random phases and we intend to distribute a computer package, based on the *MULTAN* system, to implement this and perhaps one or two of the other refinement functions as well. The computer time necessary for these parameter-shift refinement programs is not excessive and, as it turns out, for the second group of functions which includes ψ_D the timings are least. As an example, for the structure CH3PC2H5 (Table 2) with 350 reflexions and 10 050 relationships, the refinement time was about 4 min per trial on a Telefunken TR440 and would take about one half of that time or less on a DEC System 10 computer. Perhaps it should be said that while there is no strong indication that these refinement procedures are markedly better than those already in use they are different, are comparable in power and offer an alternative approach for solving difficult structures.

We wish to express our gratitude to the Computing Centre of the University of Ulm for the use of its facilities and to the Deutches Forschungsgemeinschaft and North Atlantic Treaty Organisation for support of our collaborative activities.

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A Strategy for Combining Restrained Least Squares with Computer Graphics in the Refinement of Protein Structures

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Abstract

A method of combining interactive molecular graphics with restrained least-squares refinement is described. This enables a researcher using the interactive computer graphics terminal to weight the shifts applied to the individual atoms in following cycles of refinement according to the confidence in their positions. The weights are incorporated into the least-squares procedure using the Marquardt factor which was originally introduced to handle the ill-conditioned case [Moss & Morffew (1982). *Comput. Chem.* **6**, 1–3; Marquardt (1963). *SIAM J. Appl. Math.* **11**, 431–441]. The described refinement strategy has been imlemented at Birkbeck College, London, and, using avian pancreatic polypeptide (APP) as test data, some refinements have been carried out. In order to compare the results of these refinements, histograms have been drawn that show the distribution of distances between the corresponding atoms in the different models. These histograms show that the model is improved by including the 'confidence' weighting. An improvement of 7·5% was achieved in the mean distance between the corresponding atoms in the fully refined model and a

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model resulting from four cycles of restrained least squares. When the 'confidence' weighting was included this improvement rose to 14·0%.

Introduction

Restrained least squares was first used by Waser (1963) and has subsequently been used in several refinement programs. Hendrickson & Konnert (1980) first applied this technique to protein structures and the procedure described here, *RESTRAIN* (Moss & Morffew, 1982), has many similarities with the Hendrickson & Konnert program in the way that restraints are applied to structural stereochemistry.

Interactive molecular graphics has been used for many years now (for example, Levinthal, 1966; Barry & North, 1972), and in recent years has become common in the molecular biology laboratories. Typically, a model is first built into a map on the graphics system and then refined using a batch program. When the model requires alterations that cannot be made by the refinement procedure, the researcher returns to the graphics system and manipulates the model into a new map. This may have to be repeated several times in the course of a refinement.

When restrained least squares is used in conjunction with an interactive graphics system the effects of the two parts of the refinement may work against each other. The refinement program will often tend to move the best placed atoms away from their positions as well as improving the poorly placed atoms. Such effects can be minimized by adding a weighting function to the refinement program that reflects the user's confidence in the atom positions. Weighting of this type causes the best placed atoms to have small shifts applied and the poorly placed atoms to have larger shifts applied.

Methods

The function minimized by *RESTRAIN* is given by

$$M = W_f(|F_o| - |F_c|)^2 + W_d(D_t - D_c)^2;$$

where W_f and W_d are the weighting factors; $|F_o|$ and $|F_c|$ are the observed and calculated structure amplitudes; D_t and D_c are the target and calculated interatomic distances.

The normal equations used in *RESTRAIN* take the form

$$(\mathbf{A} + m\langle \mathbf{A}_{ii} \rangle \mathbf{I})\,\delta x = -\mathbf{J}'f;$$

where $\mathbf{J}$ = the Jacobian matrix; m = the Marquardt factor; $\mathbf{I}$ = the identity matrix; $\langle \mathbf{A}_{ii} \rangle$ = the mean value of the diagonal elements of $\mathbf{A}$; $\mathbf{A} = \mathbf{J}'\mathbf{J}$; δx = the shifts in the coordinates; $\mathbf{J}'f$ = the weighted gradient vector.

This method has been found to be able to handle ill-conditioning encountered in protein crystallography when: (*a*) there are too few observations; (*b*) atoms occupy special positions. The individual atom weights that are set up on the graphics system replace the identity matrix, making the normal equations become

$$(\mathbf{A} + \langle \mathbf{A}_{ii} \rangle \mathbf{W})\,\delta x = -\mathbf{J}'f,$$

where $\mathbf{W}$ = the diagonal weight matrix.

