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Refinement of X-Ray Data on Proteins. I. Adjustment of Atomic Coordinates to Conform to a Specified Geometry*

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The atomic coordinates of a protein derived from x-ray-diffraction studies yield bond lengths and bond angles which depart significantly from those obtained from the crystal structures of the individual amino acids. A method is described for adjusting the dihedral angles (ϕ_i , ψ_i , χ_j) of the backbone and side chains by a least-squares approximation to the x-ray coordinates so that the computed structure conforms to those derived from crystal structures of its constituent amino acids. This structure may then be used as a starting point for conformational energy calculations. The performance of various minimization procedures in the least-squares computations is described, and a comparison of the adjusted structure with the x-ray structure is made in terms of deviations of the atom positions and dihedral angles from their original values. The method is applied here to the x-ray coordinates of lysozyme and chymotrypsin.

INTRODUCTION

The possibility of using conformational energy calculations to refine the atomic coordinates of a protein, obtained from x-ray data at lower than atomic resolution, has been pointed out earlier [1]. In procedures in use in our laboratory, the chain is first generated by variations of dihedral angles, keeping the geometry (i.e., the bond angles, bond lengths, and planar *trans*-peptide group) fixed and maintaining as close a fit as possible to the x-ray data. Atomic overlaps and other errors in the

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x-ray data are then reduced in a second stage by minimizing the conformational energy. The restriction to fixed geometry is then relaxed in a third stage of energy minimization.

The refinement procedure of Diamond [2] is similar to our first stage, the most important difference being that he also provides for variation in the bond angle $\tau(NC^{\alpha}C')$ at each C^{α} atom. The method of Levitt and Lifson [3] applies the conformational energy calculation directly to the x-ray data (or to the results of a Diamond refinement of the data), but uses flexible geometry. Our use of rigid geometry in the initial stages is based on the desire to explore the possibility that x-ray data may be fit without allowing the protein structure to depart significantly from currently accepted "standard" polypeptide geometry. The use of fixed geometry significantly reduces the computation time of the subsequent energy-minimization step by limiting the number of variables.

In this paper, we discuss the first stage, the fitting of the atomic coordinates to the x-ray data as closely as possible. The second stage, the application of conformational energy calculations, will be presented in a subsequent paper [4]. In computing the deviations of a chain of given geometry from the x-ray data, the function to be minimized is a complicated function of the dihedral angles. Therefore, we also examine the application and rate of convergence of several minimization procedures which differ in their efficiencies, for a function of this type. The method is applied here to hen egg-white lysozyme and to the *B* and *C* chains of bovine pancreatic tosyl-α-chymotrypsin.

METHOD

Since the positions of the backbone atoms of a polypeptide chain are constrained by the positions of neighboring backbone atoms, we may expect that their coordinates (as deduced by x-ray data) would be more accurate than those of the side-chain atoms. In many crystal structure determinations, the positions of the side-chain atoms beyond the C^{β} are obscured because of partial or complete rotational freedom (thermal motion) about the single bonds of the side chain. For these reasons, we fit the x-ray data with a polypeptide chain of specified geometry in two steps. In the first step, we consider those atoms whose positions depend only on the backbone dihedral angles ϕ_i and ψ_i [5], viz., the N, C^{α}, C', O, and C^{β} atoms. In the second step, these atoms are maintained fixed in their optimized positions, and the side-chain dihedral angles χ_j [5] are adjusted to obtain the best fit to the positions of the side-chain atoms. In both steps, the independent variables are the dihedral angles, which are adjusted to obtain a least-squares fit of the polypeptide chain to the x-ray data.

