THE COMBINATION OF MACROMOLECULAR AND DIRECT METHODS

Fan Hai-fu

Institute of Physics, Chinese Academy of Sciences, Beijing, China

Notation

$\underline{H}$ the reciprocal vector of the reflection with index hkl

$\underline{F}_{\underline{H}}$ the structure factor at the reciprocal lattice point $\underline{H}$

$F_{\underline{H}}$ the modulus of $\underline{F}_{\underline{H}}$

$\varphi_{\underline{H}}$ the phase of $\underline{F}_{\underline{H}}$

$\underline{E}_{\underline{H}}$ the normalized structure factor

$E_{\underline{H}}$ the modulus of $\underline{E}_{\underline{H}}$

$\underline{F}_{\underline{H},A}$ the diffraction contribution from the real-part scattering of the anomalous scatterers, i.e.

$$\underline{F}_{\underline{H},A} = \sum_{A=1}^{N_A} (f_A + \Delta f'_A)\exp(i2\pi \underline{H}\cdot\underline{r}_A)$$

$\underline{F''}_{\underline{H},A}$ the diffraction contribution from the imaginary-part scattering of the anomalous scatterers, i.e.

$$\underline{F''}_{\underline{H},A} = \sum_{A=1}^{N_A} i\,\Delta f''_A \exp(i2\pi \underline{H}\cdot\underline{r}_A)$$

$\sigma_n = \sum_j Z_j^n$, Z_j is the atomic number of the jth atom in the unit cell, n is an integer equal to 2 or 3

$\sigma_u = \sum_u Z_u^2/\sigma_2$, Z_u is the atomic number of the uth atom belonging to the unknown part of the unit cell

Subscripts:

A the anomalous scattering atoms

R the replacing atoms of an isomorphous pair

N the atoms in the native protein

p the atoms of the partial structure with known positions in the unit cell

u the atoms of the unknown part of the unit cell

Introduction

Since the 1960's attempts have been made to combine direct methods with the single isomorphous replacement (SIR) and the one-wave-length anomalous scattering(OAS) methods (Coulter, 1965; Fan Hai-fu, 1965; Karle, 1966). Such a combination would be important for the structure analysis of proteins due to the possibilities of reducing the number of heavy-atom de-

rivatives needed for solving a protein structure, saving the time in data collection thus enhancing the effective lifetime of the protein crystal and simplifying the process of the structure determination. However, during the last decade, procedures proposed had not been as successful as expected. Recently some progresses have been achieved. Hauptman (1982a,b) integrated the probabilistic theory of the triplet structure invariants with the SIR and OAS techniques. Giacovazzo (1983) reported a similar theory. Karle (1983; 1984a,b) proposed simple rules for the evaluation of triplet structure invariants from SIR or OAS data. All these methods have been successful in deriving large number of reliable triplet structure invariants using error free data. An alternative procedure has also been proposed (Fan, Han, Qian & Yao, 1984; Fan, Han & Qian, 1984; Fan & Gu, 1985; Yao & Fan, 1985; Qian, Fan & Gu, 1985), which, by the test calculations using theoretical as well as experimental data, has been proved to be efficient in breaking the enantiomorphous phase ambiguities in SIR or OAS method yielding large number of reliable individual phases.

1. Enantiomorphous phase ambiguities in SIR and OAS methods

In the SIR case , for a given $\underline{H}$ we have

$$\underline{F}_{\underline{H},D} - \underline{F}_{\underline{H},N} = \underline{F}_{\underline{H},R} \cdot \tag{1.1}$$

The moduli of $\underline{F}_{\underline{H},D}$ and $\underline{F}_{\underline{H},N}$ can be obtained experimentally. Accordingly the parameters of the replacing atoms can be found and $\underline{F}_{\underline{H},R}$ be calculated. Consequently, we have two ways for drawing the triangle of (1.1) leading to a phase doublet for both $\underline{F}_{\underline{H},D}$ and $\underline{F}_{\underline{H},N}$ in the phase-vector diagram, Fig. 1.

