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Structural Basis of Hierarchical Multiple Substates of a Protein. V: Nonlocal Deformations

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ABSTRACT **Distances between centers of gravity of individual residues are compared among the minimum energy conformations derived from the record of the Monte Carlo simulation of conformational fluctuations in the native state of a globular protein, bovine pancreatic trypsin inhibitor. It is found that local deformations originating from the multiplicity of local conformations cause deformations of the whole structure of the molecule in various ways, which can be classified into two types. Type 1: When a local deformation occurs in a region consisting of a few residues near the surface of the molecule, the whole shape of the molecule responds by deforming elastically. The magnitude of this deformation is in the range of thermal fluctuations calculated by the harmonic approximation around a single minimum. Type 2: We have observed one case belonging to the second type in which local deformations occur cooperatively in an extended region. This region goes across the whole molecule and divide the remaining parts into two. Atom packing changes in and around the extended region of local deformations. For this reason deformation in this region is plastic. Relative location and orientation between the divided two parts change very much. Deformation of the whole shape in this case, associated with the plastic deformation in an extended region, demonstrates that protein molecules have a flexibility beyond the harmonic limit.**

Key words: protein conformation, dynamics, Monte Carlo simulation, conformational energy, minimization, hierarchy in dynamics, conformational heterogeneity, flexibility, trypsin inhibitor

INTRODUCTION

Thermal fluctuations of native conformation of a small globular protein, bovine pancreatic trypsin inhibitor (BPTI), have been simulated by the Monte Carlo method,[1] and many minimum energy conformations (MECs) have been obtained by energy minimization starting from a number of sampled conformations.[2] In the third and fourth papers in this series we have shown that differences in local conformations in a pair of MECs are clustered in a few or several regions and that topology of atom packing changes only in and around these regions. How do the local deformations occurring within such regions affect the structure of the whole molecule? This is the question we address in this paper.

METHODS

Minimum Energy Conformations

The same nine MECs are studied here as in the previous papers.[3,4]

Map of Change in Interresidue Distances

Here we focus attention on the folded structures of the main chain, because it represents well the whole shape of the molecule. Because we are now not interested in atomic details of local conformations but in the overall shape of the molecule, we take the center of gravity of main chain atoms in each residue to represnt the position of the residue. Thus, interresidue distances are defined by distances between the centers of the gravity of individual residues.

Differences between corresponding interresidue distances in a pair of MECs are calculated and are expressed in Figure 1. From these maps we can see changes in interresidue distances systematically. We address the following two specific subjects by using these maps. (1) Nonlocal deformations of locally isomorphic segments. In the third paper in this series we carried out a systematic analysis of similarity of conformations of all possible segments in a pair of MECs. If conformation of a certain segment in a pair of MECs is similar, it is defined as ismorphic. If all possible short segments consisting of four consecutive residues within a certain segment are isomorphic between a pair of MECs, the segment is defined as locally isomorphic. In other words, a segment is locally isomorphic if it is isomorphic locally

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everywhere. A locally isomorphic segment may be isomorphic as a whole. Then it is simply an isomorphic segment. But a locally isomorphic segment may not be isomorphic as a whole, but be deformed in the overall shape. Such situations can be detected easily by the maps in Figure 1. (2) Relative movements between two isomorphic or locally isomorphic segments.

Stereodrawings of Displacements Vectors of Positions of Residues

Centers of gravities of corresponding residues in a selected set of isomorphic or locally isomorphic segments in a pair of MECs are superposed and resulting displacements vectors are shown in Figure 2. Figure 1a–e and Figure 2a–e are drawn for the same pairs of MECs. These stereodrawings help us see directly deformation of locally isomorphic segments and relative movements among the selected set of segments.

RESULTS

Deformations of Locally Isomorphic Segments

We present some examples of nonlocal deformations of locally isomorphic segments and describe how these segments are deformed.