The basic outline of the mathematical procedure (the same for both backbone

and side-chain atoms) is the following. A set of dihedral angles is first computed from the given set of atomic coordinates. Then, using a specified geometry and this set of dihedral angles, a starting conformation is generated with the aid of transformation matrices described elsewhere [6]. The position and orientation of the *N*-terminal residue is determined in such a way that the calculated starting conformation is properly oriented with respect to the x-ray coordinates by either of two procedures: (1) The plane of the *N*, C^{α}, and C^{β} atoms of the *N*-terminal residue of this computed conformation is made to coincide with that of the same atoms from the x-ray data, or (2) The plane of coincidence is that containing the C^{α} atoms of three widely spaced residues. In either procedure, the C^{α} atom of the *N*-terminal residue (taken as the origin of the coordinate system) in the computed conformation coincides with that of the x-ray structure, but the other corresponding atoms do not coincide, in general, even though the planes do. Once the position and orientation of the first residue is determined, it is fixed throughout the refinement procedure. In this fixed coordinate system, the position of any atom with respect to the origin is represented by vectors $\mathbf{r}_i$ and $\mathbf{r}_{i0}$, respectively, where the former corresponds to the *i*-th atom in the computed conformation and the latter to its position in the x-ray structure. We define the deviation, $\mathbf{a}_i$, of the position of the *i*-th atom in the computed structure from that in the x-ray structure by

$$\mathbf{a}_i = \mathbf{r}_i - \mathbf{r}_{i0} \tag{1}$$

and minimize the function

$$F = \sum_{i=1}^{N} \| \mathbf{a}_i \|^2, \tag{2}$$

where *N* is the number of atoms in the backbone (including the C^{β} atom) or individual side chain, respectively. The length of the vector $\mathbf{a}_i$ (designated $\| \mathbf{a}_i \|$) is a function of the variable dihedral angles (ϕ_i, ψ_i) and/or χ_j on the *N*-terminal side of atom *i*. In the case of minimization procedures which involve the use of gradients, the derivatives of *F* with respect to the dihedral angles are evaluated as a sum of scalar-product contributions from the various atoms *k*, viz.,

$$D_j = \sum_{k=l}^{N} (\mathbf{c}_j \times \mathbf{b}_{kj}) \cdot \mathbf{a}_k , \tag{3}$$

where D_j is the derivative of *F* with respect to the *j*-th dihedral angle, *l* is the first atom whose position is altered by variation of the *j*-th angle, $\mathbf{a}_k$ is given by Eq. (1), $\mathbf{b}_{kj}$ is a vector connecting atom *k* to one end of the bond about which rotation takes place, and $\mathbf{c}_j$ is a vector whose components are the direction cosines of that bond. The summation in Eq. (3) is carried out over all atoms whose positions are

affected by variation of the j-th dihedral angle. This gradient expression holds for the case where the N-terminal end of the chain is fixed in space, as in the present method. Equation (3) has the property that the gradient values D_j of the dihedral angles at the N-terminal end of the chain tend to be larger than those at the C-terminal end. However, since more atoms are moved by changing the former angles, the number of constraints is larger; hence, the dihedral angles near the N-terminal end are not subject to greater variation than those near the C-terminal end. Thus, one starts with the initial computed conformation and varies the dihedral angles to minimize F, the input data in the minimization procedure being the current value of F (and, in those procedures using gradients, the current value of D_j for each dihedral angle). The output from each iteration of the minimization procedure is a new set of dihedral angles which yields a lower (and ultimately the minimum) value of F.

To conserve computer time and space, the fitting to the backbone was carried out for twenty-residue segments. The number of terms in Eq. (3) for D_j, and the size of certain arrays used in some of the minimization procedures are proportional to the square of the number of residues, thus making a single computation for the entire protein impractical although not impossible. Because the chain is adjusted in segments, we expect our results to deviate slightly from the true least-squares fit to the entire protein. However, subsequent stages of refinement (as outlined in the Introduction) should allow any such errors to be corrected. Twenty residues proved to be a sufficiently large number to provide conformational correlations over many units. Longer segments might present difficulties because variations of the dihedral angles in the central region of the chain produce amplified movements of the atoms near the C-terminal end of the chain; thus, the dihedral angles in the central region would tend to vary very little. On the other hand, the use of shorter segments would introduce the error of end effects into a relatively higher proportion of residues. In order to minimize end effects, each new twenty-residue segment was made to overlap the previous segment by a variable number of residues. We have found that an overlap of two residues is generally sufficient, as evidenced by the fact that the magnitudes of the deviations in Fig. 2 and 3 (presented later) show no obvious trends which can be related to the ends of segments. Thus, since the backbone is divided into twenty-residue segments, there is no limitation on the overall chainlength which can be treated by this procedure.