In the OAS case, we have

$$\underline{F}_{\underline{H}}^{+} = \underline{F}_{\underline{H}} + \underline{F}''_{\underline{H},A} \tag{1.2}$$

and

$$\underline{F}_{\underline{H}}^{-*} = \underline{F}_{\underline{H}} - \underline{F}''_{\underline{H},A} \cdot \tag{1.3}$$

Here $\underline{F}_{\underline{H}}$ is the contribution of both the normal scattering and the real part anomalous scattering from the whole unit cell. $\underline{F}_{\underline{H}}^{-*}$ denotes the conjugate of $\underline{F}_{\underline{H}}^{-}$. It follows from (1.2) and (1.3) that

$$\underline{F}_{\underline{H}}^{+} - \underline{F}_{\underline{H}}^{-*} = 2\underline{F}''_{\underline{H},A} \cdot \tag{1.4}$$

The moduli of $\underline{F}_{\underline{H}}^{+}$ and $\underline{F}_{\underline{H}}^{-*}$ can be obtained experimentally and then $\underline{F}''_{\underline{H},A}$ can be derived. Hence we also have two ways for drawing the triangle of (1.4) leading to an enantiomorphous phase doublet for $\underline{F}_{\underline{H}}$ as shown in Fig. 2.

Both the phase doublets in SIR and OAS case can be expressed in the

generalized form

$$\varphi_{\underline{H}} = \varphi'_{\underline{H}} \pm \left| \Delta\varphi_{\underline{H}} \right| . \qquad (1.5)$$

In the case of SIR:

$$\varphi'_{\underline{H}} = \varphi_{\underline{H},R}$$

If $\varphi_{\underline{H}}$ denotes the phase of a reflection from the native protein, then

$$\Delta\varphi_{\underline{H}} = \Delta\varphi_{\underline{H},N} = \pm \cos^{-1}((F^2_{\underline{H},D} - F^2_{\underline{H},R} - F^2_{\underline{H},N})/2F_{\underline{H},R}F_{\underline{H},N}) \qquad (1.6)$$

If $\varphi_{\underline{H}}$ denotes the phase of a reflection from the derivative, then

$$\Delta\varphi_{\underline{H}} = \Delta\varphi_{\underline{H},D} = \pm \cos^{-1}((F^2_{\underline{H},D} + F^2_{\underline{H},R} - F^2_{\underline{H},N})/2F_{\underline{H},R}F_{\underline{H},D}) \qquad (1.7)$$

In the case of OAS:

$$\varphi'_{\underline{H}} = \varphi''_{\underline{H},A} ,$$

where $\varphi''_{\underline{H},A}$ is the phase of $F''_{\underline{H},A}$. We can also write

$$\varphi'_{\underline{H}} = \varphi_{\underline{H},A} + \omega_{\underline{H}} ,$$

where $\varphi_{\underline{H},A}$ is the phase of $F_{\underline{H},A}$, while $\omega_{\underline{H}}$ is the phase difference between $F''_{\underline{H},A}$ and $F_{\underline{H},A}$. If there is only one kind of anomalous scatterer in the unit cell, then $\omega_{\underline{H}} = \pi/2$. We have for the OAS case (see Blundell & Johnson, 1976)

$$\Delta\varphi_{\underline{H}} = \pm \cos^{-1}((F^+_{\underline{H}} - F^-_{\underline{H}})/2F''_{\underline{H},A}) . \qquad (1.8)$$

