LETTERS TO THE EDITOR

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Location of the sulfur atoms from the phased anomalous map using native protein data can be very helpful in tracing the peptide chain

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The location of heavy atoms belonging to the native form of a protein is a well-known technique (Rossmann, 1961; Strahs & Kraut, 1968) and relies on the combined use of normal electron density maps and maps based on the anomalous differences. While the normal map is made with mean structure amplitudes phased either by the techniques of multiple isomorphous replacement or molecular replacement, the anomalous map is obtained from the difference between Bijvoet pairs of reflections using the same phases minus 90° (Pepinsky & Okaya, 1956). This difference in phasing results from the fact that the main part of the anomalous difference (in the present case of the form $|F(hkl)|-|F(h\bar{k}\bar{l})|$) comes from the imaginary contribution to the scattering, $\Delta f''$, and thus only atoms with a significant $\Delta f''$ will appear in this density.

For heavy atoms the imaginary contribution to the anomalous scattering is sufficiently large to make it easy to find these atoms, but in principle a similar approach is feasible for second-row atoms (Hendrickson & Sheriff, 1987). We have recently observed that this is so for sulfur and data collected in a routine manner on a diffractometer with a position-sensitive detector.

The molecule under study is an 80-residue hydrophobic soya bean protein (Lehmann, Pebay-Peyroula, Cohen-Addad & Odani, 1989). The space group is $P2_12_12_1$ with one molecule per asymmetric unit. All data were collected to at least 2.8 Å on an Enraf–Nonius FAST diffractometer with the only precaution being that the *b* axis, the needle axis of the crystal, was aligned along the spindle axis of the instrument. In general this gave two simultaneous independent observations for a structure amplitude, namely one Bijvoet pair. The multiple isomorphous replacement phases were obtained from two (nearly) single-site heavy-atom derivatives, namely $PtCl_4^{2-}$ and UO_2^{2+}.

The protein contains two pairs of S—S bonds distributed around Cys28-Cys29 and Cys43-Leu44-Cys45. This gave rise to two snake-nest regions of density leading to a considerable number of different interpretations for the chain tracing. A map based on the anomalous differences in the native data was therefore calculated. All data were used, and had a mean anomalous difference corresponding to 70% of the mean estimated error. The map revealed only four sausage-shaped peaks with peak heights of 7.8, 7.8, 7.4 and 7.4 times the average fluctuation in the map. The four peaks fitted exactly into densities in the two regions which could be interpreted as S—S bridges, and this unambiguously gave the location of residues 28, 29, 43, 44 and 45. The allocation of the remaining four Cys residues, 8, 14, 67 and 77, was then based on comparison of chain lengths with the distances between the disulfur groups as well as on various aspects of the density distribution, and the chain could then be traced. Refinement of the structure is now well underway.

By comparing the size of the sulfur peaks with similar peaks derived from the heavy-atom data it was also possible to get a first estimate of the site-occupation factors for the different heavy atoms used. In the present case the integrated peaks for $PtCl_4^{2-}$ and UO_2^{2+} from the phased anomalous maps were 559 and 1499. By comparison with the observed mean value for an S—S group of 234, and by use of 0.557, 0.702, 6.925 and 13.409 electrons for $\Delta f''$ of S, Cl, Pt and U (*International Tables for X-ray Crystallography*, 1974, Vol. IV, p. 149), the site-occupation factors for the two groups were estimated to be 0.38 and 0.53, respectively.

Attempts were also made to locate the sulfur atoms in the phased anomalous maps for the heavy-atom derivatives. The reason for this was that a special effort had been made to obtain error-free differences for these data. Some sulfur peaks were found, but in general they were swamped by a good number of other peaks, in agreement with similar observations by Sheriff & Hendrickson (1987). We did not pursue this using the 'imaginary Fourier synthesis' proposed by these workers (Hendrickson & Sheriff, 1987), as we did not expect to find any identifiable ions in the solvent.

We also tried to locate the sulfur atoms from Patterson maps to see whether the structure could have been solved

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according to the same principles as used for crambin, where the anomalous scattering from sulfur was used as the sole means to solve a small protein structure using high-resolution data (Hendrickson & Teeter, 1981), but this failed.

In conclusion we therefore note that the information derived from the phased anomalous map of the native data, which is very easily calculated, can be of great help in the early part of the analysis. Moreover, it can be used in a first estimation of absolute values for the site-occupation factors, it might help locating counter ions (Hendrickson & Sheriff, 1987), and, finally, the anomalous scattering from sulfur can at this stage improve the phase information (Hendrickson, Smith & Sheriff, 1985), in line with other derivatives.

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