The fitting to the side chains was carried out separately for each side chain, after the best fit to the backbone was obtained. In the case of side chains which are symmetrically branched at a nonplanar atom (valine and leucine), the minimization must be carried out twice, once with the atoms beyond the branch point as given in the x-ray data and once with the labeling of their positions interchanged. This precaution is necessary because the minimization procedure occasionally will lead to an alternative minimum which deviates from the best conformation by 180°;

the first or second minimum is chosen, depending on which fits closest to the x-ray coordinates. This procedure is unnecessary if the side chains are planar at the branch point (e.g., aspartate and phenylalanine), since their conformations are identical after rotation by 180° (this is also true of asparagine and glutamine, since the *O* and *N* atoms are usually indistinguishable in the electron density maps).

The minimization procedures tested for this problem were Rosenbrock's method [7], a modified partan method [8], Gauss least squares [9], the Fletcher–Reeves method of conjugate gradients [10], and the Fletcher–Powell modification [11] of Davidon's method [12]. The latter three make use of gradients. We have also tested Diamond's modification [2] of Gauss' least-squares method, which compensates for the nonlinearity of the derivatives of the function to be minimized. Of all the methods tried here, the Davidon procedure appeared to be the best for this problem, for reasons to be discussed below.

The x-ray coordinates of hen egg-white lysozyme were obtained from Phillips [13], and those of bovine pancreatic tosyl-α-chymotrypsin were taken from the paper of Birktoft et al. [14]. Both sets of coordinates were measured directly from the rigid-wire models.

Two sets of geometry were used in the fitting. In the first, the bond lengths and bond angles summarized by Scheraga [1] were used, and the $\tau(NC^{\alpha}C')$ angle was the same for each residue. In the second set, the bond lengths and bond angles of the backbone were the same for each residue, except that the $\tau(NC^{\alpha}C')$ angle and the side-chain geometry were different for each residue (in accord with x-ray data for the specific amino acids and related compounds [15]).

RESULTS

Comparison of Minimization Procedures

The various minimization procedures were tested by using them to fit the first twenty-residue backbone portion of lysozyme to the x-ray coordinates (without inclusion of the side chains beyond the C^{β} atoms). The two criteria by which the results were assessed were the speed of convergence and the final (asymptotic) value of the root-mean-square (rms) deviation of the calculated coordinates from the x-ray data. The omission of the side chains (for the reason stated at the beginning of the Method section) also led to a reduction in computation time during the fitting to the backbone. Since the initial superposition of the planes of the calculated and observed coordinates is inaccurate, the first few residues tend to absorb this error, and therefore the first twenty-residue segment will yield a somewhat higher asymptotic rms value than subsequent segments. The results from the various minimization procedures are shown in Fig. 1. First of