Notice that $\varphi_{\underline{H}}$ in this case is defined as the phase of

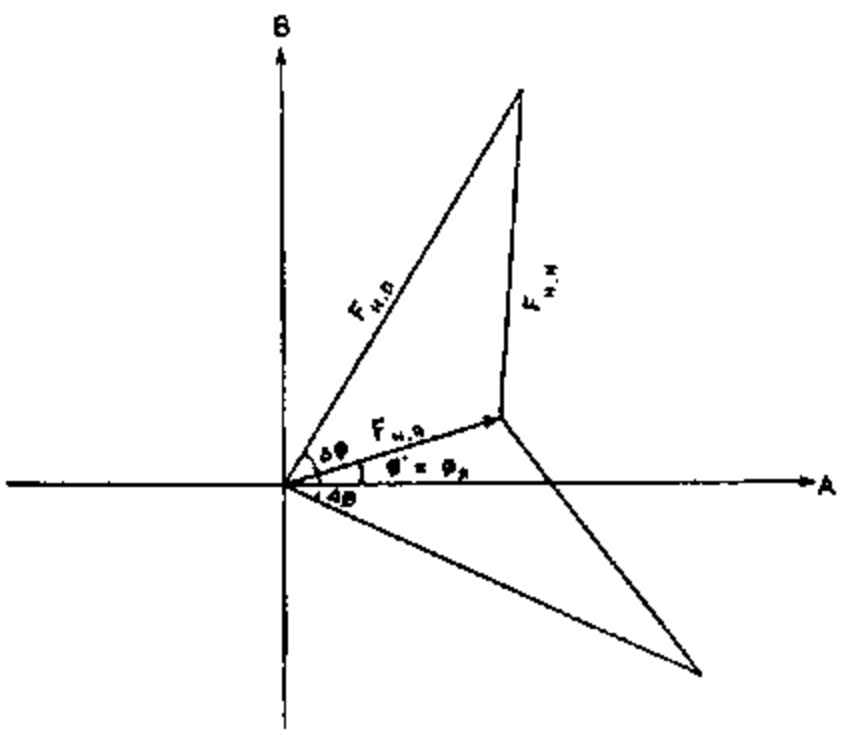
$$F_{\underline{H}} = (F^+_{\underline{H}} + F^{-*}_{\underline{H}})/2 .$$

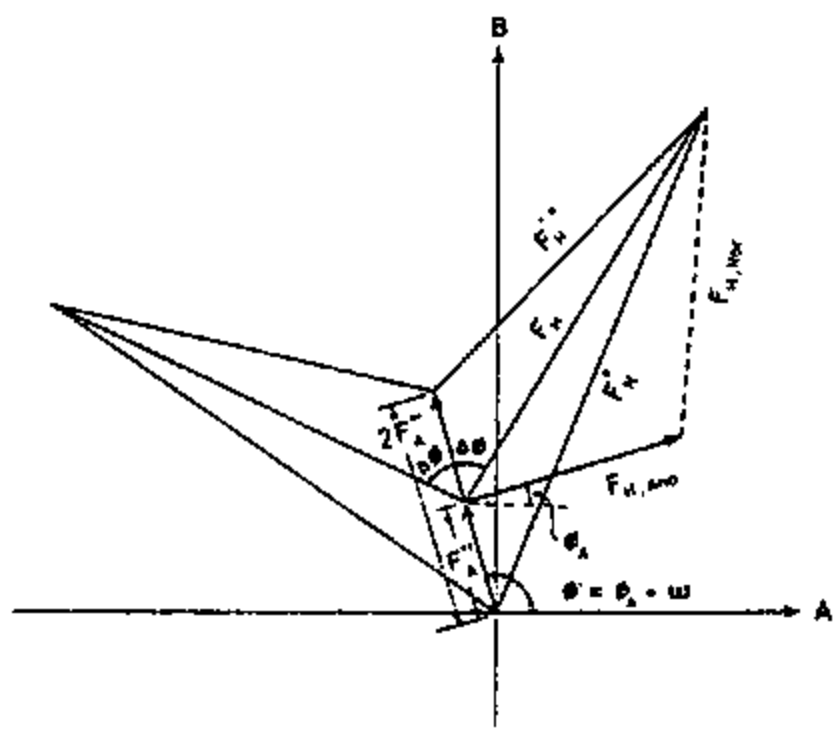
Figure 1

Enantiomorphous phase-doublet in SIR case

Figure 2

Enantiomorphous phase-doublet in OAS case





II. The probability for $\Delta\varphi_{\underline{H}}$ to be positive

With the expression $\varphi_{\underline{H}} = \varphi'_{\underline{H}} + \Delta\varphi_{\underline{H}}$, The Cochran distribution (Cochran, 1955) can be modified to give (Fan, Han, Qian & Yao, 1984):

$$P_{Cochran}(\Delta\varphi_{\underline{H}}) = (2\pi I_o(\alpha'))^{-1}\exp(\alpha'\cos(\Delta\varphi_{\underline{H}} - \beta')) \quad , \quad (2.1)$$

