Water molecules and the incoherent nuclei within the crystal will change their distribution during a period of three to four years as a result of seasonal changes embodying a temperature variation of about 30°C. The argument advanced by Agrawal (1974) that paracrystallinity is shown only by fibrous crystals is incorrect in view of the fact that non-fibrous crystals have also been found to exhibit paracrystalline distortions (Urban & Hosemann, 1972). It can also be added that based on paracrystalline disorder the appearance of rounded and polygonal and other arbitrarily shaped spots can be easily explained, as indicated in our paper, by taking the fluctuations of a more involved type than that described in the said paper (Tiwari, Prasad & Srivastava, 1973).

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Precision lattice constant determination: erratum. By W. L. BOND, *W. W. Hansen Laboratories of Physics, Stanford University, Stanford, California, U.S.A.*

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A corrected version is given of equation (10) in Bond [*Acta Cryst.* (1960). **13**, 814–818].

In the paper by Bond (1960), equation (10) should read

$$x=(\tfrac{1}{2}w)^2 \cot 2\theta\{(2+\sin^2 2\theta)/(2-\sin^2 2\theta)\}$$

where x and w are in radians.

This value should be added to the apparent Bragg angle. For $\theta > 45°$, $\cot 2\theta$ is negative, so the correction is to be subtracted.

Reference

BOND, W. L. (1960). *Acta Cryst.* **13**, 814–818.

Acta Cryst. (1975). A**31**, 698

Preliminary refinement of protein coordinates in real space. By R. J. FLETTERICK* and H. W. WYCKOFF, *Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut* 06520, *U.S.A.*

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A simple unconstrained steepest-descent procedure is described for the preliminary refinement of protein coordinates in real space. The method is illustrated by application to ribonuclease S.

The typical procedure used to determine the atomic coordinates of proteins from single-crystal X-ray diffraction analysis makes use of the optical comparator described by Richards (1968) to build a skeletal model of the protein into the electron density map. The positions of the component atoms are then carefully measured from this model and transformed into a suitable reference frame for comparison with the Fourier map. The atomic coordinates are thus subject to systematic errors at each stage in this procedure. These errors might, for example, arise from a misplaced reference origin, rotational shifts, shears and non-orthogonality of the reference axes. The random errors related to the difficulty in fitting the skeletal model in the poorly defined regions of the electron density map and the exact disposition of the bond angles and dihedral angles within the skeletal model are exceedingly difficult problems and will not be considered here, as they have been treated by Diamond (1971).

The systematic errors are, however, amenable to treatment by a simple unconstrained steepest-descent procedure which is outlined below. The results of this analysis are presented for ribonuclease S.

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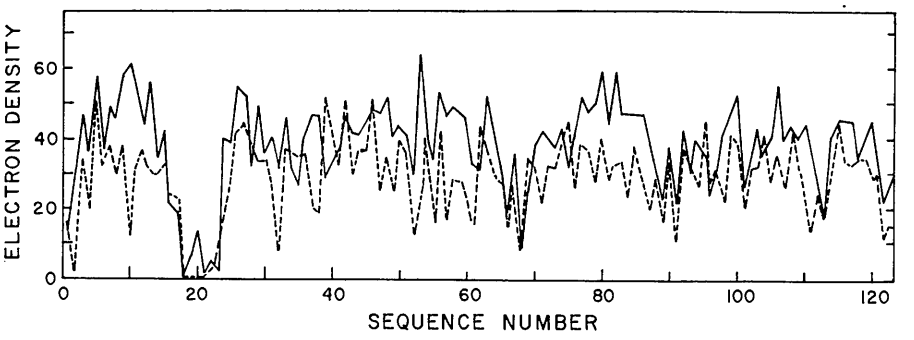

Fig. 1. A comparison of the summed electron density for the ribonuclease S polypeptide backbone and C_β atoms before (dashed line) and after (solid line) two refinement cycles. The abscissa is the sequence number for the chain. The electron density scale is arbitrary.