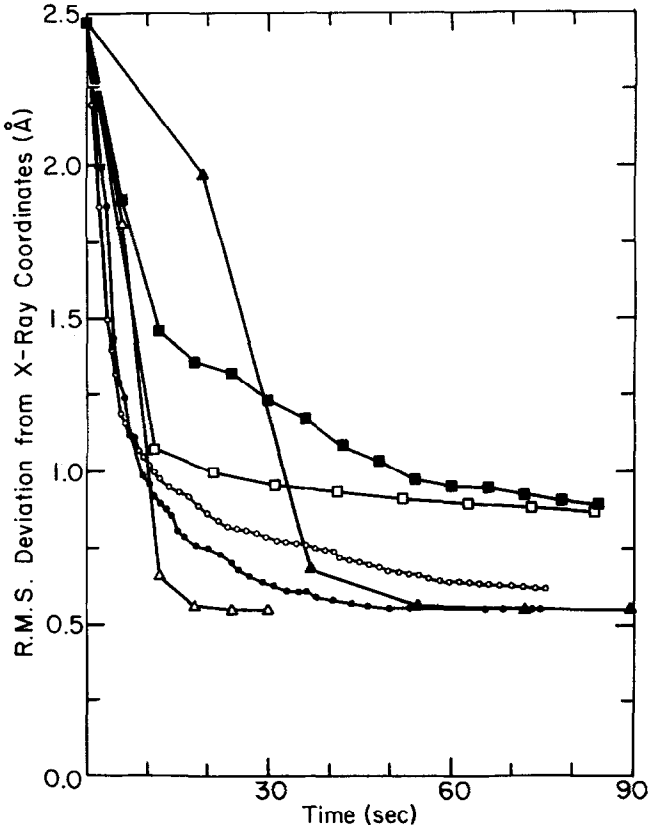

FIG. 1. Comparison of rates of convergence of various minimization procedures, for fitting a given geometry to the x-ray data for the backbones (including C^β) of the first twenty residues of lysozyme. ■ Rosenbrock, □ Modified partan, ○ Conjugate gradients, ▲ Diamond, ● Davidon, △ Gauss. The points represent the rms deviation for each iteration. For clarity, about half of the points have been omitted from the curves for the Davidon and Conjugate gradient methods.

all, it can be seen that the Rosenbrock method, the modified partan method and the method of conjugate gradients did not converge within the arbitrarily allotted time and, at the time of termination, the rms deviations from the x-ray coordinates were larger than those of the other methods. All of the remaining procedures led to the same conformation for this test case, with comparable rms deviations. For example, Diamond's method[1] [but with fixed $\tau(NC^\alpha C')$] and Davidon's procedure

[1] The control parameters used for Diamond's method were optimized in preliminary trials, and the values used were $C_1 = 1.11 \times 10^{-1}$ and $C_2 = 1.11 \times 10^{-5}$. The number of degrees of freedom (f_{max}) was set equal to the number of bonds in the structure (cf. Ref. [2]).

led essentially to the same structure in about the same length of time, all of the computed dihedral angles differing by less than one degree for both methods. A second comparison of the Diamond and Davidon methods was made for the next twenty-residue segment of lysozyme (residues 18–37). Again the two methods converged to almost the same conformation with a maximum difference of three degrees between the computed dihedral angles. However, Diamond's method was slower for the second segment, requiring eight iterations (instead of five as needed for the first segment), whereas Davidon's procedure required the same time for both segments. However, these time differences, as well as the twofold smaller size of the Davidon computer program, are not overly important, and it appears that both procedures operate with comparable efficiency.

By eliminating the filtering and reversion modifications (which are required when the coordinates of some residues are missing from the x-ray data set) from the Diamond method, the latter becomes essentially the Gauss least-squares method. The results of such a procedure are also shown in Fig. 1. This procedure led to essentially the same result as that obtained by the Davidon and Diamond methods for the test case, but at a faster rate. Despite this apparently greater efficiency of the Gauss method in this test case, we prefer the Davidon minimization procedure over the Gauss method, since the latter dictates excessively large changes of the dihedral angles during the first cycle and, as a result, tends to skip over some (possibly favorable) local minima when the initial approximation is poor; this sometimes results in a higher asymptotic rms value than was obtained with the Davidon and Diamond methods, both of which use smaller steps in the initial iterations.