where $I_o(\alpha')$ is the zero order modified Bessel function of the first kind with α' as argument,

$$\alpha' = \left\{ \left[\sum_{\underline{H}'} K_{\underline{HH}'} \sin(\varphi'_3 + \Delta\varphi_{\underline{H}'} + \Delta\varphi_{\underline{H}-\underline{H}'}) \right]^2 + \left[\sum_{\underline{H}'} K_{\underline{HH}'} \cos(\varphi'_3 + \Delta\varphi_{\underline{H}'} + \Delta\varphi_{\underline{H}-\underline{H}'}) \right]^2 \right\}^{\frac{1}{2}} ,$$

$$\tan\beta' = \frac{\sum_{\underline{H}'} K_{\underline{HH}'} \sin(\varphi'_3 + \Delta\varphi_{\underline{H}'} + \Delta\varphi_{\underline{H}-\underline{H}'})}{\sum_{\underline{H}'} K_{\underline{HH}'} \cos(\varphi'_3 + \Delta\varphi_{\underline{H}'} + \Delta\varphi_{\underline{H}-\underline{H}'})} ,$$

$$\varphi'_3 = -\varphi'_{\underline{H}} + \varphi'_{\underline{H}'} + \varphi'_{\underline{H}-\underline{H}'} \quad , \quad K_{\underline{HH}'} = 2\sigma_3\sigma_2^{-3/2} E_{\underline{H}} E_{\underline{H}'} E_{\underline{H}-\underline{H}'} .$$

In the same way, Sim's distribution (Sim, 1959) can be modified as

$$P_{Sim}(\Delta\varphi_{\underline{H}}) = (2\pi I_o(x))^{-1}\exp(x\cos(\Delta\varphi_{\underline{H}} - \delta_{\underline{H}})) \quad , \quad (2.2)$$

where $x = 2E_{\underline{H}}E_{\underline{H},p} / \sigma_u$; $\delta_{\underline{H}} = \varphi_{\underline{H},p} - \varphi'_{\underline{H}}$.

Combination of (2.1) and (2.2) gives the total probability distribution of $\Delta\varphi_{\underline{H}}$ as (Fan & Gu, 1985)

$$P(\Delta\varphi_{\underline{H}}) = (2\pi I_o(\alpha))^{-1}\exp(\alpha\cos(\Delta\varphi_{\underline{H}} - \beta)) \quad , \quad (2.3)$$

where

$$\alpha = \left\{ \left(\sum_{\underline{H}'} K_{\underline{HH}'} \sin(\varphi'_3 + \Delta\varphi_{\underline{H}'} + \Delta\varphi_{\underline{H}-\underline{H}'}) + x\sin\delta_{\underline{H}} \right)^2 + \left(\sum_{\underline{H}'} K_{\underline{HH}'} \cos(\varphi'_3 + \Delta\varphi_{\underline{H}'} + \Delta\varphi_{\underline{H}-\underline{H}'}) + x\cos\delta_{\underline{H}} \right)^2 \right\}^{\frac{1}{2}} , \quad (2.4)$$

$$\tan\beta = \frac{\sum_{\underline{H}'} K_{\underline{HH}'} \sin(\varphi'_3 + \Delta\varphi_{\underline{H}'} + \Delta\varphi_{\underline{H}-\underline{H}'}) + x\sin\delta_{\underline{H}}}{\sum_{\underline{H}'} K_{\underline{HH}'} \cos(\varphi'_3 + \Delta\varphi_{\underline{H}'} + \Delta\varphi_{\underline{H}-\underline{H}'}) + x\cos\delta_{\underline{H}}} . \quad (2.5)$$

Since $|\Delta\varphi_{\underline{H}}|$ is a known quantity, when phase-doublet information is available, the probability that $\Delta\varphi_{\underline{H}}$ has a positive sign can be derived from (2.3):

$$P_+(\Delta\varphi_{\underline{H}}) = \frac{1}{2} + \frac{1}{2}\tanh\left\{ \sin|\Delta\varphi_{\underline{H}}| \left(\sum_{\underline{H}'} K_{\underline{HH}'} \sin(\varphi'_3 + \Delta\varphi_{\underline{H}'} + \Delta\varphi_{H-H'}) + x\sin\delta_{\underline{H}} \right) \right\} . \quad (2.6)$$

On the other hand, by maximizing (2.3) we have $\Delta\varphi_{\underline{H}} = \beta$. Hence we can calculate the 'most probable' value of $\Delta\varphi_{\underline{H}}$ from (2.5). A systematic

method using (2.5) and (2.6) to break the enantiomorphous phase ambiguity will be described in the following section.