It is assumed that the starting coordinate set has been carefully measured and is expressed in the same reference frame as the electron density map. Let ϱ_T represent the total sum of the electron density recorded at each of the identified atomic positions, $\mathbf{X}_i$, for N atoms and define the set $\mathbf{X}_i'$ such that ϱ_T is a maximum: then

$$\varrho_T(\max)=\sum_{i=1}^{N}\varrho(\mathbf{X}_i')\,. \qquad (1)$$

We assume that the two coordinate sets $\mathbf{X}_i$ and $\mathbf{X}_i'$ are related by the non-singular linear inhomogeneous transformation

$$\begin{pmatrix}X'\\Y'\\Z'\end{pmatrix}_i=\begin{pmatrix}a_{11}&a_{21}&a_{31}\\a_{12}&a_{22}&a_{32}\\a_{13}&a_{23}&a_{33}\\b_1&b_2&b_3\end{pmatrix}^T\begin{pmatrix}X\\Y\\Z\\1\end{pmatrix}_i \quad (i=1,N)\,. \qquad (2)$$

The 4×3 matrix in equation (2) is composed of a general translation vector **b** coupled with the 3×3 matrix **A** which can be considered to represent simultaneously corrections for general rotation, shears, expansions and non-orthogonal reference axes for the N atomic positions.

Equation (2) can be solved for the a_{ij} and b_i by determining the left-hand side using the method of steepest descent. The usual difficulties encountered with this method are of no concern here owing to the favorable shape of the electron density distribution near the approximately correct atomic positions. The steepest-descent method requires calculation of the gradient of the electron density, ϱ, at each $\mathbf{X}_i$; this is easily computed from the Fourier map by re-evaluating the electron density for each of the atomic positions at the point $\mathbf{X}_i+\Delta\mathbf{X}$. Thus, in an orthogonal grid system, the gradient of ϱ is

$$\nabla\varrho(\mathbf{X}_i)=\frac{\Delta\varrho(X_i)}{\Delta X_i}\mathbf{i}+\frac{\Delta\varrho(Y_i)}{\Delta Y_i}\mathbf{j}+\frac{\Delta\varrho(Z_i)}{\Delta Z_i}\mathbf{k}\,. \qquad (3)$$

For $N/12\gg 1$ rather large random errors can be accepted in evaluating the gradient. The electron density at the Fourier grid point nearest the atomic position could be successfully used in order to avoid the computational expense required for interpolation between grid points.

The coordinate set $\{\mathbf{U}_i\}$ is defined by shifting each member of the set $\{\mathbf{X}_i\}$ along the direction of the gradient by an amount proportional to $|\nabla\varrho(\mathbf{X}_i)|$,

$$\mathbf{U}_i=\mathbf{X}_i+C\nabla\varrho(\mathbf{X}_i) \qquad (4)$$

where the quantity C is a constant which determines the absolute magnitude of a_{ij} and b_i. Now the set $\{\mathbf{U}_i\}$, aside from the random local errors, maximizes ϱ_T. It is not difficult to show therefore that the least-squares best solution of (2) is given by

$$\begin{pmatrix}a_{11}&a_{21}&a_{31}\\a_{12}&a_{22}&a_{32}\\a_{13}&a_{23}&a_{33}\\b_1&b_2&b_3\end{pmatrix}=(\mathbf{M}^T\mathbf{M})^{-1}\mathbf{M}^T\begin{pmatrix}U_1&V_1&W_1\\U_2&V_2&W_2\\.&.&.\\.&.&.\\U_N&V_N&W_N\end{pmatrix} \qquad (5)$$

where

$$\mathbf{M}=\begin{pmatrix}X_1&Y_1&Z_1&1\\X_2&Y_2&Z_2&1\\.&.&.&.\\.&.&.&.\\X_N&Y_N&Z_N&1\end{pmatrix}.$$

Application to ribonuclease S

The coordinates for ribonuclease S (Wyckoff, Tsernoglou, Hanson, Knox, Lee & Richards, 1970) were re-read after some readjustment of the peptide chain to improve the fit with the electron density map. Equation (5) was used to cast these coordinates into the same reference frame as that used in the above reference. 50 of the C_α atomic positions which were calculated to have been displaced the least, $<0{\cdot}4$ Å, were used to compute the transformation matrix.

Shifts of 0·4 Å in each of the coordinate components were used in equation (3) to calculate the gradient at 567 atomic centers; these included all the atoms in the polypeptide backbone and C_β except for residues 1, 32, 62, 124 and 18–23. The value of the constant C was arbitrarily set at 0·25; the transformation matrix in (5) corresponding to this value of C is

$$\begin{pmatrix}1{\cdot}005&0{\cdot}004&0{\cdot}004\\0{\cdot}005&1{\cdot}004&0{\cdot}006\\-0{\cdot}011&-0{\cdot}005&1{\cdot}005\\0{\cdot}060&-0{\cdot}274&0{\cdot}129\end{pmatrix}.$$

This matrix applied to the entire coordinate set produced an average shift of 0·44 Å; the origin of most of this shift is obviously in the translation element b_2. It is interesting to note that this element corresponds to the direction orthogonal to the Fourier map sections. A second cycle gave an average shift of 0·191 Å.

