C α -based torsion angles: A simple tool to analyze protein conformational changes

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Abstract

A simple method is presented for the analysis of protein conformational changes based on the comparison of torsion angles defined by four consecutive C α atoms. The technique was applied successfully to proteins that undergo hinge motion and shear motion. In the case of both MBP and LAO, which represent examples of hinge motion, the plot of the differences in C α -torsion angles between the open and closed forms of the proteins helped us to formulate a more thorough description of the conformational change: a large displacement of one domain with respect to the other where one of the domains does not behave like a rigid body but exhibits some degree of flexibility. The analysis of citrate synthase, which is an example of shear motion, shows that the largest differences in C α -torsion angles between the open and closed conformations are clustered around residues that belong to segments connecting α -helices, whereas the helices themselves appear to be rigid; this is in agreement with previous results obtained by detailed least-squares superpositions (Lesk AM, Chothia C, 1984, *J Mol Biol* 174:175–191).

Keywords: α -carbon torsion angle; protein conformational change; protein crystal structure; protein 3D-structure

The most important result in X-ray crystallography (and NMR) of proteins is the elucidation of structure–function relationships, of which conformational changes are often a key part. Allosteric enzymes are examples of proteins that adopt different conformations upon binding of their substrates (inhibitors) and/or effectors. Other proteins are known to undergo significant conformational changes as part of signaling processes or interaction with other proteins. The changes in conformation may range from small local variations to large displacements of entire domains. Methods of describing the conformational changes are important for their understanding.

The usual first step in comparing different forms of a particular protein is the least-squares superposition of the two corresponding C α -traces (or backbones). This procedure requires the choice of the segment(s) to be aligned, as well as the level of agreement between the structures. It is preferable in many cases to begin instead with a property that is only dependent on the internal coordinates of each form of the molecule.

We present here a method based on dihedral angles defined by four consecutive C α atoms, which thus makes use of the information contained in the respective C α traces. This technique is a convenient tool that easily and without bias can identify

most regions of the protein where conformational changes take place.

Materials and methods

Atomic coordinates for the proteins studied were taken from the Brookhaven Protein Data Bank (PDB; Bernstein et al., 1977), except for the cases of a liganded and an unliganded mutant maltose-binding protein where the structures were solved and refined in this laboratory (B.H. Shilton & S.L. Mowbray, deposited with the PDB with identification codes 1mpc and 1mpd). Given a set of coordinates of a protein, for each residue i , a dihedral angle $\alpha(i)$ was defined as C $\alpha(i-1)$ -C $\alpha(i)$ -C $\alpha(i+1)$ -C $\alpha(i+2)$. A vector, P , normal to the plane determined by vectors C $\alpha(i-1)$ -C $\alpha(i)$ and C $\alpha(i+1)$ -C $\alpha(i)$, and a vector, Q , normal to the plane determined by vectors C $\alpha(i)$ -C $\alpha(i+1)$ and C $\alpha(i+2)$ -C $\alpha(i+1)$ were first calculated. The angle between P and Q gives the value of the dihedral angle $\alpha(i)$. When looking down the vector C $\alpha(i)$ -C $\alpha(i+1)$, a clockwise rotation of the vector C $\alpha(i+2)$ -C $\alpha(i+1)$ with respect to vector C $\alpha(i-1)$ -C $\alpha(i)$ is taken as positive. A program to perform these calculations is available from the authors. Alternatively, a list of C α -torsion angles can be obtained with the program for secondary structure definition DSSP (Kabsch & Sander, 1983), where they are labeled C α -virtual angles. However, some versions of DSSP ignore residues that do not have all the backbone coor-

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dinates and thus will not perform any calculation on coordinate sets containing only C α atoms. The difference between the C α -torsion angle profiles of any two protein structures was calculated with our program.

The average coordinate error associated with crystal structures limits the accuracy of the C α -dihedral angle values. For example, a coordinate error ϵ_r in the position of C $\alpha(i+2)$ gives a maximum angular shift $\epsilon_\alpha = \tan^{-1}(\epsilon_r/3.8)$, where 3.8 corresponds to the average C α -C α distance in Ångströms. Extending the ideas of Baldwin and Chothia (1979), the average uncertainty for a C α -torsion angle profile, $\epsilon_\alpha(p)$, can be approximated by $\sqrt{2}\epsilon_\alpha$. In the comparison of the profiles of two identical structures, a and b, with average angular errors $\epsilon_\alpha(a)$ and $\epsilon_\alpha(b)$ respectively, the expected RMS difference (RMSD) σ is approximately equal to

$$\sqrt{0.5[\epsilon_\alpha(a) + \epsilon_\alpha(b)]^2 + 0.5[\epsilon_\alpha(a) - \epsilon_\alpha(b)]^2}.$$

Differences in C α -torsion angles ($\Delta\alpha$) greater than or equal to 2σ were considered significant, whereas those below this level were taken as noise.

