

(Received 4 January 1966)

The method of combining isomorphous replacement and anomalous scattering data for locating the positions of heavy atoms in protein derivatives was first tried in Professor Dorothy Hodgkin's laboratory at Oxford (Harding, 1962). $|F_P|$ and $|F_{HP}|$, the structure amplitudes of the protein and the heavy-atom protein, can be combined with $|F(+)|$ and $|F(-)|$, the structure amplitudes of direct and inverse reflexions of the heavy-atom protein, to give the heavy-atom contributions $|F_H|^2$. A synthesis with $|F_H|^2$ as coefficients will contain the heavy-atom Patterson vectors alone. Recently Kartha & Parthasarathy (1965) and Matthews (1965) have given expressions which are approximate, for $|F_H|^2$ in terms of $|F_P|$, $|F_{HP}|$, $|F(+)|$ and $|F(-)|$. An exact expression for $|F_H|^2$ in terms of other measurable quantities has been derived in this note. A method for placing the two sets of data $|F_P|$ and $|F_{HP}|$ on a common scale has also been suggested.

Expression for $|F_H|^2$ From the triangle OAB (Fig. 1)

$$|F_{HP}|^2 = |F_P|^2 + |F_H|^2 + 2|F_P||F_H| \cos \varphi. \quad (1)$$

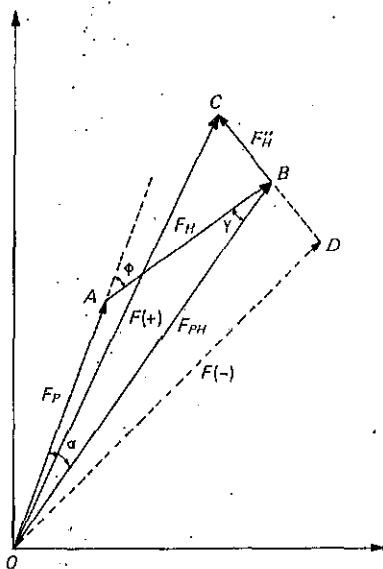


Fig. 1.

From the triangle OCB ,

$$|F(+)|^2 = |F_{HP}|^2 + |F_H''|^2 - 2|F_{HP}||F_H''| \cos(\gamma + \pi/2)$$

or

$$|F(+)|^2 = |F_{HP}|^2 + |F_H''|^2 + 2|F_{HP}||F_H''| \sin \gamma. \quad (2)$$

Combining equations (1) and (2), we have

$$|F(+)|^2 = |F_P|^2 + |F_H|^2 + |F_H''|^2 + 2|F_P||F_H| \cos \varphi + 2|F_{HP}||F_H''| \sin \gamma. \quad (3)$$

Now from the triangle OAB

$$|F_{HP}| \sin \gamma = |F_P| \sin \varphi. \quad (4)$$

Combining equations (3) and (4), we have

$$|F(+)|^2 = |F_P|^2 + (1 + 1/k^2)|F_H|^2 + 2|F_P||F_H| \cos \varphi + (2/k)|F_P||F_H| \sin \varphi, \quad (5)$$

where $k = |F_H|/|F_H''|$. For one type only of anomalous scatterers (heavy atom), k is also equal to f'/f'' , f' and f'' being real and imaginary parts of the scattering factor. It must be noted that k is a function of $\sin \theta$.

Similarly

$$|F(-)|^2 = |F_P|^2 + (1 + 1/k^2)|F_H|^2 + 2|F_P||F_H| \cos \varphi - (2/k)|F_P||F_H| \sin \varphi. \quad (6)$$

Subtracting equation (6) from (5)

$$\Delta I = |F(+)|^2 - |F(-)|^2 = (4/k)|F_P||F_H| \sin \varphi \quad (7)$$

or

$$2|F_P||F_H| \cos \varphi = \pm [4|F_P|^2|F_H|^2 - (k/2)^2(\Delta I)^2]^{1/2}. \quad (8)$$

Adding equation (5) to (6) gives

$$|F_m|^2 = \frac{1}{2}[|F(+)|^2 + |F(-)|^2] = |F_P|^2 + (1 + 1/k^2)|F_H|^2 + 2|F_P||F_H| \cos \varphi; \quad (9)$$

strictly speaking $|F_m|^2$ is not equal to $|F_{HP}|^2$. The approximation $|F_m|^2 \approx |F_{HP}|^2$ (footnote on page 746 of Kartha & Parthasarathy, 1965) holds good only for large values of k . Eliminating $\cos \varphi$ from equation (9) with the help of (8), we have

$$(1 + 1/k^2)^2|F_H|^4 - 2|F_H|^2[(1 + 1/k^2)\{|F_m|^2 - |F_P|^2\} + 2|F_P|^2] + [|F_m|^2 - |F_P|^2]^2 + (k/2)^2(\Delta I)^2 = 0. \quad (10)$$