III. Treatment of errors of the starting phases in the presence of enantiomorphous phase ambiguity

For more details on this subject the reader is referred to Fan, Han & Qian (1984).

1. The 'best phase relationship'

Following Blow & Crick (1959), we can introduce into direct methods the concepts of 'best phase' and 'figure of merit' for individual reflections. A best normalized structure factor is defined as

$$\underline{E}_{\underline{H}\text{best}} = m_{\underline{H}} E_{\underline{H}} \exp(i\varphi_{\underline{H}\text{best}}) \quad , \tag{3.1}$$

where $$\underline{m}_{\underline{H}} = m_{\underline{H}} \exp(i\varphi_{\underline{H}\text{best}}) = \int \exp(i\varphi_{\underline{H}}) P(\varphi_{\underline{H}}) d\varphi_{\underline{H}} \quad .$$

A triplet phase relationship which consists of the best normalized structure factors is called the 'best phase relationship'.

2. Expressions of the best phase and the figure of merit for a single reflection with enantiomorphous phase ambiguity

Defining $\Delta\varphi_{\underline{H}\text{best}} = \varphi_{\underline{H}\text{best}} - \varphi'_{\underline{H}}$, the $\Delta\varphi_{\underline{H}\text{best}}$ and $m_{\underline{H}}$ can be expressed as follows

$$\tan(\Delta\varphi_{\underline{H}\text{best}}) = 2(P_+(\Delta\varphi_{\underline{H}}) - \tfrac{1}{2})\sin|\Delta\varphi_{\underline{H}}| / \cos\Delta\varphi_{\underline{H}} \quad , \tag{3.2}$$

$$m_{\underline{H}} = \exp(-\sigma^2_{\underline{H}}/2)\left\{\left[2(P_+ - \tfrac{1}{2})^2 + \tfrac{1}{2}\right](1-\cos 2\Delta\varphi_{\underline{H}}) + \cos 2\Delta\varphi_{\underline{H}}\right\}^{\frac{1}{2}} \quad . \tag{3.3}$$

where $\sigma_{\underline{H}}$ is related to the experimental error and can be derived from the standard deviation D of the 'lack of closure error' (Blow & Crick, 1959).

$m_{\underline{H}}$ may be regarded as a measure of reliability of $\Delta\varphi_{\underline{H}\text{best}}$. As can be seen, there are three factors included in the expression of $m_{\underline{H}}$:

$\exp(-\sigma^2_{\underline{H}}/2)$	a measure of the sharpness of the experimental distribution of $\varphi_{\underline{H}}$
$(P_+ - \frac{1}{2})^2$	a measure of the bias of $\Delta\varphi_{\underline{H}}$ towards positive or negative. It reaches the maximum value when P_+ equals 0 or 1
$\cos 2\Delta\varphi_{\underline{H}}$	a measure of the closeness of the two possible phases, $\varphi^+_{\underline{H}} = \varphi'_{\underline{H}} + \lvert\Delta\varphi_{\underline{H}}\rvert$ and $\varphi^-_{\underline{H}} = \varphi'_{\underline{H}} - \lvert\Delta\varphi_{\underline{H}}\rvert$. It reaches the maximum vlue when $\Delta\varphi_{\underline{H}}$ equals 0 or π.

Either of the last two factors will have no effect on $m_{\underline{H}}$ when the other

one reaches the maximum value.