The results were plotted using the Macintosh program Cricket Graph (Cricket Software, Malvern, Pennsylvania). The molecular graphics program O (Jones & Kjeldgaard, 1995) was used for inspection of the protein three-dimensional structures and for least-squares superposition of pairs of structures. Following the work of Baldwin and Chothia (1979), the expected RMSD for the superposition of two identical structures was calculated with

$$\sqrt{0.5[\epsilon_r(a) + \epsilon_r(b)]^2 + 0.5[\epsilon_r(a) - \epsilon_r(b)]^2},$$

where $\epsilon_r(a)$ and $\epsilon_r(b)$ are the average coordinate errors of structures a and b, respectively. Coordinate errors for the structures determined in this laboratory were estimated by using a Luzzati plot (Luzzati, 1952). A value of 0.2 Å was assumed for high-resolution structures taken from the PDB when the coordinate error was not reported.

Results

The method was applied to analyze examples of proteins undergoing conformational changes such as hinge motion (which produces mainly rigid body domain shifts) and shear motion (which is composed of changes in small units). To assess the effects of crystal-packing forces and resolution on the C α -torsion angle difference profile, the cases of independent refinement of the same protein at two different resolutions and of nearly identical structures crystallized in different space groups were first examined.

Identical, independently refined structures at two different resolutions

The periplasmic glucose/galactose-binding protein (GBP) is involved in sugar transport and chemotaxis in gram-negative bacteria. The protein contains 309 amino acid residues; it is organized in two globular domains linked by a hinge consisting of three segments of polypeptide chain. Two models of GBP from *Salmonella typhimurium* (GBP-S) were compared: a structure of the protein bound to glucose, refined to a resolution

Fig. 1. $\Delta\alpha$ profiles for pairs of GBP structures. The 2σ cutoff 12.1° is shown; residues with $|\Delta\alpha|$ values above this level are indicated. Segments on the upper part of the figure show the location of the two structural domains and the connecting hinge (1, 2, and h, respectively). **A:** Independently refined models of the protein from *Salmonella typhimurium*: 3gbp complex with glucose, 2.4 Å resolution and 1gca complex with galactose, 1.7 Å resolution. **B:** Nearly identical proteins crystallized in different space groups: 1gca crystallized in space group C2 and the *Escherichia coli* GBP, 2gbp 1.9 Å resolution, crystallized in space group P2₁.

of 2.4 Å, deposited in the PDB with identification code 3gbp (Mowbray et al., 1990) and a structure of the protein bound to galactose, refined to a resolution of 1.7 Å (1gca; Zou et al., 1993). The unit cell dimensions in the two cases were the same within experimental error. The average coordinate error is estimated to be 0.2 Å for both models. The two backbones are essentially identical; they can be superimposed with an RMS fit of 0.15 Å after application of a 0.3-Å rigid body translation. The small differences observed are attributed solely to coordinate error (see discussion in Zou et al. [1993]). A plot of the difference in C α -torsion angles between these two structures is presented in Figure 1A; the graph shows the level of noise present when only coordinate error and different resolution of the structures are considered. Only one peak, at residue 199 (-14.8°), is above the 2σ cutoff (12.1°). Residue 199 is part of a reverse turn on the surface of the molecule where the conformation is better defined in the high-resolution electron density map.

Nearly identical structures that differ in crystal packing

The high-resolution GBP-S structure (1gca, crystallized in space group C2) was next compared to a very similar GBP from *Esch-*

erichia coli (GBP-E) that displays 94% sequence identity. The latter structure, available in the PDB as coordinate set 2gbp (Vyas et al., 1988), was crystallized in space group $P2_1$ and refined to a resolution of 1.9 Å. The $\Delta\alpha$ profile for this pair of structures is shown in Figure 1B. The largest differences in $C\alpha$ -torsion angles are found at the N- and C-termini and at residues 28, 31, 108, 109, 176, 276, 277, and 287; all of them are above the 2σ cutoff (12.1°). Residues 108 and 109 are located in the first segment of the hinge and can be correlated to a slight difference in the relationship of the domains in the two proteins (Zou et al., 1993). The peaks at residues 276 and 277 reflect different orientations of the peptides in the two proteins, namely the main-chain O atoms of their respective residues 276 point in opposite directions (Zou & Mowbray, 1994). All of the remaining peaks correspond to loop and other surface regions involved in small movements related to crystal contacts in either or both proteins or to the sites of sequence changes (see Fig. 7B in Zou et al. [1993]).