Fig. 1 shows a comparison of the average electron density summed for the backbone atoms and C_β plotted against the sequence numbers before and after the two refinement cycles. There is an obvious improvement in continuity of the electron density along the polypeptide backbone; in addition, the average electron density at each atomic site increased 32% for the backbone atoms and 22% for the side-chain atoms. In spite of the fact that no stereochemical constraints were applied, the bond lengths and bond angles remained relatively unchanged under the above transformation; the average values of the C_α–C′, C′–O and C_α–N bonds are 1·536, 1·218 and 1·466 Å respectively.

The coordinates produced by this procedure have been listed by Richards & Wyckoff (1973) and used by them to prepare full drawings of the structure. They are also on file in the Protein Data Bank, designated set 6D (T. F. Koetzle, Department of Chemistry, Brookhaven National Laboratory, Upton, New York 11973).

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Single-crystal diffractometer data: economizing in background counting times.

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A method is described for economizing the time spent in background counting during a single-crystal data collection with a computer-controlled diffractometer. The percentage time saved for a reflexion depends on the peak-to-background ratio whereas the fraction of peak counting time spent on the background depends, in addition, on the background level and on the precision required for the reflexion intensity.

It is possible to reduce the total time spent measuring single-crystal intensity data, without sacrificing precision, by economizing on the background counting time. The procedure enables the time allocated to peak and background measurements to be varied so that for a given reflexion the gain in precision obtained by a small increase in peak measurement time off-sets the loss in precision suffered by a larger decrease in the background measurement time. The percentage time saved on a reflexion depends on the peak-to-background ratio. The method is equally applicable to scan-type or peak-height measurements.

It has been shown (Grant, 1973) that single-crystal intensity data can be collected to a prestated counting statistics precision p' on a computer-controlled single-crystal diffractometer. Before each reflexion is measured fully, it is first measured for a short trial time, with equal times q on the peak and background to give counts of I_q and B_q respectively. To give the required precision the total counting times for both peak and background should be $t'=f'q$ where

$$f'=\frac{I_q+B_q}{p'^2(I_q-B_q)^2}\ . \qquad (1)$$

If both peak and background are measured for further times $(f'-1)q$ then the required precision would be obtained and the total time spent on this reflexion would be $2f'q$.

If the diffractometer uses a step-scan technique then the peak is measured for the further time by a step scan in which the time per step is increased by the factor $(f-1)$ over that for the trial step scan. If a continuous scan technique is used then repeated fast scans of the peak for a total time $(f-1)q$ are made, if a fast scan of time q is used for the trial measurement.

It is possible to economize on the time spent on a reflexion, particularly if the peak-to-background ratio is large, by measuring the background for a shorter time than that for the peak without reducing the overall precision of the measurement. If, after the trial time q, the time $t=fq$ is calculated for a precision p better than p', then

$$f=\frac{I_q+B_q}{p^2(I_q-B_q)^2} \qquad (2)$$

and if the peak is measured for a further time $(f-1)q$, the background can be measured for a shorter time without changing the overall precision from p' and peak time will be increased slightly. Under this arrangement the total peak counts expected will be $I=fI_q$ and the total background counts expected will be $B=B_q+(f-1/n)B_q$, where the background subsequent to the trial time is measured for one nth of the remaining peak time.

The precision p' of such a measurement is

$$\frac{\sigma(N)}{N}=\frac{(I+r^2B)^{1/2}}{I-rB}=\frac{(I_q+rB_q)^{1/2}}{f^{1/2}(I_q-B_q)}\ ,$$

where the background is measured for one rth of the total peak time, *i.e.*

$$r=\frac{f}{\left[1+\dfrac{(f-1)}{n}\right]}\ . \qquad (3)$$

Let $p'/p=y$ where $y>1$; then

$$y=\frac{(I_q+rB_q)^{1/2}}{f^{1/2}(I_q-B_q)}\cdot\frac{f^{1/2}(I_q-B_q)}{(I_q+B_q)^{1/2}}=\frac{(I_q+rB_q)^{1/2}}{(I_q+B_q)^{1/2}}\ .$$

Writing this in terms of the peak-to-background ratio $x(=I_q/B_q)$,

$$y=\left(\frac{x+r}{x+1}\right)^{1/2}$$

or

$$r=y^2(x+1)-x\ . \qquad (4)$$