If $P_+ = P_- = \frac{1}{2}$, (3.2) reduce to

$$\Delta\varphi_{\underline{H}\text{best}} = \begin{cases} 0 & \text{if } \mathrm{SIGN}(\cos\Delta\varphi_{\underline{H}}) = 1 \\ \pi & \text{if } \mathrm{SIGN}(\cos\Delta\varphi_{\underline{H}}) = -1 \end{cases}$$

or
$$\exp(i\varphi_{\underline{H}\text{best}}) = \mathrm{SIGN}(\cos\Delta\varphi_{\underline{H}})\exp(i\varphi'_{\underline{H}}) \quad . \tag{3.4}$$

Meanwhile, (3.3) reduce to

$$m_{\underline{H}} = \exp(-\sigma^2_{\underline{H}}/2)\left|\cos\Delta\varphi_{\underline{H}}\right| \quad . \tag{3.5}$$

Substituting (3.4) and (3.5) into (3.1), one obtains

$$E_{\underline{H}\text{best}} = \exp(-\sigma^2_{\underline{H}}/2)\cos\Delta\varphi_{\underline{H}}E_{\underline{H}}\exp(i\varphi'_{\underline{H}}) \tag{3.6}$$

This is the 'best' normalized structure factor which could be obtained at the begining from the corresponding doublet. In other words, when enantiomorphous ambiguities are present, the best way to start a direct method process is to use the averaged value of the phase doublet as the starting phase and use the weight, $\exp(-\sigma^2_{\underline{H}}/2)\left|\cos\Delta\varphi_{\underline{H}}\right|$, for the correspoding $E_{\underline{H}}$.

3. Procedure for breaking enantiomorphous phase ambiguities

By replacing the $E_{\underline{H}}$, and $E_{\underline{H}-\underline{H}'}$, with their 'best' values, we can modify (2.5) and (2.6) to give

$$P_+(\Delta\varphi_{\underline{H}}) = \frac{1}{2} + \frac{1}{2}\tanh\left\{\sin\left|\Delta\varphi_{\underline{H}}\right|\left[\sum_{\underline{H}'} m_{\underline{H}'}\, m_{\underline{H}-\underline{H}'}\, K_{\underline{H}\underline{H}'} \cdot \sin(\varphi'_3 + \Delta\varphi_{\underline{H}'\text{best}} + \Delta\varphi_{\underline{H}-\underline{H}'\text{best}}) + x\sin\delta_{\underline{H}}\right]\right\} \tag{3.7}$$

and
$$\tan(\Delta\varphi_{\underline{H}}) = \frac{\sum_{\underline{H}'} m_{\underline{H}'}\, m_{\underline{H}-\underline{H}'}\, K_{\underline{H}\underline{H}'} \sin(\varphi'_3 + \Delta\varphi_{\underline{H}'\text{best}} + \Delta\varphi_{\underline{H}-\underline{H}'\text{best}}) + x\sin\delta_{\underline{H}}}{\sum_{\underline{H}'} m_{\underline{H}'}\, m_{\underline{H}-\underline{H}'}\, K_{\underline{H}\underline{H}'} \cos(\varphi'_3 + \Delta\varphi_{\underline{H}'\text{best}} + \Delta\varphi_{\underline{H}-\underline{H}'\text{best}}) + x\cos\delta_{\underline{H}}} \tag{3.8}$$

Now the enantiomorphous phase ambiguities can be broken by the following procedure:

Starting from (3.2) and (3.3) a set of $\Delta\varphi_{\underline{H}\text{best}}$ and $m_{\underline{H}}$ are first calculated with $P_+ = \frac{1}{2}$. The $\Delta\varphi_{\underline{H}\text{best}}$ and $m_{\underline{H}}$ are then substitued into (3.7) to calculate a new set of $P_+(\Delta\varphi_{\underline{H}})$, which will mostly differ from $\frac{1}{2}$ considerably and the enantiomorphous phase ambiguities will thus be broken. The above process can be carried out iteratively. If not only the signs but also the magnitudes of $\Delta\varphi_{\underline{H}}$ are to be refined, then (3.8) should be involved.

IV. Results on the test calculation

1. Data used in the test

Error free and experimental data from three known proteins were used for the test:

a) Insulin, which crystallizes in space group R3 with a=82.5 c=34.0Å, $\gamma=120^{\circ}$ and Z=9. There are ~6400 independent reflections at 1.9Å resolution.

b) Avian pancreatic polypeptide (APP), which crystallizes in space group C2 with a=34.18 b=32.92 c=28.44Å, $\beta=105.30^{\circ}$ and Z=4. There are ~ 2100 independent reflections at 2.1Å resolution.

c) Rice ferricytochrome C (RFC), which crystallizes in space group $P6_1$ with a=43.78 c=110.05Å, $\gamma=120^{\circ}$ and Z=6. there are ~6000 independent reflections at 2Å resolution.

