qui est le plus général, les directions des axes de rotation de P et P' sont à 30° les unes des autres.

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The Minimization of Errors in the Molecular Replacement Structure Solution; the Effect of the Errors on the Least-Squares Refinement Progress

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Abstract

The molecular replacement method (MR) is likely to cause significant systematic errors in the analysed structure. These errors are not always corrected automatically during least-squares refinement. It is therefore crucial that both the orientation and translation parameters are optimized before refinement is undertaken. The techniques used to achieve this depend either on the rigid-body refinement concept or on a six-dimensional R-factor minimization and all are expensive and time consuming. A simple procedure to refine the molecular replacement structure solution parameters is shown. It uses the existing restrained least-squares method, requires no new software, and is shown to be very effective.

Introduction

The molecular replacement method (MR) has recently become an increasingly popular alternative to the multiple isomorphous replacement method (MIR) of solving macromolecular crystal structures. Both methods yield model structures which may be subsequently refined using various crystallographic least-squares procedures. However, the nature of errors introduced with each of the models may be different.

The atomic coordinates derived from the MIR electron density map will suffer mainly from random errors with serious faults confined to less well resolved (and in most cases external) residues with comparatively high temperature factors. During the refinement of actinidin (Baker & Dodson, 1980), starting with a MIR model, the root mean square (r.m.s.) difference between the starting and final sets of atomic coordinates was 0·5 Å. Considering the theoretical radius of convergence which is about half a bond length (0·6 Å), one can expect such models to refine successfully, although manual interaction during refinement is still essential if standard crystallographic

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least-squares refinement techniques are used. It has been shown recently that molecular dynamics calculations with incorporated crystallographic refinement can overcome even more substantial barriers and effectively provide a fully refined structure from an automatic run (Brünger, Kurijan & Karplus, 1987).

In the case of model structures obtained through MR calculations even small errors in the rotation and translation parameters may lead to systematic shifts of large parts of the structure relative to true positions. Such a situation may cause severe problems during least-squares refinement. In this paper I present a simple technique for the refinement of the rotation and translation parameters of the starting model obtained from the MR calculations; this approach may also serve as a useful alternative to the time-consuming procedure of rigid-body refinement (Sussman, Holbrook, Church & Kim, 1977; Huber & Schneider, 1985), or stepwise variation of group parameters and calculation of crystallographic R values (Bode, Fehlhammer & Huber, 1976; Grau, Rossmann & Trommer, 1981).

1. The semioxyhaemoglobin structure

The structure of human deoxyhaemoglobin (Hb) crystals (space group $P2_12_12_1$, $a=95{\cdot}78$, $b=97{\cdot}78$, $c=65{\cdot}49$ Å) grown from solutions of poly(ethylene glycol) (PEG) of molecular weight 6000 was initially solved using the MR method by Ward, Wishner, Lattman & Love (1975) at 3·5 Å resolution. Two Hb crystal forms from this solvent were subsequently analysed at 3·5 Å resolution using a more elaborate approach (Derewenda, Dodson, Dodson & Brzozowski, 1981). When 2·1 Å resolution synchrotron data were collected at LURE (Paris) on this form the results of previous calculations were used to position the model of human Hb (Fermi, 1975) in the unit cell of the PEG crystal. The structure was then refined by a variety of techniques (Agarwal, 1978; Dodson, Isaacs & Rollet, 1976; Hendrickson & Konnert, 1979; Jack & Levitt, 1978). Considerable time was spent on manual rebuilding of the model to fit consecutive electron density maps. The structure has been proved to be that of semioxygenated haemoglobin {Hb[$(O_2)_2$]} (Brzozowski *et al.*, 1984). The model has since been refined; the conventional crystallographic R factor is 0·205.

2. The accuracy of MR structure solution parameters in semioxyhaemoglobin

Once a structure is fully refined, it is easy to check the accuracy of the initial rotation and translation parameters (established by the MR calculations) by simple least-squares matching of the search model on the final structure. Any discrepancies between the values of the rotation and translation parameters obtained in this way and those established by MR calculations may be taken as a measure of errors in the MR solution. For clarity I shall henceforth refer to the model obtained by MR calculations as the MR model, and to the model obtained by least-squares matching as the LSQ model. The two differ only in respect to their positions in the unit cell. The LSQ model illustrates the best possible (in terms of the least-squares) solution of the MR technique.