Hinge motion

Structures of two other periplasmic binding proteins provide clear examples of large domain shifts: the maltose-binding protein (MBP), which is active in the transport of maltose and in chemotaxis, and the lysine/arginine/ornithine-binding protein (LAO), active in transport. Both MBP and LAO consist of two similar domains connected by a hinge of three and two polypeptide segments, respectively. The large conformational change associated with the binding of the corresponding ligand seems to be crucial for proper recognition of these proteins by the membrane-bound receptors and permeases.

MBP contains 370 amino acid residues; its hinge is made up by the segments of the polypeptide chain near residues 111, 260, and 312. The structures of an open conformation and of a closed conformation of a mutant protein, both solved and refined in this laboratory (B.H. Shilton & S.L. Mowbray, unpubl. results) to resolutions of 2.1 Å and 2.4 Å, respectively, were used in the comparison. The open MBP was crystallized in space group $P1$, whereas the crystals of the closed one displayed the symmetry of space group $P2_12_12_1$. The estimated coordinate error is 0.22 Å for both structures. A plot of the differences in $C\alpha$ -torsion angles between the two conformations as a function of residue number is shown in Figure 2A. The peaks with $|\Delta\alpha| \geq 2\sigma$ (13.3°) are present at residues 78, 80, 81, 99, 100, 108, 109, 111, 121, 172, 175, 244, 312, 313, 326, and 332. Of these, residues 108, 109, and 111 are in the first strand of the hinge and residues 312 and 313 belong to the third hinge segment; these changes thus can be correlated to the large relative movement of the domains undergone by the molecule. Residues 172 and 175 are part of a loop of the C-terminal domain located on the side of the hinge that comes into contact with the N-terminal domain (near residues 99 and 100) upon opening of the molecule. This contact of portions of the N- and C-terminal domains might be important in stabilizing the open conformation in solution. The 170–175 loop is disordered in the closed form of the protein (B.H. Shilton & S.L. Mowbray, unpubl. results). The only cases for which the high $C\alpha$ -torsion angle differences are attributed to different crystal packing occur at residues 78–81 and 120–122.

The presence of several peaks (244, 326, and 332) in the non-hinge region of the C-terminal domain, which are not explained by crystal packing, is indicative of the fact that the conforma-

tional change observed is not simply a rigid body motion of one domain with respect to the other but a more complex one involving smaller subdomain motions as well. A least-squares superposition for each pair of equivalent domains separately gives an RMS fit of 0.32 Å for 161 $C\alpha$ atoms of the N-terminal domain (comprised of residues 1–110 and 261–311) and 0.70 Å for 209 $C\alpha$ atoms of the C-terminal domain (residues 111–260 and 312–370). The RMS fit observed for the superposition of the second domains is higher than would be expected (0.32 Å) if the two domains were structurally identical at the present level of coordinate error. Inspection of the superposed C-terminal domains at the graphics workstation shows two main features: the different conformations of segments 171–176 and 328–329, and the small displacements of helices 229–239 and 316–326. Thus, as suggested by the $\Delta\alpha$ plot, residues 244, 326, and 332 seem to act as pivots for the small movements of these helices.

LAO contains 238 amino acid residues; its hinge is made up by residues 89–92 and 186–194 (Oh et al., 1993). Two coordinate sets were obtained from the PDB: 2lao for the unliganded open conformation of the protein (1.9 Å resolution; Kang et al., 1992; Oh et al., 1993) and 1lso for the lysine-bound closed form of the protein (1.8 Å resolution; Oh et al., 1993). The crystals of open and closed LAO were not isomorphous. A plot of $\Delta\alpha$ as a function of residue number for the two conformational states of the protein is shown in Figure 2B. Peaks above the 2σ