From the fact that the Gauss, Davidon and Diamond methods converge rapidly to the same conformation, it appears that the starting coordinates are sufficiently close to those of the x-ray data, so that the derivative function is almost linear in the dihedral angles. Aside from these comparisons, all minimizations in our computations are carried out by Davidon's method. With this procedure, the minimum is reached (as in Fig. 1) in fewer than 50 iterations for the backbone of a twenty-residue segment. If the minimum is not detected after 50 cycles, the rate of change of the conformation in subsequent cycles will generally be so small that the minimization is halted to save computer time. The average time required for minimization is about 75 sec for the backbone of a twenty-residue segment, and about 20 sec for the side chains of 100 residues, on the IBM 360/65 computer.[2]

[2] The Fortran IV programs for fitting the backbone atoms (48K memory requirement on the IBM 360/65) and for fitting the side-chain atoms (54K memory requirement) have been deposited as Document No. NAPS-01623 with the ASIS National Auxiliary Publication Service, c/o CCM Information Corp., 909 3rd Ave., New York, N.Y. 10022. A copy may be secured by citing the document number and by remitting $2.00 for microfiche or $5.00 for photocopies. Advance payment is required. Make checks or money orders payable to: ASIS–NAPS.

Backbone Fitting

After minimization, the deviations of the coordinates of the backbone atoms from the x-ray coordinates are those shown in Table I, expressed both as root-mean-square and as maximum deviations. In lysozyme I and II, the geometries used were those summarized by Scheraga [1] and by Momany et al. [15], respectively. In lysozyme III, the former geometry was used, but the fit was made only to the C' and O atoms. It can be seen that comparable results were obtained for the first two cases, while the deviations for lysozyme III are significantly higher for all atoms except the C' and O atoms.

The results for the backbones are also expressed in terms of plots against residue number of the rms deviations of all backbone atoms in each residue of lysozyme I [Fig. 2(B)], and in terms of the deviations of the dihedral angles ϕ and ψ for each

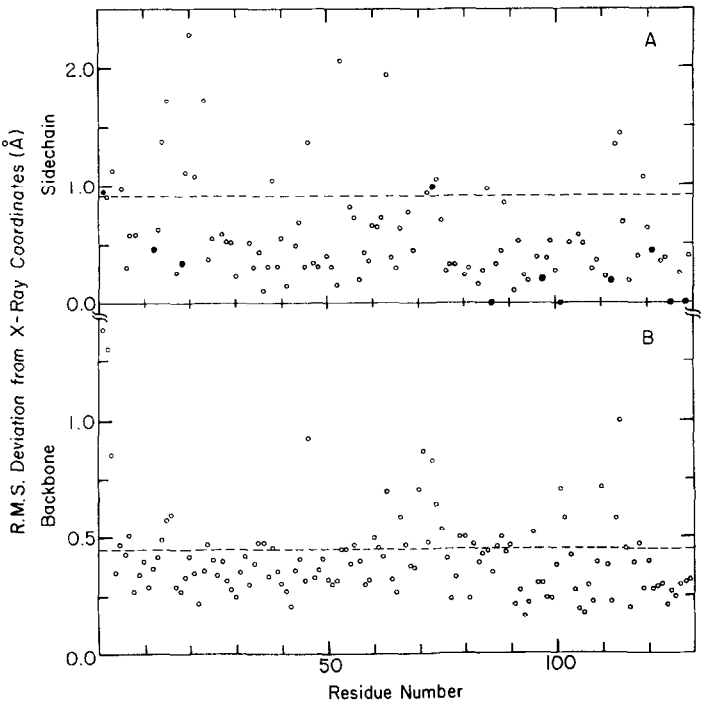

FIG. 2. Root-mean-square deviations of the computed coordinates of lysozyme I from the x-ray coordinates. (A) Side-chain atoms; (B) Backbone atoms. The horizontal dashed lines represent the rms values over all side chains (not including C^β) and backbones, respectively. The solid circles in (A) designate residues for which some or all of the coordinates of the side-chain atoms were not reported in the x-ray data. No points are plotted in (A) for glycine, alanine and proline, which have no movable atoms beyond the C^β.

