Rossmann (1961) and Kartha (1961) independently showed that it was possible to compute usable phases from a single isomorphous replacement (SIR) derivative. This contradicted the previously accepted notion that it was necessary to have at least two isomorphous derivatives to be able to determine a noncentrosymmetric reflection's phase (Harker, 1956). Hence, currently, the procedure used to correlate origins in different derivatives is to compute SIR phases from the first compound and apply them to a difference electron-density map of the second heavy-atom derivative. Thus, the origin of the second derivative will be referred to the arbitrarily chosen origin of the first compound. More important, however, the interpretation of such a 'feedback' difference Fourier is easier than that of a difference Patterson. Hence, once one heavy-atom derivative has been solved for its heavy-atom sites, the solution of other derivatives is almost assured. This concept is examined more closely in the following section.

2.3.3.5. *Interpretation of isomorphous difference Pattersons*

Difference Pattersons have usually been manually interpreted in terms of point atoms. In more complex situations, such as crystalline viruses, a systematic approach may be necessary to analyse the Patterson. That is especially true when the structure contains noncrystallographic symmetry (Argos & Rossmann, 1976). Such methods are in principle dependent on the comparison of the observed Patterson, $P_1(\mathbf{x})$, with a calculated Patterson, $P_2(\mathbf{x})$. A criterion, C_P, based on the sum of the Patterson densities at all test vectors within the unit-cell volume V, would be

$$C_P = \int_V P_1(\mathbf{x}) \cdot P_2(\mathbf{x})\, \mathrm{d}\mathbf{x}.$$

C_P can be evaluated for all reasonable heavy-atom distributions. Each different set of trial sites corresponds to a different P_2 Patterson. It is then easily shown that

$$C_P = \sum_{\mathbf{h}} \Delta_{\mathbf{h}}^2 E_{\mathbf{h}}^2,$$

where the sum is taken over all $\mathbf{h}$ reflections in reciprocal space, $\Delta_{\mathbf{h}}^2$ are the observed differences and $E_{\mathbf{h}}$ are the structure factors of the trial point Patterson. (The symbol E is used here because of its close relation to normalized structure factors.)

Let there be n noncrystallographic asymmetric units within the crystallographic asymmetric unit and m crystallographic asymmetric units within the crystal unit cell. Then there are L symmetry-related heavy-atom sites where $L = nm$. Let the scattering contribution of the ith site have a_i and b_i real and imaginary structure-factor components with respect to an arbitrary origin. Hence, for reflection $\mathbf{h}$

$$E_{\mathbf{h}}^2 \left(\sum_L a_{\mathbf{h}i}\right)^2 + \left(\sum_L b_{\mathbf{h}i}\right)^2 = L + \sum_{i\neq j}^{N}\sum^{N} (a_{\mathbf{h}i}a_{\mathbf{h}j} + b_{\mathbf{h}i}b_{\mathbf{h}j}).$$

Therefore,

$$C_P = \sum_{\mathbf{h}} \Delta_{\mathbf{h}}^2 \left[L + 2 \sum_{i\neq j}\sum (a_{\mathbf{h}i}a_{\mathbf{h}j} + b_{\mathbf{h}i}b_{\mathbf{h}j}) \right].$$

But $\sum_{\mathbf{h}} \Delta_{\mathbf{h}}^2$ must be independent of the number, L, of heavy-atom sites per cell. Thus the criterion can be re-written as

$$C_P' = \sum_{\mathbf{h}} \Delta_{\mathbf{h}}^2 \left[\sum_{i\neq j}\sum (a_{\mathbf{h}i}a_{\mathbf{h}j} + b_{\mathbf{h}i}b_{\mathbf{h}j}) \right]. \qquad (2.3.3.3)$$

More generally, if some sites have already been tentatively determined, and if these sites give rise to the structure-factor components $A_{\mathbf{h}}$ and $B_{\mathbf{h}}$, then

$$E_{\mathbf{h}}^2 = \left(A_{\mathbf{h}} + \sum_N a_{\mathbf{h}i}\right)^2 + \left(B_{\mathbf{h}} + \sum_N b_{\mathbf{h}i}\right)^2. \qquad (2.3.3.4)$$