Table 1. *Comparison of the rotation (Eulerian α, β and γ) and translation ($\mathbf{t}_x$, $\mathbf{t}_y$, $\mathbf{t}_z$) parameters obtained from MR calculations (Derewenda et al., 1981) and a posteriori by least-squares fitting of the 2·5 Å resolution model of Fermi (1975) onto the final structure using main-chain atoms*

	MR	LSQ	Error
	$\alpha\beta_1$ dimer		
α (°)	66·00	67·10	−1·10
β (°)	−124·00	−123·07	−0·30
γ (°)	161·00	163·10	−2·10
$\mathbf{t}_x$ (Å)	4·12	3·44	0·68
$\mathbf{t}_y$ (Å)	1·25	1·32	−0·07
$\mathbf{t}_z$ (Å)	15·22	15·42	−0·20
	$\alpha_2\beta_2$ dimer		
α (°)	68·00	67·30	0·70
β (°)	56·00	56·40	−4·40
γ (°)	−164·00	−165·30	1·30
$\mathbf{t}_x$ (Å)	3·59	3·47	0·12
$\mathbf{t}_y$ (Å)	1·39	1·46	−0·07
$\mathbf{t}_z$ (Å)	15·60	15·45	0·15

Table 1 compares the rotation and translation parameters of the MR and LSQ models of Hb[$(O_2)_2$] in PEG crystals. As in the initial MR calculations, the dimers were treated separately. It is clear that the MR method gave a fairly accurate solution, with orientation errors generally within 1·5 Å [except for γ of dimer(1)] and translation parameters correct to 0·2 Å [except for $\mathbf{t}_x$ of dimer(1)].

Even though errors in rotation and translation parameters provide a useful measure of the accuracy of the MR solution, subsequent least-squares refinement depends on the actual distances of the model atomic positions from the true ones. The r.m.s. difference between the atomic coordinates of the MR model and the final refined structure of Hb[$(O_2)_2$] is 0·73 Å for the $\alpha_1\beta_1$ dimer and 0·83 Å for $\alpha_2\beta_2$. The LSQ model yields significantly lower values of 0·43 and 0·46 Å respectively. The latter figures represent the differences resulting from different crystal packing, inaccuracies in the search model (refined at lower resolution by real-space technique) and conformational changes induced by ligation. The former higher r.m.s. values incorporate discrepancies resulting from rotation and translation function errors.

Fig. 1 shows a comparison of mean residue differences between the atomic positions of each of the models and the final atomic positions established following the crystallographic refinement (only the

α_1 chain is shown). As was expected the errors introduced with the MR model affect mostly those parts which differ in the LSQ and MR models, and exceed the error of the LSQ model on average by a factor of two.

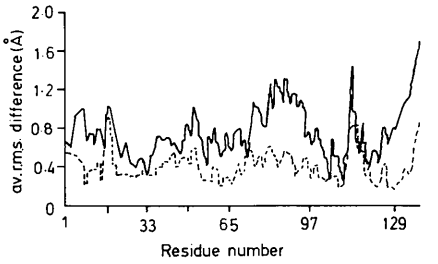

Fig. 1. Mean-residue r.m.s. difference between the MR model and the refined final structure (solid line), and between the LSQ model and the refined structure (dashed line).