1000 largest E's and 60000 strongest $\sum_2$ relationships from each structure were used in the following test calculations.

The data of the three proteins were kindly provided by Drs G. Dodson and E. Dodson, Prof. T. Blundell and Prof. N. Tanaka respectively.

2. Test for the accuracy of the probability formula

The probability formula (2.6), which forms the base of the present method, is not rigorous in the mathematical sense. Hence a test on the accuracy of the formula is highly deserved. Error free SIR and OAS data of insulin were used for this purpose. In the test, error free quantities, including the correct signs of $\Delta\varphi_{\underline{H}}$, and $\Delta\varphi_{\underline{H}-\underline{H}'}$, were substituted into the right-hand side of (2.6) then compare the result on left-hand side with the true sign of $\Delta\varphi_{\underline{H}}$. A correct result will contribute no error to the phase, while a wrong result will contribute an error of $2\left|\Delta\varphi_{\underline{H}}\right|$ to the corresponding phase. The results are summarized in Table 1. It turns out that (2.6) is accurate enough for practical purpose in both SIR and OAS cases.

3. Application to phasing SIR data with replacing atoms in non-centrosymmetric arrangement

Error free insulin SIR data were used. With $P_+=\frac{1}{2}$, values of $\Delta\varphi_{\underline{H}best}$ and $m_{\underline{H}}$ were calculated using(3.2) and (3.3) respectively and then substituted into (3.7) to calculate a new set of P_+. Most of them differ from $\frac{1}{2}$ considerably. With the new set of P_+, one more cycle of iteration led to further improvement on the reliability. The results are listed in Table 2. It shows that the method is capable of deriving large number of initial phases with high reliability.

Table 1.

Results on testing the accuracy of (2.6) using error free SIR and OAS data of insulin

	OAS		SIR	
Group	%	ER	%	ER
1	99.5	1	100	0
2	97.8	3	98.5	1
3	95.8	4	95.3	4
4	90.3	6	90.1	7
5	83.7	8	82.7	9

Table 2.

Result on phasing the error free SIR data of insulin

	SIR	
Group	%	ER
1	93.5	7
2	89.8	11
3	82.5	16
4	77.1	18
5	72.6	18

The reflections were arranged in descending order of $\left|P_+ - \frac{1}{2}\right|$ and then cumulated into 5 groups. The groups numbered 1,2,3,4 and 5 contain the top 200, 400, 600, 800 and all reflections respectively.

% Percentage of reflections with the signs of $\Delta\varphi_{\underline{H}}$ correctly determined in the test.

ER Averaged error of phases (in degrees).

4. Application to phasing SIR data with replacing atoms in centrosymmetric arrangement

Error free SIR data of APP were used in this test. Two kinds of phase ambiguities simultaeously occured in this case. One is inherent in the SIR method, this can be resolved as in the above example. The other comes from the centrosymmetric arrangement of the replacing atoms, this causes the term $\sin(\varphi_3' + \Delta\varphi_{\underline{H}'best} + \Delta\varphi_{\underline{H}-\underline{H}'best})$ to be identical with zero at the begining of iteration. Hence the calculation using (3.7) will result in a set of P_+ always equal to $\frac{1}{2}$ leaving the phase ambiguities unresolved. In order to overcome this difficulty, a multi-solution procedure with random starting sign sets (Yao & Fan, 1985) was used. With this method the signs of $\Delta\varphi_{\underline{H}}$ were assigned randomly to positive or negative with an associated probability P_+ equal to 0.6 or 0.4 respectively. Then the signs were refined by the iterative calculation using (3.2), (3.3) and (3.7). The result is to be described by Yao Jia-xing in this Winter School. Although some methods have been proposed to resolve the phase ambiguities in SIR case (Blow & Rossmann, 1961; Wang, 1981,1984), none of them was capable of phasing a set of SIR data with the replacing atoms in a centrosymmetric arrangement. Hence the method described here may be a useful complement to the others.