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TABLE I

Root-Mean-Square and Maximum Deviations of Computed Coordinates from x-ray Coordinates (in Angstrom Units)

Protein	N	C^{α}	C'	O	C^{β}	Backbone rms deviation	Backbone maximum	Side-chain rms deviation	Side-chain maximum	Overall rms deviation
Lysozyme I[a]	0.36	0.36	0.33	0.57	0.58	0.45	1.79	0.91	3.86	0.64
Lysozyme II[b]	0.37	0.37	0.35	0.62	0.59	0.48	2.26	0.92	3.91	0.66
Lysozyme III[c]	0.52	0.59	0.36	0.45	0.97	0.61	2.35	—	—	—
Chymotrypsin B[a]	0.35	0.35	0.29	0.59	0.59	0.45	2.08	0.71	3.70	0.56
Chymotrypsin C[a]	0.34	0.33	0.33	0.52	0.56	0.43	2.35	0.74	2.40	0.55

[a] Using the geometry summarized by Scheraga [1].
[b] Using the geometry of Momany *et al.* [15].
[c] Using the same geometry as in (*a*), but fitting only the C' and O atoms.

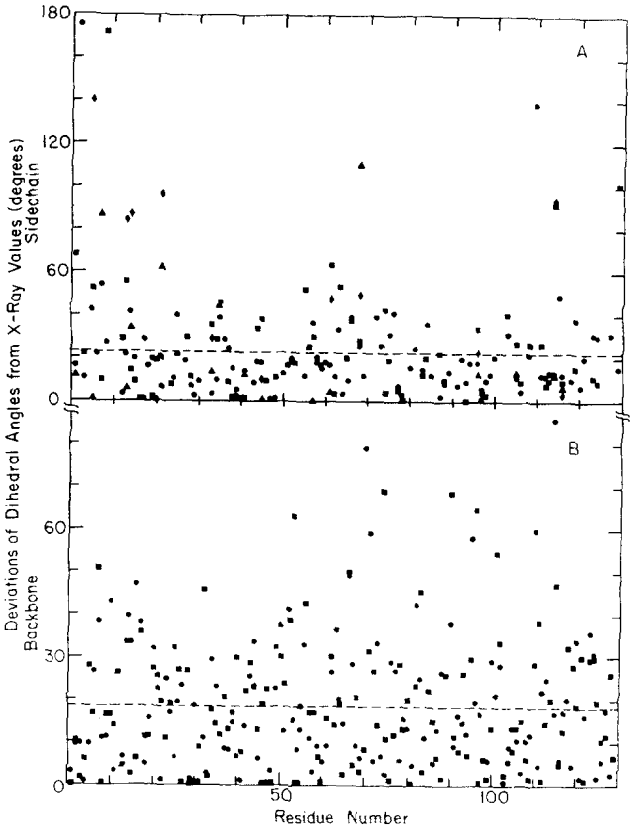

FIG. 3. Deviations of the computed dihedral angles from the x-ray values of lysozyme I. (A) Side-chain angles: $\bullet\chi_1$, $\blacksquare\chi_2$, $\blacktriangle\chi_3$, $\blacklozenge\chi_4$; (B) Backbone angles: $\blacksquare\phi$, $\bullet\psi$. Points were omitted for those side-chain angles where the coordinates of atoms defining that angle were not reported in the x-ray data. The horizontal dashed lines represent the overall average deviations for all side-chain and backbone dihedral angles, respectively.

residue [Fig. 3(B)]. The deviations in the coordinates appear to be small, while those in the dihedral angles are quite large. However, as can be seen from Fig. 4(A), (B), (C) the computed dihedral angles (except for glycine) essentially all lie within the 5 kcal energy-contour diagram for glycyl-*L*-alanine [16].

Side-Chain Fitting

The deviations of the computed side-chain conformations of lysozyme I (for all atoms beyond C^β) from the x-ray values are plotted against residue number in Figs. 2(A) and 3(A), showing the coordinates and dihedral angles, respectively.