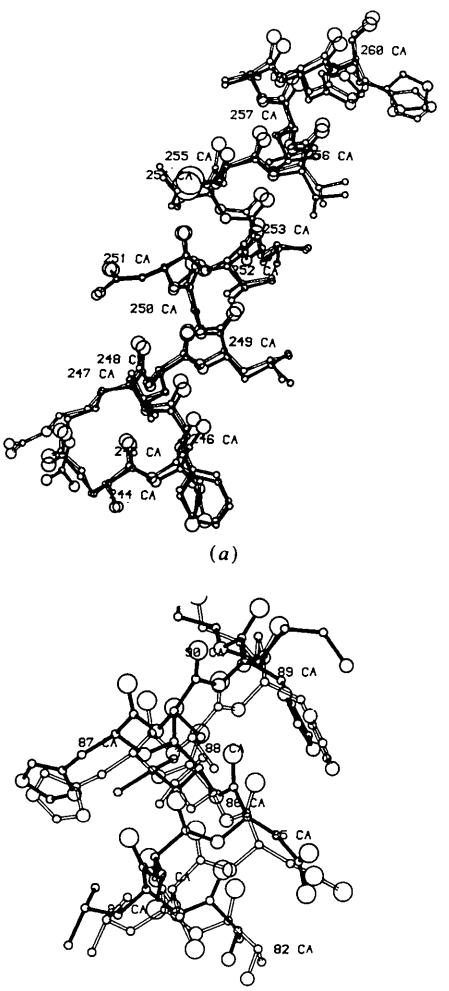

Fig. 2. A comparison of the MR model with the final structure: (*a*) the β_1 *G* helix, and (*b*) the α_1 *F* helix; the open bonds and atoms represent the MR structure.

The direction of the displacement is also an important factor in refinement progress. For example, if a helix is rotated and translated by half a bond length, the refinement is less likely to cope with the distortion than in the case of a displacement perpendicular to the helix axis. Fig. 2 shows two fragments of the $Hb[(O_2)_2]$ molecule and compares them with an analogous fragment from the MR model. It will be shown that a displacement of the nature shown for the *F* helix of the α_1 chain (Fig. 2*b*) can be a source of extreme difficulties in the course of refinement.

3. The effects of MR solution errors on the course of least-squares refinement

The efficiency of the least-squares refinement depends upon the quality of the phase-angle estimates obtained from the initial set of coordinates. If their values are close to the correct ones, the difference Fourier synthesis should reveal well defined gradients and accordingly convergence should be fast. Fig. 3 shows the quality of the $2F_o - F_c$ maps calculated using the LSQ and MR models to obtain the values of F_c and α_c. The detail shown is the *F* helix of the α_1 chain.

Fig. 3 shows that the MR model is (at least in the fragment shown) sufficiently displaced to disturb the phases in such a way that the map reveals significant 'ghosting', *i.e.* reappearance of the initial erroneous model and the resulting electron density spreads over the model and the true positions. The LSQ model is basically free from these faults; the density is sharper and the true positions are more clearly visible.

In order to determine the influence of the starting set of coordinates on the course of the refinement, both models were subjected to least-squares restrained refinement by the Konnert–Hendrickson method with incorporated fast Fourier transform. The

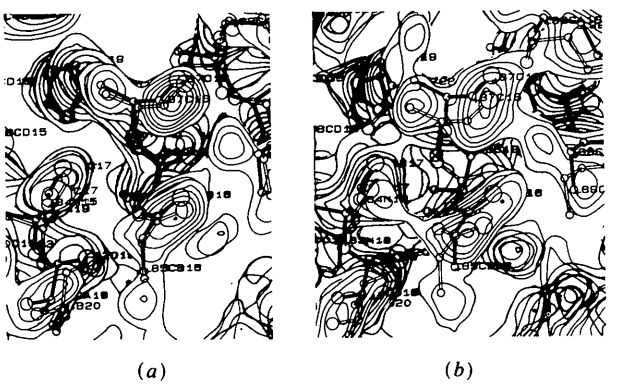

Fig. 3. $2F_o - F_c$ Fourier synthesis electron density maps calculated with the values of F_c and phase angles derived from (*a*) the actual MR model, and (*b*) the LSQ model; open bonds and atoms represent model parameters, full bonds and atoms show the refined structure.

refinement of the MR model was repeated because initially it was carried out with other techniques and therefore its progress could not be readily compared with the results obtained from current refinement procedures.