Birkbeck College has an interactive graphics facility which is running the FRODO system (Jones, 1978). This system has been adapted so that a file of weights is included in the molecule data format. The weights range between 0 and 8; 0 indicating the researcher has no confidence in the atom position and 8 indicating that the researcher is very sure that the atom is correctly placed. When a new model is being inspected, each atom begins with the default value 4. The weights are passed to *RESTRAIN* in the atom coordinate file for the refinement cycle.

Results

Experiments that compare the effects of different refinement techniques are very difficult to assess. Most difficult is the presentation of the results so that the whole picture is evident. The experiments described here, using APP data (Wood, Pitts, Blundell, Tickle & Jenkins, 1977) concentrate upon the effects of four cycles of refinement under the same conditions, with the same initial model and the same weighting coefficient for the terms in the residual. The results of the refinement cycles are given as histograms which show the frequency of Δ, the distances between the resulting atom coordinates and the coordinates of the fully refined model.

Two refinement experiments were carried out, the resolution of the data used being 2·1 Å. The refinements were: (*a*) a pure restrained least squares (no Marquardt factor); (*b*) a restrained least squares with individual atom weights. The individual atom weights were assigned according to the distance of the atoms in the initial model from the corresponding atoms in the fully refined model. Fig. 1 shows schematically the derivation of the results in this experiment. The two models produced by refinement, *A* and *B*, have been compared to the fully refined model (0·98 Å) which is assumed to be much nearer to the true structure than any of the others. This is reflected in the *R* factors for the three models which were 0·174 for the fully refined model, 0·304 for model *A* and 0·301 for model *B*.

A problem occurred in showing the results as histograms, in that they only showed the frequency of distances up to 0·78 Å. There were larger values of Δ that could not be shown on this scale. When a model was improved, some points on the new histogram had

been beyond the range of the previous histogram making direct comparison difficult. This has been overcome by only displaying the 400 lowest values of Δ. The statistics given are for the whole model.

Fig. 2 shows the first of the histograms which compares the initial model with the fully refined structure. Mean Δ = 0·282 Å and the standard deviation = 0·298 Å.

Fig. 3 shows the comparison between the model produced from *RESTRAIN* without the Marquardt factor (model *A*) and the fully refined structure. There has been some cleaning up of the model and this is shown as a shift to the left on the histogram. The improvement is reflected in the statistics, mean Δ = 0·261 Å and the standard deviation = 0·273 Å.

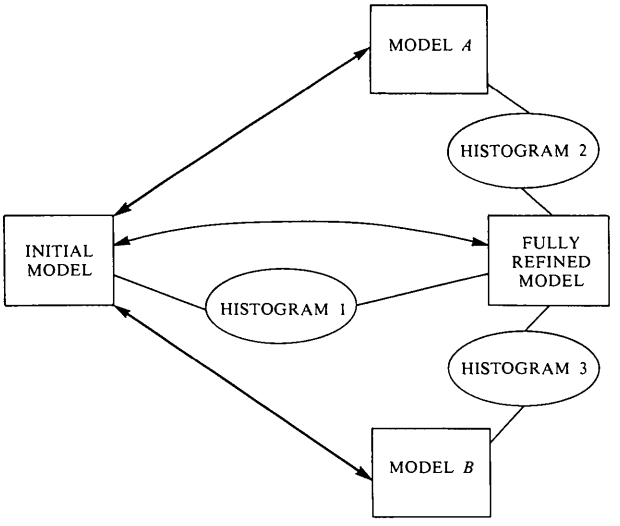

Fig. 1. A schematic diagram illustrating the relationship between the different models of APP generated by (1) the full refinement, (2) four cycles of restrained least-squares refinement at 2·1 Å, and (3) four cycles of restrained least squares with individual atom weighting.

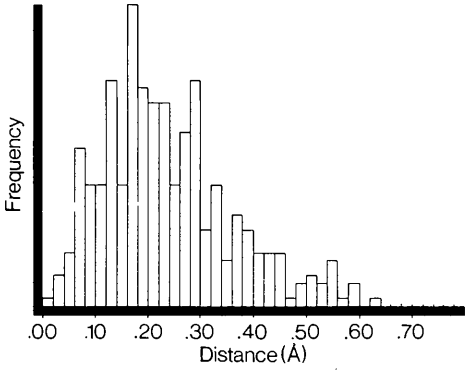

Fig. 2. The first histogram comparing the initial model with the fully refined model.

Fig. 4 shows the comparison between model *B*, using the individual atom weights, and the fully refined model. Again, there has been an improvement in the model, mean Δ = 0·243 Å and the standard deviation = 0·267 Å.

The improvement in mean Δ between the initial model and model *A* is 7·5% and between the initial model and model *B* is 14·0%.

The mean contributions to the normal equations were 1·84 for the structure amplitudes in both refinement experiments, 0·26 for the restraints in the final cycle of the experiment without individual atom weights, and 0·37 for the restraints in the final cycle of the experiment with individual atom weights.