Following the same procedure as above, it follows that

$$C_P' = \sum_{\mathbf{h}} \Delta_{\mathbf{h}}^2 \left[(A_{\mathbf{h}}a_{\mathbf{h}} + B_{\mathbf{h}}b_{\mathbf{h}}) + \sum_{i\neq j}\sum (a_{\mathbf{h}i}a_{\mathbf{h}j} + b_{\mathbf{h}i}b_{\mathbf{h}j}) \right], \qquad (2.3.3.5)$$

where $a_{\mathbf{h}} = \sum_{i=1}^{L} a_{\mathbf{h}i}$ and $b_{\mathbf{h}} = \sum_{i=1}^{L} b_{\mathbf{h}i}$.

Expression (2.3.3.5) will now be compared with the 'feedback' method (Dickerson *et al.*, 1967, 1968) of verifying heavy-atom sites using SIR phasing. Inspection of Fig. 2.3.3.4 shows that the native phase, α, will be determined as $\alpha = \varphi + \pi$ (φ is the structure-factor phase corresponding to the presumed heavy-atom positions) when $|\mathbf{F}_N| > |\mathbf{F}_H|$ and $\alpha = \varphi$ when $|\mathbf{F}_N| \ll |\mathbf{F}_H|$. Thus, an SIR difference electron density, $\Delta\rho(\mathbf{x})$, can be synthesized by the Fourier summation

$$\begin{aligned}\Delta\rho(\mathbf{x}) &= \frac{1}{V}\sum m(|\mathbf{F}_{NH}| - |\mathbf{F}_N|)\cos(2\pi\mathbf{h}\cdot\mathbf{x} - \varphi_{\mathbf{h}}) \\ &\quad \text{from terms with } \Delta_{\mathbf{h}} = |\mathbf{F}_{NH}| - |\mathbf{F}_N| > 0 \\ &\quad + \frac{1}{V}\sum m(|\mathbf{F}_{NH}| - |\mathbf{F}_N|)\cos(2\pi\mathbf{h}\cdot\mathbf{x} - \varphi_{\mathbf{h}} - \pi) \\ &\quad \text{from terms with } \Delta_{\mathbf{h}} < 0 \\ &= \frac{1}{V}\sum m|\Delta_{\mathbf{h}}|\cos(2\pi\mathbf{h}\cdot\mathbf{x} - \varphi_{\mathbf{h}}),\end{aligned}$$

where m is a figure of merit of the phase reliability (Blow & Crick, 1959; Dickerson *et al.*, 1961). Now,

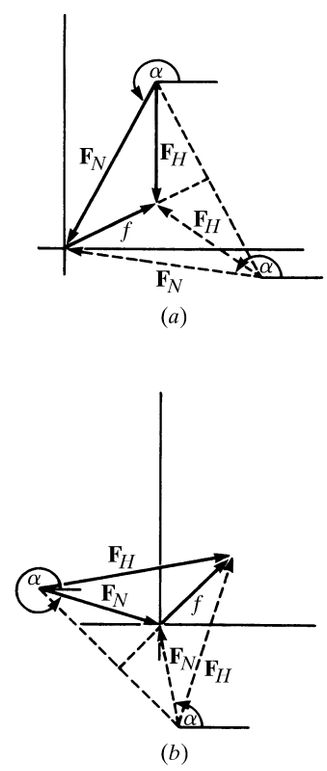

Fig. 2.3.3.4. The phase α of the native compound (structure factor $\mathbf{F}_N$) is determined either as being equal to, or 180° out of phase with, the presumed heavy-atom contribution when only a single isomorphous compound is available. In (*a*) is shown the case when $|\mathbf{F}_N| > |\mathbf{F}_{NH}|$ and $\alpha \simeq \varphi + \pi$. In (*b*) is shown the case when $|\mathbf{F}_N| < |\mathbf{F}_{NH}|$ and $\alpha = \varphi$, where φ is the phase of the heavy-atom structure factor $\mathbf{F}_H$.

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references