Both models seem to refine satisfactorily. It has to be remembered that ordinarily one would only be dealing with a MR model and would judge the progress of the refinement mainly by the decrease in the value of the R factor and by the overall stereochemistry of the refined model. The MR model converges with an R factor of 0·249 and with the r.m.s. variation on the C–C bonds 0·045 Å. At this point one would normally be satisfied with the progress of automatic refinement. Nonetheless an electron density map calculated at this stage reveals serious problems in certain parts of the structure. This situation was actually encountered during the initial $Hb[(O_2)_2]$ structure refinement. The differences were at the time assumed to result primarily from crystal packing, ligation effects and inaccuracies in the search model. Accordingly all regions exhibiting significant errors were then rebuilt manually.

Fig. 4 shows the effect of the present refinement on residues from the F helix of the α_1 chain. Even though the atomic positions have shifted half way towards the correct positions the refinement converged at this point in a false minimum. This illustrates well that systematic errors in coordinates cannot be readily corrected, although significant improvement is achieved.

Careful monitoring of the progress of the automatic refinement in various parts of the model reveals that there are regions which converge quickly, with small overall shifts and comparatively low individual isotropic temperature factors (B values). An inspection of an electron density map may show parts with well resolved groups of atoms or even individual atoms (depending on the resolution of the diffraction data used) as well as regions of diffuse density where the model atoms are clearly displaced, although there may be no clear indication of how one should correct their positions. Fig. 5 shows the progress of the refinement of B values in the MR and LSQ models and compares them with the final B values from the refined structure. In the case of the MR model the temperature factors exhibit much more pronounced variations even in regions where local secondary structure implies stability, *i.e.* individual helices. In those regions which are known to be in error the temperature factors exhibited a tendency to increase steadily as the refinement proceeded.

4. The refinement of the MR structure solution parameters using restrained least-squares refinement

The observations described in the above paragraph have clearly indicated that whereas the parts of the model structure within the radius of convergence could refine properly and exhibit low final tem-

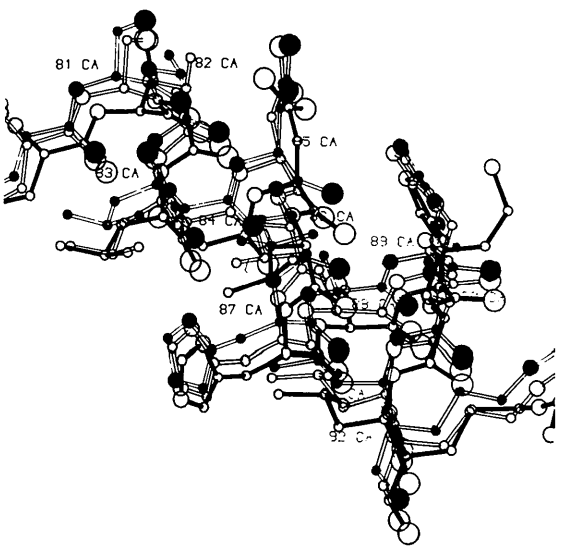

Fig. 4. The progress of least-squares refinement of the MR model in comparison with the final coordinates: open bonds and full atoms – starting (MR) model; open bonds and open atoms – refined MR model; open atoms and full bonds – final coordinates. The detail shown is the α_1 F helix.

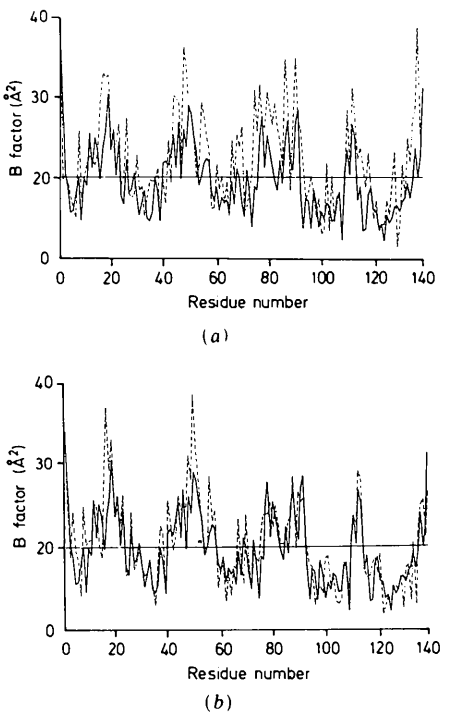

Fig. 5. The progress of the refinement of individual temperature factors (B factors) and a comparison with the final refined values: (*a*) the MR model (*b*) the LSQ model. In both diagrams the line drawn at $B = 20$ Å^2 represents the starting point of the refinement.

