$$\mathbf{J} = \begin{bmatrix} \dfrac{\partial F_1}{\partial \rho_1} & \dfrac{\partial F_1}{\partial \rho_2} & \cdots & \dfrac{\partial F_1}{\partial \rho_n} \\ \dfrac{\partial F_2}{\partial \rho_1} & \dfrac{\partial F_2}{\partial \rho_2} & \cdots & \dfrac{\partial F_2}{\partial \rho_n} \\ \vdots & \vdots & \ddots & \vdots \\ \dfrac{\partial F_m}{\partial \rho_1} & \dfrac{\partial F_m}{\partial \rho_2} & \cdots & \dfrac{\partial F_m}{\partial \rho_n} \end{bmatrix}, \tag{15.1.5.5}$$

ε is a vector of residuals to equation (15.1.5.3) for a trial solution, $\rho(\mathbf{x})$, and $\delta\rho(\mathbf{x})$ is a vector of shifts to the density. Hence, the solution for $\rho(\mathbf{x})$ is achieved in an iterative manner,

$$\rho^{i+1}(\mathbf{x}) = \rho^{i}(\mathbf{x}) + \delta\rho(\mathbf{x}). \tag{15.1.5.6}$$

Therefore, the problem of solving a system of nonlinear equations (15.1.5.3) is transformed into solving a system of linear equations (15.1.5.4), which forms one cycle of Newton–Raphson iteration.

If there are more equations than unknowns ($m > n$), the unknowns are obtained through a least-squares solution to equations (15.1.5.4),

$$\mathbf{J}^T\mathbf{J}\delta\rho(\mathbf{x}) = -\mathbf{J}^T\varepsilon. \tag{15.1.5.7}$$

Theoretically, the above system of equations could be solved by matrix multiplication and inversion, *i.e.*

$$\delta\rho(\mathbf{x}) = -\left(\mathbf{J}^T\mathbf{J}\right)^{-1}\mathbf{J}^T\varepsilon. \tag{15.1.5.8}$$

However, the amount of calculation involved in setting up the normal matrix of least squares is huge for the problem presented by protein structures. This can be completely avoided by using the conjugate-gradient technique for solving the system of linear equations.

15.1.5.2.1. *The conjugate-gradient method*

The conjugate-gradient method does not require the inversion of the normal matrix, and therefore the solution to a large system of linear equations can be achieved very quickly.

Starting from a trial solution to equations (15.1.5.4), such as a null vector,

$$\delta\rho_0(\mathbf{x}) = \mathbf{0}, \tag{15.1.5.9}$$

the initial residual is

$$\mathbf{r}_0 = -\mathbf{J}^T(\varepsilon - \mathbf{J}\delta\rho_0(\mathbf{x})) \tag{15.1.5.10}$$

and the initial search step is

$$\mathbf{p}_0 = \mathbf{r}_0. \tag{15.1.5.11}$$

The iterative process is as follows. The new shift to the density is

$$\delta\rho_{k+1}(\mathbf{x}) = \delta\rho_k(\mathbf{x}) + \alpha_k\mathbf{p}_k, \tag{15.1.5.12}$$

where

$$\alpha_k = \mathbf{r}_k^T\mathbf{p}_k/\mathbf{q}_k^T\mathbf{q}_k \tag{15.1.5.13}$$

and

$$\mathbf{q}_k = \mathbf{J}\mathbf{p}_k. \tag{15.1.5.14}$$

The new residual is

$$\mathbf{r}_{k+1} = \mathbf{r}_k - \alpha_k\mathbf{s}_k, \tag{15.1.5.15}$$

where

$$\mathbf{s}_k = \mathbf{J}^T\mathbf{q}_k. \tag{15.1.5.16}$$

The next search step which conjugates with the residual is

$$\mathbf{p}_{k+1} = \mathbf{r}_{k+1} + \beta_k\mathbf{p}_k, \tag{15.1.5.17}$$

where

$$\beta_k = -\mathbf{r}_{k+1}^T\mathbf{s}_k/\mathbf{q}_k^T\mathbf{q}_k. \tag{15.1.5.18}$$

The process is iterated by increasing k until convergence is reached, when

$$|\mathbf{r}_{k+1} - \mathbf{r}_k| \Rightarrow 0.$$

The number of iterations required for an exact solution is equal to the number of unknowns, because the search vector at each step is orthogonal with all the previous steps. However, a very satisfactory solution can normally be reached after very few iterations. This makes the conjugate-gradient method a very efficient and fast procedure for solving a system of equations. Note that the normal matrix never appears explicitly, although it is implicit in (15.1.5.10) and (15.1.5.16). The inversion of the normal matrix and matrix multiplication is completely avoided. Most of the calculation comes from the formation of the matrix-vector products in (15.1.5.10), (15.1.5.14), and (15.1.5.16). These can be expressed as convolutions and can be performed using FFTs, thus saving considerably more time.

The solution to $\delta\rho(\mathbf{x})$ at the end of conjugate-gradient iteration is substituted into equation (15.1.5.6) to get a new solution for $\rho(\mathbf{x})$. The solution to the system of nonlinear equations (15.1.5.3) is obtained when the Newton–Raphson iteration has reached convergence.