Fig. 2. Differences in $C\alpha$ -torsion angles between open and closed forms of two proteins that undergo hinge motion. In each case, segments on the upper part of the graph indicate the location of the two structural domains and the connecting hinge (1, 2, and h, respectively). The 2σ levels for each example are shown; residues with $|\Delta\alpha| \geq 2\sigma$ are labeled in both plots. **A:** Mutant MBP $2\sigma = 13.3^\circ$. **B:** LAO $2\sigma = 12.1^\circ$.

cutoff (12.1°) occur at residues 4, 90, 92, 93, 140–142, 156–160, 183, 189–191, and 193. Residues 90–93 and 189–193 are located in the first and second segments of the hinge, respectively, and so these differences in C α -torsion angles are correlated with the large relative shift of the domains upon opening of the protein. (Oh et al. [1993] describe this as a change in the ψ angles of residues 90, 92, and 193, and a change in the ϕ angles of residues 190 and 193.) Differences in crystal packing between the two structures are observed at the N-terminus, reflected by the peak at residue 4, and around residue 141, which is located at the lip of the binding cleft.

A set of peaks in Figure 2B (residues 156–160 and 183) that do not belong to the hinge segments cannot be explained by differences in crystal packing. This result suggests that, as observed in MBP, subdomain motion might accompany the primary domain movement taking place upon opening of the protein molecule. Hence, further analysis of the two structures was carried out using the graphics program O. A least-squares superposition of the domains aligned independently gives RMS fits of 0.34 Å for 135 C α atoms (1–89, 193–238) of the first domains and 0.69 Å for 103 C α atoms (90–192) of the second domains. The RMS fit for the superposition of the second domains is significantly higher than would be expected if the domain had moved as a rigid body; at the present level of coordinate error, the expected value is 0.28 Å. Like the C α angle difference, this fact is indicative of some degree of flexibility in the second domain. Display of the superimposed second domains on the graphics workstation shows small displacements in helices, especially the region 161–177; as suggested by the $\Delta\alpha$ profile, the stretch 156–160 and residue 183 seem to be responsible for these movements.

Shear motion

Citrate synthase is a well-studied example of a mechanism of conformational change described as helix interface shear (Lesk & Chothia, 1984; Chothia & Lesk, 1985; Gerstein et al., 1994). The enzyme catalyzes the condensation of acetyl coenzyme A and oxaloacetic acid to form citric acid in the citric acid cycle. It is a dimeric protein made of two identical polypeptide chains each containing 437 amino acid residues. The crystal structures of a pig- and a chicken-heart enzyme in various complexes were first determined by Huber and coworkers (Remington et al., 1982; Wiegand et al., 1984). The monomer is organized in two domains, a large one containing 15 α -helices (labeled A–M, S, and T) and a small one made of 5 α -helices (labeled N–R); the active site is located in the cleft between the domains. Two main conformational states of the citrate synthase monomer were observed depending on its ligation state: a closed conformation when citrate and acetyl coenzyme A are bound, and an open conformation when only citrate is bound (Remington et al., 1982).

Two coordinate sets from the PDB were used in the present study: 1cts (open conformation, 2.7 Å resolution, space group $P4_12_2$; Remington et al., 1982) and 2cts (closed conformation, 2.0 Å resolution, space group $C2$; Remington et al., 1982). From the information given by the authors, average coordinate errors of 0.35 Å and 0.20 Å were assumed for 1cts and 2cts, respectively. The $\Delta\alpha$ profile for these structures is presented in Figure 3. Values of torsion-angle differences greater than the 2σ cutoff (17.2°) appear mostly clustered around residues that be-

Fig. 3. Plot of $\Delta\alpha$ versus residue number for two conformations of pig-heart citrate synthase monomer: open form 1cts and closed form 2cts. The 2σ cutoff 17.2° as well as those residues with $|\Delta\alpha|$ values above this level are shown. Dashed-line segments on the upper part of the figure indicate the helical segments of the polypeptide chain; they have been labeled according to Remington et al. (1982). Open circles mark the beginning and the end of individual helices.

long to segments connecting helices. These are found between the pairs of helices B–C (residues 45–70), H–I (residues 162–163), I–J (residues 195–201), J–K (residue 221), K–L (residue 236), M–N (residue 274), S–T (residue 418), all in the large domain, and between the pairs of helices O–P (residue 312) and Q–R (residues 366–373) in the small domain. Peaks 300, 311, 364, and 377 correspond to residues located inside the helices O, Q, and R of the small domain.