5 Application to phasing the experimental OAS data of three known proteins

Experimental OAS data of insulin, RFC and APP were used separately with the procedure described in paragraph IV.3 . All the three sets of data gave satisfactory result as shown in Tables 3, 4 and 5a respectively. In order to demonstrate the efficiency of the method, the result in Table 5a is compared with that from the SIR-OAS method, which was used by Tickle et al. (1981) to solve the APP structure. Corresponding to the 1000 reflections used with the direct method, 1000 'best phases' resulted from the SIR-OAS method were selected and arranged in descending order of the figures of merit. The reflections were then cumulated into 5 groups. The result is listed in Table 5b. It turns out that, for the selected 1000 reflections, about one half of the total number of reflections at 2.1 Å resolution, the direct phased OAS method is as good as the SIR-OAS method.

Table 3.

Result on phasing the experimental OAS data of insulin

Group	%	ER
1	93.0	33
2	94.5	35
3	94.0	37
4	93.5	38
5	92.3	41

Table 4.

Result on phasing the experimental OAS data of RFC

Group	%	ER
1	95.5	22
2	93.0	25
3	92.2	28
4	88.8	33
5	85.8	36

Table 5.

Results on phasing the experimental data of APP

(a) phased by direct method

Group	%	ER
1	95.0	24
2	90.8	30
3	91.3	29
4	89.0	32
5	86.3	36

(b) phased by SIR-OAS method

Group	%	ER
1	92.0	16
2	88.8	20
3	87.5	24
4	84.1	30
5	79.4	39

For the meaning of 'Group', % and 'ER' , see the foot-note of Tables 1 and 2.

Conclusion remarks

We are now at a stage that, direct methods are ready for use in the ab-initio phasing of SIR or OAS data. The method may still be greatly strengthened if some kind of phase extension-refinement techniques can be incorporated. It can be expected that, the combination of macromolecular and direct methods will play an important role in protein crystallography.

References

Blow, D.M. & Crick, F.H.C.(1959). Acta Cryst. 12, 794-802

Blow, D.M. & Rossmann, M.G.(1961). Acta Cryst. 14, 1195-1202

Blundell, T.L. & Johnson, L.N.(1976). "Protein Crystallography" p177. London: Academic Press

Cochran, W.(1955). Acta Cryst. 8, 473-478

Coulter, C.L.(1965). J. Mol. Biol. 12, 292-295

Fan Hai-fu (1965). Acta Physica Sinica 21, 1114-1118

Fan Hai-fu & Gu Yuan-xin (1985). Acta Cryst. A41, 280-284

Fan Hai-fu, Han Fu-son & Qian Jin-zi (1984). Acta Cryst. A40, 495-498

Fan Hai-fu, Han Fu-son, Qian Jin-zi & Yao Jia-xing (1984). Acta Cryst. A40 489-495

Giacovazzo,C.(1983). Acta Cryst. A39, 585-592

Hauptman, H.(1982a). Acta Cryst. A38, 289-294

Hauptman, H.(1982b). Acta Cryst. A38, 632-641

Karle, J.(1966). Acta Cryst. 21, 273-276

Karle, J.(1983). Acta Cryst. A39, 800-805

Karle, J.(1984a). Acta Cryst. A40, 4-11

Karle, J.(1984b). Acta Cryst. A40, 374-379

Qian Jin-zi, Fan Hai-fu & Gu Yuan-xin (1985). Acta Cryst. (in the press)

Sim, G.A.(1959). Acta Cryst. 12, 813-815

Tickle, I.J., Glover, I.D., Pitts, J.E., Wood, S.T. & Blundell, T.L.(1981) Acta Cryst. A37, C-14

Wang, B.C.(1981). Acta Cryst. A37, C-11

Wang, B.C.(1984). in "Diffraction Methods for Biological Macromolecules" a volume of "Methods in Enzymology" Ed. by H.W. Wyckoff, London: Academic Press (in the press)

Yao Jia-xing & Fan Hai-fu (1985). Acta Cryst. A41, 284-285