Short Communications

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Acta Cryst. (1966). **21**, 279

The determination of heavy atom positions in protein derivatives.

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(*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)

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

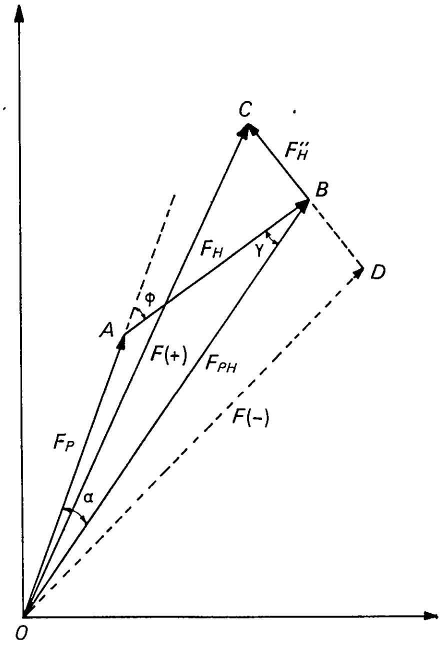

Fig. 1.

From the triangle *OCB*,

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

or

$$|F(+)|^2=|F_{HP}|^2+|F''_H|^2+2|F_{HP}||F''_H|\sin\gamma\,. \qquad (2)$$

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

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

Now from the triangle *OAB*

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

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

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

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

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

Subtracting equation (6) from (5)

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

or

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

Adding equation (5) to (6) gives

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

strictly speaking $|F_m|^2$ is not equal to $|F_{HP}|^2$. The approximation $|F_m|^2\simeq|F_{HP}|^2$ (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

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

Equation (9) is quadratric 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,

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

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

$$|F_H|^2=|F_m|^2+|F_P|^2-2[|F_m|^2|F_P|^2-(k/4)^2(\Delta I)^2]^{\frac{1}{2}}\ . \quad (12)$$

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

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

and

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

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

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

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

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

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

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

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 Coller for some discussions and Dr B.W. Matthews of the Medical Research Council, Cambridge, for kindly providing a preprint of his paper.

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Acta Cryst. (1966). **21**, 280

The crystal structure of $[Cr(H_2O)_4Cl_2]Cl\cdot 2H_2O$.[*]

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(*Received* 2 *February* 1966 *and in revised form* 2 *March* 1966)

We here compare the almost identical results of two independent three-dimensional crystal structure studies of $[Cr(H_2O)_4Cl_2]Cl\ .\ 2H_2O$, and present several supplementary illustrations. The duplication of effort was discovered only when one study was almost complete (Morosin, 1965) and the other had recently been accepted for publication (Dance & Freeman, 1965).

Experimental

Single crystals (plates bound by $\{111\}$, $\{1\bar{1}1\}$ and well developed $\{001\}$) suitable for X-ray studies were obtained by recrystallization of the commercially available salt. Weissenberg and precession photographs were used to determine the symmetry of the compound and the perfection of the specimen used for intensity measurements. Systematic absences and final agreement between observed and calculated structure factors indicate $C2/c$ to be the correct space group. Lattice constants (Table 1)[†] were obtained with Cu $K\alpha$ radiation ($\lambda K\alpha_1=1{\cdot}54050$ Å) by least-squares fit of 44 high 2θ values measured from films on which lead nitrate powder patterns ($a_0=7{\cdot}8404$ Å) were superimposed. Three-dimensional Mo $K\alpha$ intensity data were collected with the use of balanced filters and a scintillation counter. Angles were set by a semi-automatic or 'remote' control Datex module. Small absorption corrections ($\mu=19{\cdot}0$ cm^{-1}) were calculated with a modified ORABS program (Wehe, Busing & Levy, 1962) and applied for the plate of approximate dimensions $0{\cdot}6\times0{\cdot}6\times0{\cdot}15$ mm. A Patterson synthesis yielded Cr and Cl positions; subsequent Fourier synthesis indicated probable oxygen positions. Positional and thermal parameters

Table 1. *Lattice constants*

	Dance & Freeman	This work
a_0	12·053 (±2)	12·055 (±1)
b_0	6·840 (±2)	6·832 (±1)
c_0	11·640 (±2)	11·648 (±1)
β	94·169 (±5)	94·143 (±8)
V	957·0 Å^3	956·85 Å^3
D_x	1·849 g.cm^{-3}	1·849 g.cm^{-3}
D_m[*]	1·835 g.cm^{-3}	Dm 1·845 g.cm^{-3}

[*] Value of Biltz & Birk (1925).

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[*] This work was supported by the U.S. Atomic Energy Commission.

[†] Throughout this note in Tables and Figures, the value of the error corresponds to the least significant digits in the function value.