The profile indicates that a substantial fraction of the large domain remains unchanged in the two conformations. In all of its helices and the segments connecting helices A and B, C–H, and L and M, the polypeptide chain follows the same trajectory in both conformations of the protein. Hence, rearrangement of the large domain is accomplished mainly by a series of changes in each of four regions (between helices B–C, H–I, I–J, and S–T). These results are in agreement with the definition of a rigid core including helices F, G, H, I, L, M, and S by Lesk and Chothia (1984). (Helices F, G, L, and M are at the dimer's interface [Remington et al., 1982].) Indeed a least-squares superposition gives an RMS fit of 0.35 Å for the 126 α -carbon atoms of the core, whereas the expected RMS if the cores were identical at the present level of coordinate error is 0.40 Å. The small domain appears to be more flexible, including cases where conformational differences are found inside an α -helix. The locations of actual changes in the structure correlate well with those suggested by the $\Delta\alpha$ profile.

Discussion

The C α trace of a protein is a widely used representation of the protein's conformation. The C α -based torsion angles allow the information contained in the C α trace to be evaluated in a quantitative fashion. We have successfully applied the technique to several examples, ranging from nearly identical structures to cases of large conformational changes. The method has permitted us to identify regions where the directions of the polypeptide chains differ, without previous alignment of the structures.

In the case of both MBP and LAO, which represent examples of hinge-bending motion, the observed peaks are distrib-

uted sparsely along the polypeptide chain. Some of these peaks correspond to segments of the molecules that were known to be responsible for the displacement of one domain relative to the other. Moreover, peaks located in one of the domains in each protein suggested that these residues might allow for rearrangement of portions of the domain to accompany the larger motion. Inspection at the graphics workstation of the least-squares superposed open and closed conformations of both MBP and LAO confirmed that this was the case. Thus, the $\Delta\alpha$ plot helped us to formulate a more thorough description of the process: a large displacement of one domain with respect to the other, where one of the domains does not behave like a rigid body but exhibits some degree of flexibility.

The plot of the comparison of an open and a closed form of citrate synthase shows a different pattern than those of the hinge-bending examples. Clusters of peaks are found almost exclusively in the segments connecting α -helices. This is consistent with the fact that the shear motion takes place through a sum of small displacements. There is almost no motion inside the helices of the large domain, but a certain degree of flexibility is seen in the small domain. Interestingly, with the present approach, we easily gained much of the information that was obtained previously through more complicated comparison procedures (Lesk & Chothia, 1984; Chothia & Lesk, 1985).

Differences in crystal packing may be a source of signals above the 2σ cutoff in some cases. Therefore, it is important to examine the contact regions. However, the comparison of independently refined structures indicates that the level of noise is low, well below the expected RMSD. Peaks above 2σ can be present when improvement of the electron density map at higher resolution causes changes in the model; this is mainly to be expected to occur at loops and exposed regions. This technique can thus assist in the location of errors through the comparison of molecules related by noncrystallographic symmetry, of models that resulted from different refinement protocols, and of different models during the course of refinement. The only requirement for the comparison of the $C\alpha$ -torsion angle profiles of two structures is that they contain the same number of amino acid residues.

Korn and Rose (1994) have recently presented a similar method based on main-chain torsion angles (ϕ, ψ). Although the results obtained in both approaches are comparable, the level of noise due to coordinate error is lower for the $\Delta\alpha$ profile, thus making it useful even for structures known at medium and low resolution. Furthermore, due to the anticorrelation of $\psi(i-1)$ and $\phi(i)$ (McCammon et al., 1977), there may be instances where peaks present in both the $\Delta\phi$ and the $\Delta\psi$ plots do not have a counterpart in the $\Delta\alpha$ profile. In these cases, the $\Delta\phi$ and $\Delta\psi$ peaks do not represent a change in the course of the $C\alpha$ -trace, but a reorientation of the peptide in question, which in turn will not be detected by the $\Delta\alpha$ profile. Because the $C\alpha$ -torsion angle comparisons involve only one parameter (and one plot), the analysis is simpler. The difference in $C\alpha$ -torsion angles and in main-chain dihedral angles (ϕ, ψ) of the 11-residue "lid" of triosephosphate isomerase was previously used by Joseph et al. (1990) in the analysis of the motion of the loop. However, no discussion of either method or suggestions for their generalization were presented.

We propose the use of the $\Delta\alpha$ profile as a simple first tool for conformational analysis. The method can provide a quick and easy way to highlight the conformational differences between two forms of the same protein, guiding and complementing other techniques such as main-chain torsion angle differences and least-squares superposition of the appropriate portions of the structures.

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