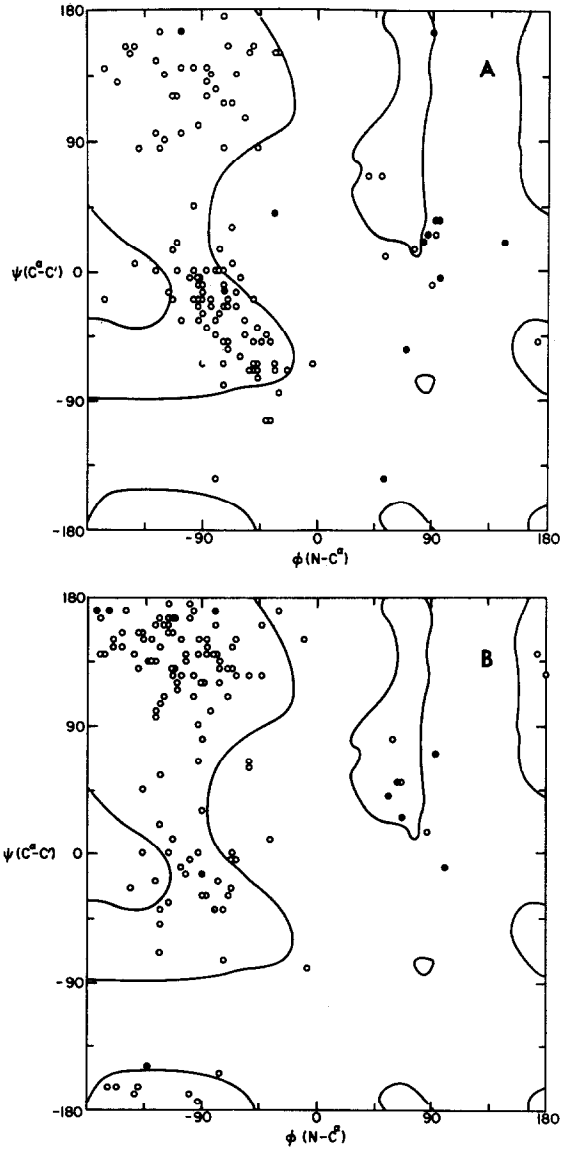

FIG. 4. Plot of computed backbone dihedral angles for Lysozyme I (A), Chymotrypsin B (B), and Chymotrypsin C (C). The solid circles denote glycine residues. The 5 kcal energy contours for glycyl-*L*-alanine [16] are included for comparison.

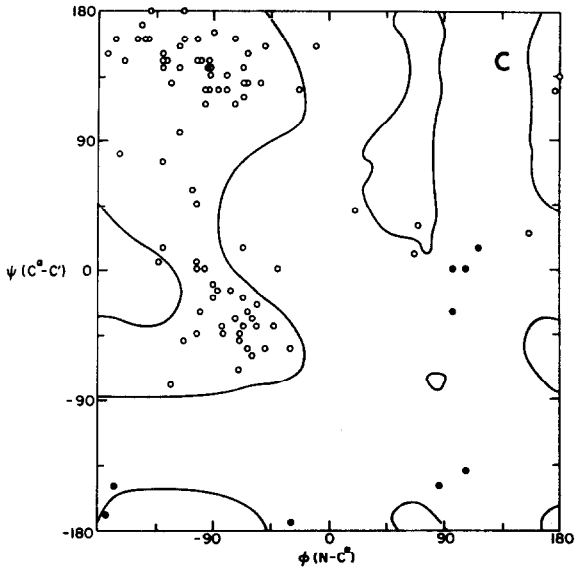

FIG. 4C.

The overall average and maximum deviations of the coordinates of the side-chain atoms for all of the structures are given in Table I, and the corresponding data for the dihedral angles are given in Table II. As might be expected, the deviations of the side-chain coordinates and angles are generally larger than those of the backbone atoms. The larger deviations found for the side-chain atoms may arise in part from our procedure of ignoring the side-chain atoms while fitting the backbone atoms. However, we think that the major origins of the larger side-chain deviations are the inaccuracies in the x-ray data for the positions of the side-chain atoms.