Table 2. *The progress of the refinement of the rotational and translational parameters of the haemoglobin dimer model positioned in the PEG semioxyHb unit cell (for details of the calculations see text)*

	0*	1†	2‡
	$\alpha_1\beta_1$ dimer		
α (°)	−1·10	−0·40	0·00
β (°)	−0·30	−0·10	−0·10
γ (°)	−2·10	−1·50	0·00
t_x (Å)	0·68	0·44	0·24
t_y (Å)	−0·07	0·03	0·07
t_z (Å)	−0·20	−0·08	−0·02
	$\alpha_2\beta_2$ dimer		
α (°)	0·70	0·40	0·30
β (°)	−0·40	0·00	−0·30
γ (°)	1·30	0·90	0·40
t_x (Å)	0·12	0·04	0·01
t_y (Å)	−0·07	−0·10	−0·11
t_z (Å)	0·15	0·12	0·05

*Original errors in the MR model.
† Error values after the first round of least-squares fitting.
‡ Error values after the second round of least-squares fitting.

perature factors, others, outside the radius of convergence, will remain in error. In order to use the information from the initial least-squares refinement to improve the overall position of the model, the following procedure was used.

Following the first round of automatic refinement (five cycles of x, y, z and three of B value refinement) of the MR model, 91 atoms with low temperature factors close to the centre of the molecule were selected from each dimer. An analogous set of 91 atoms was taken from the unrefined search model and fitted by least squares onto each refined set of atoms. Any deviation from the rotation and translation parameters obtained by MR calculations was taken as an indication of an overall rotation and/or translation of the entire group. It was assumed that this correction was necessary for the entire molecule. The initial search model of each of the dimers was therefore repositioned in the unit cell according to the new parameters and the refinement was resumed.

In this way, the first initial round of refinement was therefore used only to refine the values of rotation and translation parameters, as a form of rigid-body refinement.

Following the second round of refinement the entire procedure was repeated and new values were again used to reposition the model. Table 2 gives the details of these calculations. It was found that this simple technique brought the model to within 0·4° and 0·2 Å of the correct position as defined by the LSQ model.

5. Applications of the MR refinement method

Since the test calculations were carried out, the technique was used successfully in a number of cases. In this paper I present the details of two applications.

5.1. *The methaemoglobin refinement*

Methaemoglobin crystals obtained by oxidation of the crystals grown from PEG solutions were found to be isomorphous, within experimental error, with deoxyHb crystals. For this reason it was decided to begin the refinement with the set of deoxyHb coordinates as a starting model. The initial R-factor value was 0·46, suggesting considerable structural differences between the two forms. In spite of lengthy refinement using the Konnert–Hendrickson restrained refinement method involving various modifications (like periodic restoration of proper geometry without X-ray terms, truncation of B values to introduce sharper gradients, weakening geometric restraints *etc.*) the refinement seemed to converge with an R value of 0·28. Visual inspection of the electron density map resulted in an addition of 120 water molecules to the 84 taken from the earlier deoxyHb refinement. This had little effect on the progress of the refinement, lowering the R value to only 0·27. Another map was plotted. Fig. 6 shows a part of this map. In only a few cases does the density suggest a better position for an individual side chain or a fragment of the main chain. At this point the procedure described above was applied, even though the isomorphism of the two structures did not make it a classic case of molecular replacement.