Figure 1a is a map of changes in interresidue distances which occur in the conformational change from A320 to A180. In this pair of MECs the segment 1~ 13 is locally isomorphic. The interresidue distances between parts 1~ 4 and 9~ 13 increase. The whole structure of this locally isomorphic segment is therefore stretched. This nonlocal deformation is represented also by displacement vectors of residues in Figure 2a. The β-sheet segment, 17~ 35, is also locally isomorphic in this conformational change. The interresidue distances between parts 19~ 22 and 29~ 32 decrease, and those between parts 22~ 25 and 32~ 35 increase. This deformation is also represented by displacement vectors in Figure 2a.

On the other hand whole shapes of locally isomorphic segments are conserved in some other cases. The segment 1~ 13 is locally isomorphic also in the conformational change from A320 to A240. All of the interresidue distances in this segments change less than 0.2 Å. Thus, the whole shape of this segment does not change as much in this case (Figs. 1b and 2b) as in the conformational change from A320 to A180.

As in the examples shown above, the whole structures of the locally isomorphic segments are often deformed. The corresponding local structures are similar to each other. Therefore displacements of atoms change continuously along the chain. Recall that the molecule is deformed in a very low-frequency normal modes in such a way that displacements of atoms change continuously in space.[5] This continuity in atomic displacements is characteristic of elastic deformations. The nonlocal deformations observed in the locally isomorphic segments are elastic deformations.

Relative Displacements Between Locally Isomorphic Segments

Here we will see effects of local deformations on the relative locations and orientations between the locally isomorphic segments. The relative displacements between locally isomorphic segments are analyzed by inspecting the maps of changes in interresidue distances. Three types of modes are found in their displacements.

Mode 1: Two isomorphic segments do not change their relative location and orientation (see Fig. 1c). Changes in interresidue distances are less than 0.2 Å between the isomorphic segments 1~ 5 and 17~ 37, except for the distance between the residues 2 and 30. Between Cys-5 and Arg-17 two locally deformed segments and one isomorphic segment exist (Fig. 6 in ref. 3). Changes in interresidue distances are observed between the segments 9~ 11 and 1~ 5 and between the segments 9~ 11 and 17~ 37. The isomorphic segment 9~ 11 moves relative to both of the segments 1~ 5 and 17~ 37 (Fig. 2c). The local deformations in the segments 6~ 8 and 12~ 16 cause the displacement of segment 9~ 11, but cancel each other of their effects on the relative location and oreintation between the two isomorphic segments 1~ 5 and 17~ 37.

Mode 2: The whole structure of an isomorphic segment is deformed, and interresidue distances between the segment and another segment change. In the conformational change from A320 to A180 the locally isomorphic segment 17~ 35 (β-sheet) is deformed nonlocally (Fig. 2a). One of the β-strand, 19~ 25, moves toward the bottom and another, 29~ 35, moves toward the top. Between segments 1~ 13 and 17~ 35, distances between segments 1~ 6 and 32~ 35 increase and distances between segments 8~ 12 and 29~ 30 decrease. The whole structure of segment 1~ 13 is stretched as mentioned above. In other words, the upper half of this segment goes up and the lower half goes down. The distance changes are observed between the segments going in opposite directions and are not observed between those going in similar directions. In this mode interresidue distance changes between two locally isomorphic segments are mainly due to the internal deformations in the individual segments.

Mode 3: The whole structure of two isomorphic segments moves relative to each other. As shown in Figure 1d the isomorphic segment 1~ 5 and the locally isomorphic segment 9~ 44 move relative to each other in the conformational change from A200 to A180. The whole shape of segment 9~ 44 is almost conserved. On the other hand interresidue distances change greatly between the two segments. The changes in the distances indicate that segment

