel pairs of reflections were measured for each of the three heavy-atom compounds, and the problem was treated as if there had been a total of six heavy-atom compounds. Although the method had been found helpful to some extent, analysis of the phases showed that the anomalous-scattering data had played comparatively little part in determining the positions of the centroids of the phase probability distributions, even for reflections with apparently significant anomalous differences.

ACTN observed that this apparent contradiction is because of the fact that the anomalous differences between Bijvoet pairs of reflections measured from the same crystal are inherently more accurate than the isomorphous differences that are measured from different crystals and subject to different systematic errors (North, 1965; Phillips, 1966). Indeed, analysis of the equivalent reflections from native and derivative crystals (Section 26.1.3.6) showed that the r.m.s. error E' , corresponding to the anomalous differences, was about one-third of E , the error in the isomorphous differences. The result of incorporating this distinction in the phase program is illustrated in Fig. 26.1.3.7. Phase calculations for the new 6 Å and 2 Å maps of lysozyme were therefore carried out by using ACTN's method, with E' set at one third of E .

The data tapes containing the F values for the native and the Friedel pairs of F values for the three derivatives were used as input to a phase program written by ACTN. For acentric reflections, phase probabilities were calculated as described in the previous section, and the centroids of the distributions were determined in order to derive a 'figure of merit', which was applied to the structure amplitudes, as first proposed by Blow & Crick (1959), so as to

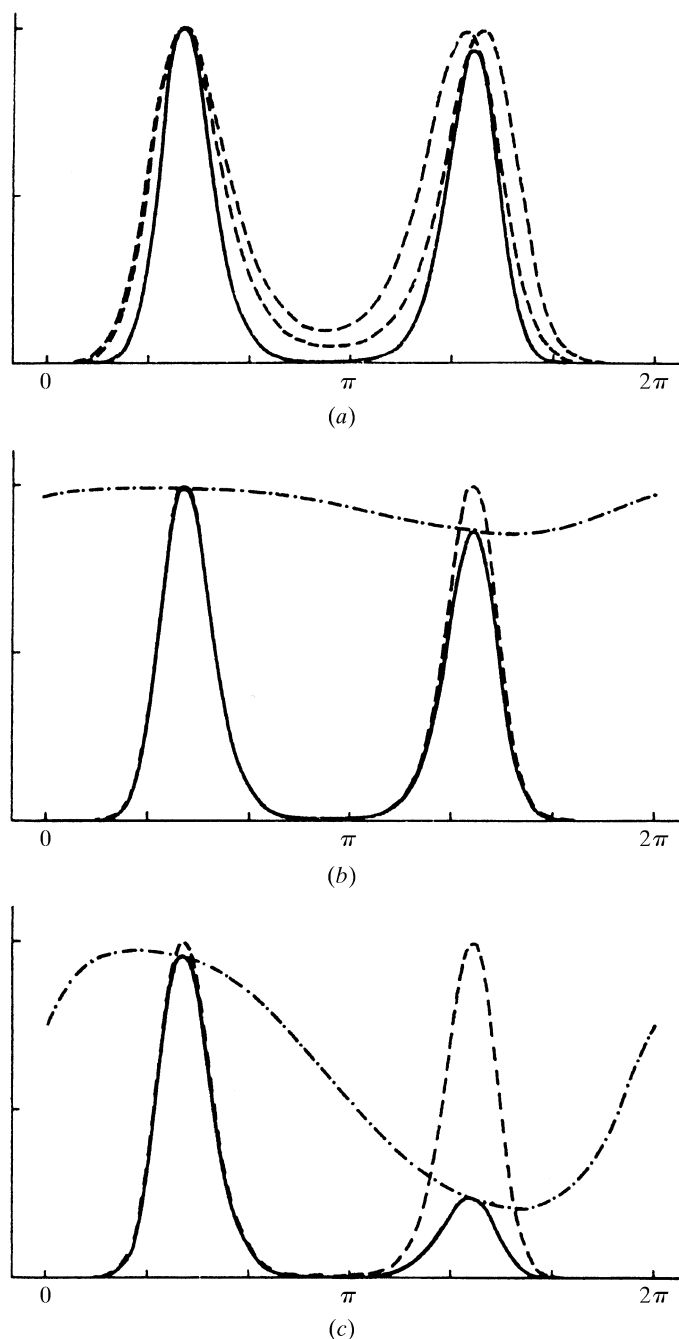


Fig. 26.1.3.7. (a) Phase probability curve for a Bijvoet pair of reflections (broken lines) with the joint probability curve (full line) derived by the method of Blow & Rossmann (1961). (b) Isomorphous-replacement phase probability curve derived from the mean of F_{PH^+} and F_{PH^-} (broken line); anomalous-scattering probability curve (chain line); and joint probability curve (full line) derived by the method of North (1965), using $E'(\text{anomalous}) = E(\text{isomorphous})$. (c) As (b), but with $E' = (1/3)E$. Reproduced with permission from North (1965). Copyright (1965) International Union of Crystallography.

produce a 'best' Fourier map. For the quite high proportion of centric reflections in the lysozyme diffraction pattern, phase probabilities were calculated by the formula appropriate to the case in which the native and derivative F 's are collinear with each other and with the vector due to the heavy atom.

The phases of the 9040 reflections were calculated on the Elliott 803B computer and had a mean figure of merit of 0.60. The variation with angle was very similar to that obtained with sperm-whale myoglobin and is shown for the centric and acentric reflections separately in Fig. 26.1.3.8.