15.1.5.2.2. *The full-matrix solution*

The equations to be solved for the electron-density shifts, $\delta\rho(\mathbf{x})$, are from the Jacobian of equation (15.1.5.2),

$$\begin{cases} (2V/N)\sum\limits_{\mathbf{y}}\rho(\mathbf{y})\psi(\mathbf{x}-\mathbf{y}) - \delta\rho(\mathbf{x}) = \Delta\rho(\mathbf{x}) \\ \delta\rho(\mathbf{x}) = \Delta H(\mathbf{x}) \end{cases}, \tag{15.1.5.19}$$

where $\Delta\rho(\mathbf{x})$ is the residual to Sayre's equation,

$$\Delta\rho(\mathbf{x}) = \rho(\mathbf{x}) - (V/N)\sum_{\mathbf{y}}\rho^2(\mathbf{y})\psi(\mathbf{x}-\mathbf{y}), \tag{15.1.5.20}$$

and $\Delta H(\mathbf{x})$ is the residual to the linear density-modification equations,

$$\Delta H(\mathbf{x}) = H(\mathbf{x}) - \rho(\mathbf{x}). \tag{15.1.5.21}$$

Starting from a trial solution of $\delta\rho_0(\mathbf{x}) = \mathbf{0}$, the initial residual vector is

$$\mathbf{r}_0(\mathbf{x}) = (2/V)\rho(\mathbf{x})\sum_{\mathbf{h}}\theta(\overline{\mathbf{h}})\Delta F(\mathbf{h})\exp(-2\pi i\mathbf{h}\mathbf{x}) - \Delta\rho(\mathbf{x}) + \Delta H(\mathbf{x}), \tag{15.1.5.22}$$

where

$$\Delta F(\mathbf{h}) = F(\mathbf{h}) - \theta(\mathbf{h})G(\mathbf{h}), \tag{15.1.5.23}$$

$$G(\mathbf{h}) = (V/N)\sum_{\mathbf{y}}\rho^2(\mathbf{y})\exp(2\pi i\mathbf{h}\mathbf{y}) \tag{15.1.5.24}$$

and

$$\Delta\rho(\mathbf{x}) = (1/V)\sum_{\mathbf{h}}\Delta F(\mathbf{h})\exp(-2\pi i\mathbf{h}\mathbf{x}). \tag{15.1.5.25}$$

Thus, only three FFTs are required to calculate the initial residual. The residual of Sayre's equation is given in equation (15.1.5.23).

The calculation of $\mathbf{q}_k$ in equation (15.1.5.14) is achieved in a similar manner using FFTs,

$$\mathbf{q}_k = \mathbf{J}\mathbf{p}_k = \left\{\frac{(1/V)\sum_{\mathbf{h}}[2a(\mathbf{h})\theta(\mathbf{h}) - b(\mathbf{h})]\exp(-2\pi i\mathbf{hx})}{p_k(\mathbf{x})}\right\} = \left[\frac{Q_k(\mathbf{x})}{p_k(\mathbf{x})}\right], \quad (15.1.5.26)$$

where the vector is partitioned as shown above, and

$$a(\mathbf{h}) = (V/N)\sum_{\mathbf{y}}\rho(\mathbf{y})p_k(\mathbf{y})\exp(2\pi i\mathbf{hy}), \quad (15.1.5.27)$$

$$b(\mathbf{h}) = (V/N)\sum_{\mathbf{y}} p_k(\mathbf{y})\exp(2\pi i\mathbf{hy}). \quad (15.1.5.28)$$

Similarly, vector $\mathbf{s}_k$ in equation (15.1.5.16) is obtained from

$$\mathbf{s}_k = \mathbf{J}^T\mathbf{q}_k = (2/V)\rho(\mathbf{x})\sum_{\mathbf{h}}\theta(\overline{\mathbf{h}})[2a(\mathbf{h})\theta(\mathbf{h}) - b(\mathbf{h})]\exp(-2\pi i\mathbf{hx}) - Q_k(\mathbf{x}) + p_k(\mathbf{x}), \quad (15.1.5.29)$$

where $Q_k(\mathbf{x})$ is defined in equation (15.1.5.26).

The remaining calculations in equations (15.1.5.12), (15.1.5.13), (15.1.5.15), (15.1.5.17) and (15.1.5.18) require either the inner product of a pair of vectors or a linear combination of vectors, both of which are very quick to calculate. Each iteration of the conjugate gradient requires four FFTs, as described in equations (15.1.5.26–15.1.5.29).

15.1.5.2.3. *The diagonal approximation*

The full-matrix solution to equation (15.1.5.4) requires a significant amount of computing, although it can be achieved using FFTs. The diagonal approximation to the normal matrix has been used as an alternative method of solution to the electron-density shift in equation (15.1.5.4) (Main, 1990*b*). As with the full-matrix calculation, it can be done entirely by FFTs and a linear combination of vectors.