TABLE II

Average Deviations of Computed Dihedral Angles from x-ray Values

Protein[a]	Backbone average	Side-chain average
Lysozyme I	18.5°	22.6°
Lysozyme II	20.2°	22.5°
Lysozyme III	20.4°	—
Chymotrypsin B	19.4°	25.0°
Chymotrypsin C	16.7°	26.5°

[a] Same geometries as indicated in Table I.

Analysis of Close Contacts

Both the errors in the x-ray data, and the procedure for initial fitting of geometry used here, can introduce overlaps between atoms. Therefore, it is of interest to examine the computed structures for possible overlaps. These are expressed in Table III in terms of the numbers of atoms separated by $\leqslant 2$ Å. It can be seen that a number of overlaps exist. However, the second-stage refinement [4], involving conformational energy calculations, should relieve these overlaps.

TABLE III

Summary of Close Contacts

Protein[a]	Number within the range of		
	0.5–1.0 Å	1.0–1.5 Å	1.5–2.0 Å
Lysozyme I	1	9	58
Lysozyme II	1	10	54
Chymotrypsin B	0	7	39
Chymotrypsin C	0	3	27

[a] Same geometries as indicated in Table I.

Discussion

The procedure described here should *not* in itself be regarded as a tool to refine x-ray data, but rather as one which will allow conformational energy calculations to be carried out with a starting conformation which has specific bond lengths and bond angles and is close to that deduced from the x-ray data. Our procedure is based on the premise that the geometry of a polypeptide chain in a protein does not depart significantly from that observed in crystal structures of its constituent amino acids. If deviations from such geometry are required in order to obtain an optimum fit to the x-ray data, after completing the conformational energy calculations with fixed geometry, they can then be introduced. In this way, the refinement calculations can be carried out with a minimum amount of computer time.

While rms deviations from the x-ray coordinates have been quoted in this paper, these should not be regarded as a criterion of "validity" of the procedure presented here. Instead, as an overall criterion of validity of the whole treatment, the conformation obtained by energy minimization [4] should be compared with x-ray data from the native protein (and not with those from the isomorphous heavy-atom

derivatives). In this way many of the errors, which might exist in the currently reported Fourier maps, would not be involved in such a comparison.

The main differences between our procedure and that of Diamond are the following. The function to be minimized here is not a quadratic function of the dihedral angles, and therefore the derivatives of the function are nonlinear. Since it is assumed in the Gauss method that the derivatives are linear in the variables, Diamond introduces several procedures to compensate for the nonlinearity in this problem. In contrast, Davidon's minimization technique exhibits quadratic convergence and performs well on other complex functions [11, 17]. In our procedure, we keep $\tau(NC^{\alpha}C')$ fixed to reduce the number of variables and consequently the computation time for the next stage of refinement, which involves energy minimization. Another feature of our method is the independent fitting of backbone and side-chain atoms to avoid introducing errors in the backbone atom positions due to the influence of the less-accurately-known positions of the side-chain atoms. Finally, we apply our backbone-minimization procedure to twenty-residue segments, whereas Diamond applies his method to segments of lengths ranging from three to seven residues. By correlating the conformation of a larger number of residues, we distribute the deviations over a larger number of atoms, and thereby avoid excessively large movements of particular atoms.

As indicated by the results for lysozyme III in Table I, our procedure is also applicable when only the C' and O coordinates (or a similar partial coordinate set) are available. Of course, the fit will improve as the data set becomes more complete. However, if the positions of only the C^{α} atoms are available, there are too many redundancies in the values of ϕ and ψ for a given C^{α}–C^{α} distance (which itself has errors in it) to enable a unique conformation to be deduced.

With slight modifications, our method can be applied to proteins for which certain regions of the x-ray structure are not well defined. Finally, the method can be used for any selected set of geometrical parameters, and with any algorithm for generating the chain.

The fitted structures obtained here are now being used as starting conformations for conformational energy calculations, to obtain refined structures of these proteins [4, 18].

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