A single deoxyHb dimer in the molecular frame was used as the search model. 261 atoms located not further than 15 Å from the origin of the molecular frame were taken from the set of refined MetHb coordinates and least-squares fitted onto an analogous set of atoms from the deoxyHb dimer. The new values of rotation and translation parameters were used to reposition the model of deoxyHb, thus discarding the results of the preceding round of refinement and using only the 'rigid-body' results. The refinement was continued for 16 cycles and the calculation was repeated with the same set of 261

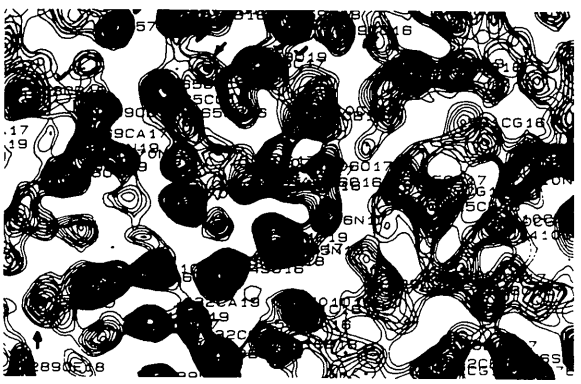

Fig. 6. A fragment of an electron density map (calculated with coefficients $2F_o - F_c$) of methaemoglobin after convergence of least-squares refinement ($R = 0{\cdot}28$); arrows show additional water molecules identified on this map.

Table 3. *The refinement of the translational and rotational parameters of semioxyHb dimers positioned in the PEG MetHb unit cell (details of calculations in the text)*

	Cycle 1	Cycle 2	Cycle 3
	$\alpha_1\beta_1$ dimer		
α (°)	67·10	67·20	67·30
β (°)	−123·70	−123·10	−123·20
γ (°)	163·10	163·30	163·30
t_x (Å)	3·44	2·83	2·71
t_y (Å)	1·32	1·48	1·49
t_z (Å)	15·42	15·35	15·33
	$\alpha_2\beta_2$ dimer		
α (°)	67·30	67·40	67·60
β (°)	56·40	56·60	56·80
γ (°)	−165·30	−165·70	−165·80
t_x (Å)	3·47	3·00	2·86
t_y (Å)	1·46	1·59	1·62
t_z (Å)	15·45	15·45	15·39

atoms. The resulting rotation and translation parameters are shown in Table 3. The first round was sufficient to bring the refinement out of the local minimum; in the second round the final R value fell to 0·211, which compares well with the results for deoxy- and semioxyHb. Table 3 shows that the molecule of MetHb is translated along the crystallographic X axis with respect to deoxyHb by approximately 0·75 Å. Fig. 7(*a*) plots r.m.s. shifts for

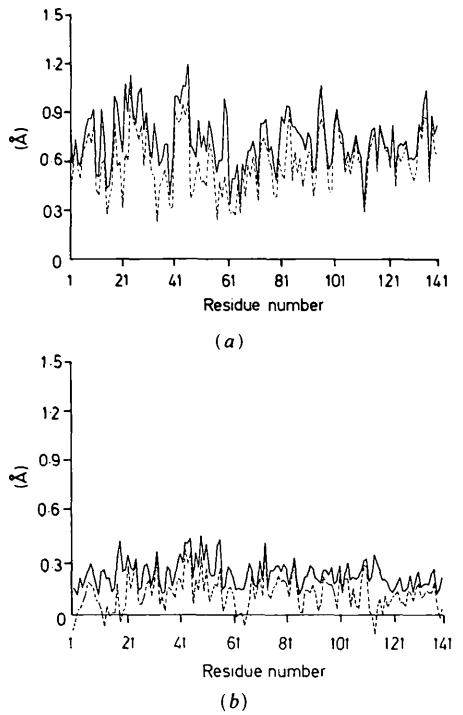

Fig. 7. A comparison of a r.m.s. shift during refinement of methaemoglobin (averaged over each residue) (solid line) with the absolute shift along the X axis (dashed line) (*a*) after the first round of refinement and (*b*) after the last round; for other details see text and Table 3.

main-chain atoms within each residue during the first round of refinement of MetHb and compares them with the mean shift along the crystallographic X axis. Although about 75% of the overall shift resulted from the concerted shift of the entire molecule in the correct direction, it was still insufficient to cope with the systematic error introduced with the starting model. Fig. 7(*b*) shows an analogous calculation for the last round of the refinement. Although a small residual translation along the X axis remains, the errors are in general randomly distributed. Such a situation allows effective least-squares refinement.