The diagonal element of the normal matrix, $\mathbf{J}^T\mathbf{J}$, in equation (15.1.5.7) is

$$d_0(\mathbf{x}) = (4/N)\rho(\mathbf{x})\left[\rho(\mathbf{x})\sum_{\mathbf{h}}|\theta(\mathbf{h})|^2 - \sum_{\mathbf{h}}\theta(\mathbf{h})\right] + 2. \quad (15.1.5.30)$$

The right-hand side of equation (15.1.5.7), $-\mathbf{J}^T\varepsilon(\mathbf{x})$, is identical to the residual vector, $r_0(\mathbf{x})$, which can be calculated from equation (15.1.5.22). Therefore, the solution to the electron-density shift, $\delta\rho(\mathbf{x})$, can be calculated from

$$\delta\rho(\mathbf{x}) = r_0(\mathbf{x})/d_0(\mathbf{x}). \quad (15.1.5.31)$$

Compared with the full-matrix solution, all the calculations involved in between equations (15.1.5.12) and (15.1.5.18) and the subsequent iterations are spared in the diagonal approximation. This makes calculation by the diagonal approximation much faster than by the full-matrix method.

15.1.6. Example

To demonstrate the effect of different constraints on phase improvement, various density-modification techniques were applied to an MIR data set for which the refined structure coordinates are available. The test structure is 5-carboxymethyl-2-hydroxymuconate isomerase, solved by Wigley *et al.* (1989). MIR phases were available to 3.7 Å, with SIR information to 2.6 Å. Density modification was used to improve and extend phases to the limit of the data at 2.1 Å. The structure includes threefold noncrystallographic symmetry.

The MIR and density-modified phases are compared by plotting the mean of the cosine of the phase error, weighted by the figure of merit and structure-factor amplitude, as a function of resolution (Zhang *et al.*, 1997),

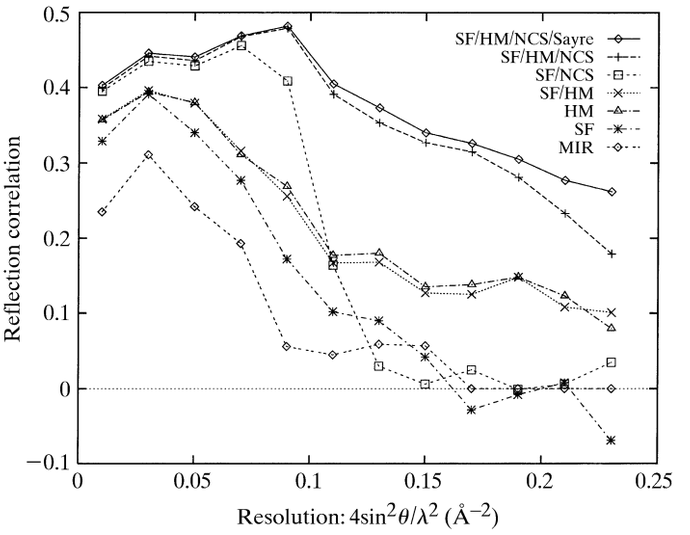

Fig. 15.1.6.1. Phase correlations after different combinations of density modifications.

$$C_f = \left\langle w|F|^2\cos(\varphi - \varphi_0)\right\rangle \Big/ \left(\left\langle w^2|F|^2\right\rangle\left\langle|F|^2\right\rangle\right)^{1/2}. \quad (15.1.6.1)$$

This phase correlation over all reflections is equivalent to map correlation. The results of density modification by various techniques, using the reflection-omit method for phase combination, are shown in Fig. 15.1.6.1.

Solvent flattening alone has slightly improved the phases at low resolution but has not lead to significant phase extension. The solvent-flattening function in Fig. 15.1.3.1 only has nonzero amplitudes close to the origin. It relates structure factors only in a very thin resolution shell. Therefore, solvent flattening is weak on phase extension.

Histogram matching alone improves the low-resolution phases and gives significant phase extension to higher resolutions. The histogram-matching function in Fig. 15.1.3.1 showed much stronger high-resolution amplitudes. Therefore, it could relate structure factors in a larger resolution shell. Moreover, there is always an ideal histogram specified at a given target resolution for phase extension. These two reasons combined make histogram matching a more powerful technique in phase extension than solvent flattening.

The combination of histogram matching and solvent flattening is slightly more powerful than histogram matching alone; since histogram matching sharpens the protein density, it implies an element of solvent flattening. Solvent flattening and averaging give a significant improvement at low resolution, but little phase extension. Averaging is powerful for phase refinement, but is weak for phase extension if no special precautions are taken. If there are flexible loop regions on the protein surface, these regions should be excluded from the molecular mask for averaging. The phasing power of averaging weakens at high resolution when the differences between NCS-related molecules become significant. Solvent flattening, histogram matching and averaging combined give a dramatic improvement at all resolutions. The addition of Sayre's equation gives a slight further improvement at high resolution.

Sayre's equation is very effective for phase refinement and extension at atomic or near atomic resolution. It becomes ineffective at low resolution or when the initial map is poor. Under these circumstances, it is better to apply other density-modification methods first to refine the phases and extend them to a higher resolution before Sayre's equation is applied. Sayre's

references