The r.m.s. deviations for the bond lengths and the restraints across bond angles were 0·03 and 0·08 Å, respectively, in the fully refined structure. In model *A* the r.m.s. deviations were 0·05 and 0·05 Å. In model *B* they were 0·06 and 0·06 Å.

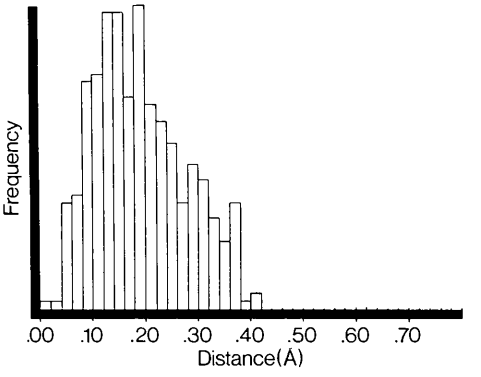

Fig. 3. This histogram shows the comparison between the model produced from four cycles of restrained least-squares refinement at 2·1 Å and the fully refined model. Comparison with Fig. 2 shows a noticeable shift to the left indicating the model has been improved.

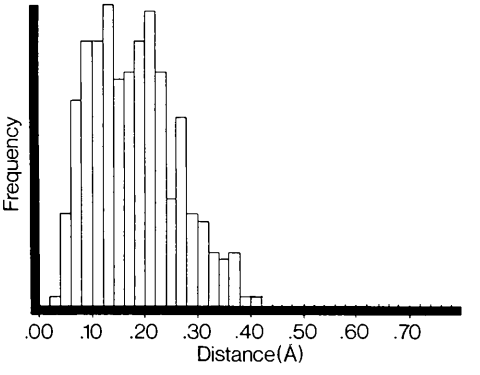

Fig. 4. This histogram compares the model produced from four cycles of restrained least-squares refinement at 2·1 Å, including the new facility for weighting individual atom shifts, with the fully refined model. Comparison with the other histograms indicates a further improvement in the model.

Concluding remarks

These results indicate that the restrained least-squares refinement method can be successfully adapted to include individual atom weighting from a molecular graphics system. In the early stages, this will be of great benefit in controlling the course of the refinement.

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Applications of High-Order Laue-Case Rocking Curves

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Abstract

Spherical-wave *Pendellösung* fringes have been used for many years to make absolute measurements of X-ray coherent scattering amplitudes. Bonse & Teworte [*J. Appl. Cryst.* (1980), **13**, 410–416] have suggested that the corresponding fringes seen in Laue-case rocking curves between two crystals with almost equal thicknesses might have important applications and they showed that agreement to within $\frac{1}{2}$% could be achieved in structure factor measurements on silicon. Two further applications are demonstrated in this paper. By a simple construction the range of double-crystal topography, using only a Lang camera, has been extended to the region $\delta d/d < 10^{-9}$. In another experiment it is found that the *Pendellösung* method can be extended to very high orders (the 10,10,0 reflection in silicon for example) so that attention can be focused, for the first time with high precision, on the coherent Bragg scattering at very high $\sin\theta/\lambda$.

Introduction

As part of our dispersive double-crystal interferometric spectrometer which is installed at the storage-ring source at Daresbury we required a two-reflection Laue-case silicon monochromator. Since the monochromator was intended for work at short wavelengths and with high orders of Bragg reflection, its testing involved new aspects which we believe have wider application. For example, in the 10,10,0 reflection of Mo $K\alpha_1$ radiation the full width at half height of the reflection curve is only 0·1″ of arc. The stability of our monochromator is better than 0·001″ of arc per day. The gradient of the reflection curve is very steep so that 1% intensity change corresponds to only 0·0005″ of arc in $\delta\theta$ or 9×10^{-10} in $\delta d/d$. For perspective we might note that the reflection widths quoted here are one hundred times narrower than those more commonly used in double-crystal topography (*e.g.* the silicon 440 reflection of Cu $K\alpha_1$) or in double-crystal diffractometry (*e.g.* the 880 reflection of Cu $K\alpha_1$ from gadolinium gallium garnet). The reflection-curve gradients are up to ten times larger than those achieved previously in double-crystal topography (Hart, 1968; Bonse & Hartmann, 1981) using oblique high-order Bragg reflections such as the 880, 844 and 12,0,0 from silicon.

Conventional double-crystal topography with separate reference and sample crystals requires quite complicated goniometers and servo control systems to achieve resolutions of 10^{-8} in $\delta d/d$. Systems with superior resolution have not so far proved feasible and would, in our experience, be extremely difficult to implement in the conventional way.

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