It is generally agreed that during the initial stage of refinement it is desirable to use only medium-resolution data, in order to increase the radius of convergence (estimated as 1/3 of the maximum resolution of the data) and therefore force the correction of larger errors first. To test this approach two refinement calculations were performed, in which the deoxyHb model was refined against MetHb data at 3·0 and 3·5 Å resolution. Although the 3·5 Å refinement produced a significant overall shift along the X axis (exceeding shifts along other axes by a factor of three) it was still less than 1/7 of the necessary translation.

5.2. *The refinement of human insulin - orthorhombic crystal form*

Orthorhombic crystals of insulin were obtained long ago, but considerable difficulties in obtaining heavy-atom derivatives prevented further structural work. The more recent developments in the molecular replacement technique allowed the use of the known structures of the so-called 2Zn and 4Zn insulins in rhombohedral ($R3$) crystals (Bentley, Dodson, Dodson, Hodgkin & Mercola, 1976) as models for the structure solution of the human hormone in orthorhombic crystals.

The actual structure solution will be described elsewhere. Here I only wish to show how the procedure outlined above facilitated automatic refinement.

The rotational and translational parameters were established by the fast rotation function and R-factor search function. A 2Zn human insulin dimer was used as a model, with the first six residues deleted from the B chain (this part of the molecule is known for its conformational variability). Table 4 shows the progress of the least-squares refinement of the MR solution. Insulin is a more flexible molecule than haemoglobin, and all the atoms of the model were used for least-squares fitting in a search for an optimum overall fit.

The changes as expressed by the differences in the Eulerian angles are large, but it must be remembered that there exists in the Eulerian space a high correlation between the values of α and γ (especially when β approaches 0 or 180°). It is probably better to

Table 4. *The progress of the refinement of the MR solution of 2Zn insulin dimer in orthorhombic crystals (for other details see text)*

	Starting values	Cycle 1	Cycle 2	Cycle 3	Cycle 4
			Rotational parameters(°)		
Eulerian:					
α	40·00	38·20	35·90	34·00	32·70
β	30·00	30·40	30·80	30·80	30·80
γ	40·00	41·30	42·90	44·70	45·70
Spherical polar:					
ω	—	130·10	132·20	129·10	128·20
φ	—	68·70	63·70	55·40	51·60
χ	—	1·00	2·50	3·20	3·80
			Translational parameters (Å)		
t_x	8·38	8·29	8·18	8·11	9·08
t_y	23·97	23·81	23·60	23·49	23·37
t_z	6·33	6·15	5·93	5·74	5·65
			R value*		
(%)	57·10	53·20	53·60	52·60	52·10

* Conventional crystallographic *R* value obtained after placing a 2Zn insulin dimer model in the orthorhombic unit cell using rotation and translation parameters from a current cycle of refinement.

express the difference between the successive models and the final structure in spherical polar coordinates; in this system χ is the sole measure of the rotational error, and with roughly spherical molecules the values of ω and φ (which define the position of the rotation axis) are less relevant. As pointed out earlier, however, the crucial indication is the overall r.m.s. distance between the model and the true positions. This has fallen from over 1·0 Å for the initial solution to 0·7 Å for the refined model (all model atoms were included in this calculation). As the latter value is approximately equal to the radius of convergence of refinement at 2·0 Å resolution, this improvement has a dramatic effect on the progress of refinement and the quality of the phasing. It should also be noted that, as was the case with haemoglobin, the least-squares fitting technique very quickly resulted in an almost perfect fit (in the least-squares sense) on the final structure.

After the last round of least-squares fitting, the model was reviewed on a computer graphics PS300 Evans & Sutherland system and following manual rebuilding of a small number of amino acids and the identification of water molecules subsequent refinement converged with an *R* value of 0·19. There is little doubt that the refinement of the MR solution parameters, as described in this paper, has substantially reduced the time needed to refine the structure.