Equation (9) is quadratic in $|F_H|^2$. Since the last term is positive and the coefficient of $|F_H|^2$ is always negative, both the roots of the above equation are always positive. The two roots are,

$$|F_H|^2 = (1 + 1/k^2)^{-2} \{ (1 + 1/k^2)|F_m|^2 + (1 - 1/k^2)|F_P|^2 \} \pm 2(1 + 1/k^2)^{-2} \{ |F_P|^2 \{ (1 + 1/k^2)(|F_m|^2 - |F_P|^2) + |F_P|^2 \} - (k/4)^2(1 + 1/k^2)^2(\Delta I)^2 \}^{1/2}. \quad (11)$$

This is a relation which gives $|F_H|^2$ in terms of $|F_m|^2$ and $|F_P|^2$. The positive and negative signs before the second term in equation (11) correspond respectively to the negative and positive values of $\cos \alpha$. In most cases, α is acute and hence only the negative sign before the second term need be considered (cf. the approximation made in the paragraph following equation (3), Kartha & Parthasarathy, 1965).

In practice, to a good approximation $1/k^2$ may be neglected as compared with unity. Then equation (11) reduces to

$$|F_H|^2 = |F_m|^2 + |F_P|^2 - 2|F_m|^2|F_P|^2 - (k/4)^2(\Delta I)^2 \quad (12)$$

Equation (11) is exact but equation (12) may be used without introducing much error (<3%) due to the approximation $1/k^2 \ll 1$.

The scale factor

In the derivation of equations (11) and (12) it is assumed that the two sets of data $|F_P|$ and $|F_{HP}|$ are on a common scale. (The absolute scales are not necessary for this method). It is well known that the Wilson's method of obtaining average intensity and hence the method of scaling (Wilson, 1942) is not valid in the case of proteins (Harker, 1953). By using the following method, accurate relative scale factors may be determined.

If the scale factors of $|F_P|$ and $|F_{HP}|$ be S_P and S_{HP} , then equations (7) and (9) can be rewritten in the form

$$|F_H| = \frac{k(\Delta I)S_{HP}^2}{4S_P|F_P| \sin \phi} \quad (13)$$

and

$$S_{HP}^2|F_m|^2 = S_P^2|F_P|^2 + (1 + 1/k^2)|F_H|^2 + 2|F_P||F_H|S_P \cos \phi \quad (14)$$

Eliminating $|F_H|$ from equation (14) with the help of (13) and neglecting $1/k^2$ in comparison with 1 gives

$$S_{HP}^2S_P^2|F_m|^2|F_P|^2 \sin^2 \phi \approx S_P^4|F_P|^4 \sin^2 \phi + (k/4)^2(\Delta I)^2S_{HP}^4 + (k/4)(\Delta I)S_{HP}^2|F_P|^2S_P^2 \sin 2\phi = 0 \quad (15)$$

If the average is taken over a large number of reflexions $\overline{\sin^2 \phi} = \frac{1}{2}$ and $\overline{\sin 2\phi} = 0$. Thus equation (15) reduces to

$$S_R^4|F_P|^4 - S_R^2|F_m|^2|F_P|^2 + (k^2/8)(\Delta I)^2 = 0 \quad (16)$$

where $S_R^2 = S_P^2/S_{HP}^2$. As k varies with $\sin \theta$ the reflexions may be divided in small intervals of $\sin \theta$ such that k does not vary appreciably in the interval chosen. The scale factor is given by

$$S_R^2 = \left[\overline{|F_m|^2|F_P|^2} + \left\{ \overline{|F_m|^2|F_P|^2}^2 - (k^2/2)(\Delta I)^2 \overline{|F_P|^4} \right\}^{1/2} \right] / 2 \overline{|F_P|^4}$$

It must be noted that the method does not need the knowledge of the total number of the heavy atoms in the unit cell.

The authors wish to thank Professor Dorothy Hodgkin and Mrs Eleanor Collier for some discussions and Dr B. W. Matthews of the Medical Research Council, Cambridge, for kindly providing a preprint of his paper.

References

- HARDING, M. M. (1962). D. Phil. Thesis, Oxford Univ.
 HARKER, D. (1953). *Acta Cryst.* **6**, 731.
 KARTHA, G. & PARTHASARATHY, R. (1965). *Acta Cryst.* **18**, 745.
 MATTHEWS, B. W. (1965). *Acta Cryst.* **20**, 320.
 WILSON, A. J. C. (1942). *Nature, Lond.* **150**, 152.