6. Discussion

During the initial refinement of the structure of semi-oxyHb almost two years were spent on the reconstruction of those parts of the molecule that were found on consecutive electron density maps to suffer from serious errors. This excessive amount of time would have been reduced considerably by the use of computer graphics rather than maps plotted on transparent sheets, but would have been almost completely eliminated had the search model been placed without any error. This extreme example illustrates the need for very careful analysis of any solution found using the molecular replacement method. One of the techniques used to refine the general orientation and position of large parts of the structure is the rigid-body refinement procedure known as *CORELS* (Sussman *et al.*, 1977). Although it was proved to be very efficient, particularly for some tRNA structures, the fact that it is extremely time consuming makes it of limited use, particularly in those laboratories where computer time is a limiting factor. An alternative method involving group refinement was developed by Huber & Schneider (1985). An *R*-factor minimization approach, in which the crystallographic *R* factor is computed during a stepwise variation of positional parameters of one or more rigid groups (Bode *et al.*, 1976; Grau *et al.*, 1981) was also proposed, but does not seem to be an efficient way of reaching the proper solution in multiparameter space. The method presented here and used with success in several cases may serve as a useful alternative. It uses only the well known retrained least-squares refinement software and the corrections of rotation and translation parameters in calculations shown in this paper were obtained from only a few cycles of least-squares refinement, which on the VAX-11/750 may take up to 2 h for a protein as large as haemoglobin (4556 non-hydrogen atoms). In the case of haemoglobin two dimers were refined independently, but the procedure can also be applied to refine a number of separate domains or chains. The refinement of MetHb shows that even in the case of seemingly isomorphous crystals it is worthwhile to check the general position of the starting model prior to final least-squares refinement.

Since this paper was written, a number of crystal structures solved at York by molecular replacement have been refined using the protocol documented here. Most notably, a crystal structure of concanavalin A with a complete tetramer occupying the asymmetric unit was correctly positioned, reducing the initial *R* value from 0·52 to 0·38 (at 3·0 Å resolution) (Derewenda *et al.*, 1988). Each of the four monomers was refined independently. In a similar fashion recombinant porcine myoglobin (with two molecules per asymmetric unit) was refined, reducing the *R* value from 0·56 to 0·41; as before, the molecules were treated independently (Tom Oldfield, personal communication).

The success of this technique suggested to us its use as a method to identify a correct MR solution. It is well known that any atomic model, however wrong,

may be 'successfully' refined by least-squares methods to an R value of approximately 0·35, or even lower, at about 2·0 Å resolution. We have observed, however, that correct solutions almost invariably yield to the technique documented in this paper; least-squares refinement of incorrect solutions produces no overall shifts and hence global refinement of the orientation and/or position of the molecule produces no results.

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On the Use of the Term 'Absolute' in Crystallography

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Abstract

The concept of 'absolute structure' as introduced by Jones [*Acta Cryst.* (1984), A**40**, 660-662] is discussed, and the term 'absolute' is extended to cases where the structure has been related to some external macroscopic physical property. The non-centrosymmetric crystal classes are divided into seven distinct categories for which specific terms are proposed: *structural chirality*, when the 'absolute structure' is determined for crystals in classes 1, 2, 3, 4, 6, 222, 422, 32, 622, 23 and 432; *absolute chirality*, when the 'absolute structure' is linked to a chiral property such as optical rotation; *absolute polarity*, when the 'absolute structure' is linked to a polar property such as pyroelectricity; *absolute morphology*, when the 'absolute structure' is linked to the crystal habit: in this case there may be two further subdivisions - *absolute chiral morphology* (or *absolute enantiomorphism*) to describe a link to the hand of the habit, and *absolute polar morphology* to describe a link to the polar nature of the habit. It is further recommended that the term *absolute configuration* should be reserved only for molecular species and not for crystal structures.

It has been pointed out several times in recent years (*e.g.* Rogers, 1981; Jones, 1984*a*, *b*; Glazer &

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