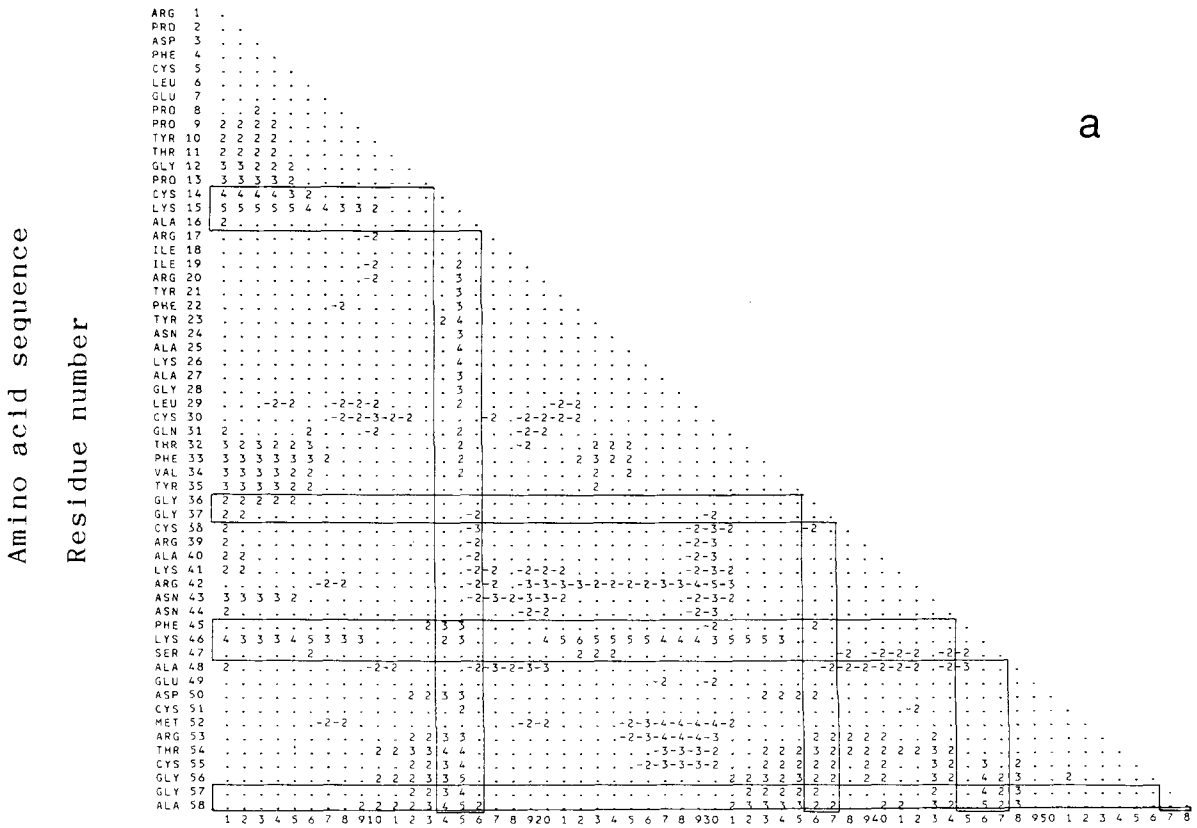


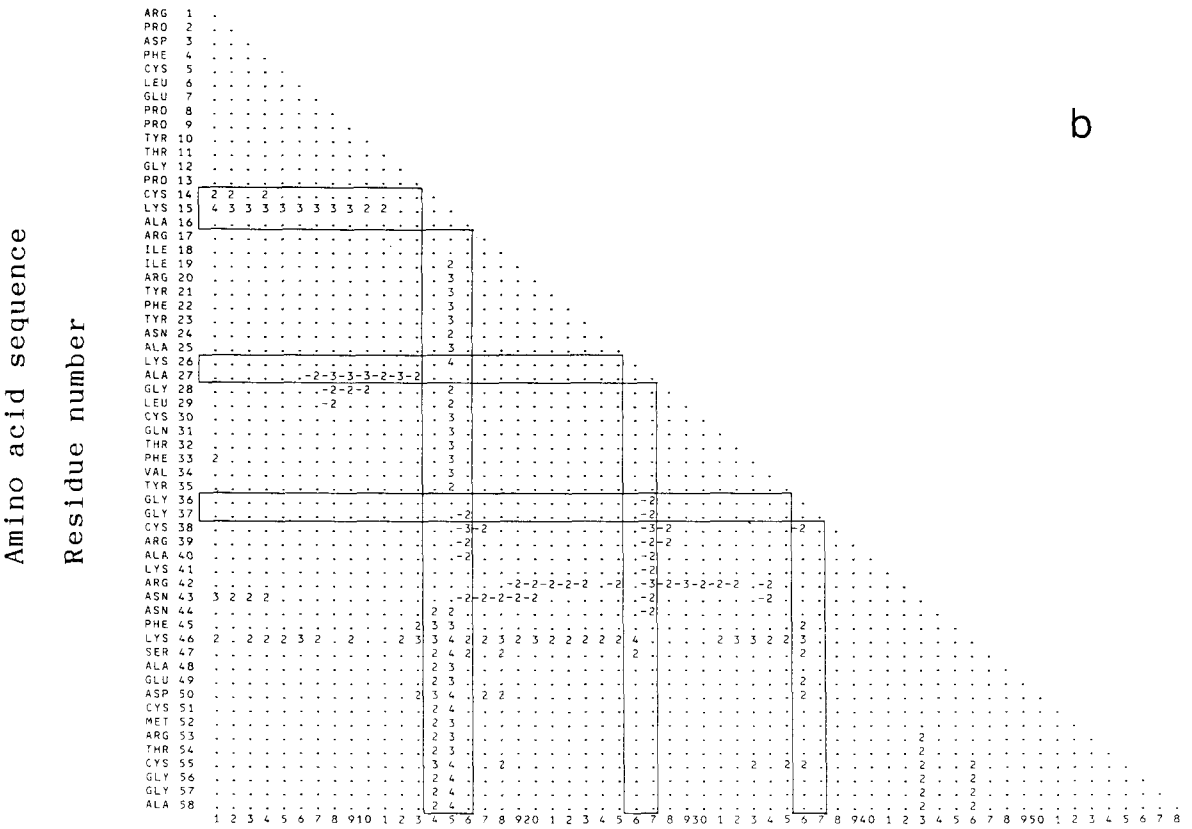
Fig. 1. Maps showing changes in interresidue distances for conformational changes: from A320 to A180 (**a**), from A320 to A240 (**b**), from A320 to A280 (**c**), from A200 to A180 (**d**), and from B440 to A240 (**e**). Changes of interresidue distances are divided by 0.1 Å, except for **e** in which they are divided by 0.5 Å, and then are truncated. The resulting number for the pair of residues *i* and *j* is put at position (*i*,*j*) in the map. Changes less than 0.2 Å are represented by dots. The positions of locally deformed main chain segments are marked by boxing residue-pair positions involving a residue in the locally deformed segments.

1~ 5 gets farther away from the upper half of the molecule consisting of the segments 9~ 20 and 33~ 44, and gets nearer to the bottom of the molecule, 27~ 29 (Fig. 2d). The two segments 1~ 5 and 9~ 44 move relatively, as if they are rigid bodies. Other examples of this mode are the relative displacement between segments 1~ 5 and 9~ 11, and that between 9~ 11 and 17~ 37 in the case shown in Figure 1c. In Figure 1e the map for the conformational change B440 to A240 is given. Large changes (≥ 0.5 Å) are observed in interresidue distances between isomorphic segments. There are examples of large relative displacements between the segments also in this mode. This map will be analyzed in detail later.

What is the mechanism of nonlocal deformations in mode 3? Are they also elastic deformations? In Figure 2d we show the change of topology of atom packing caused by local deformations which occur in a region between isomorphic segment 1~ 5 and locally isomorphic segment 9~ 44 in the conformational change from A200 to A180. This example indicates that relative displacements between the isomorphic and locally isomorphic segments are not elastic but plastic. In the deformation of mode 3 each locally isomorphic segment behaves like a rigid body. Therefore the two segments change their relative locations more easily than they change their individual shapes elastically.

Intergroup Conformational Changes

Here we analyze the differences between the two groups of MECs from the viewpoint of relative displacements among locally isomorphic segments. The root-mean-square deviations (RMSD) of C^{α} atoms of residues 5–55 between a pair of MECs in the same group are in the range from 0.17 to 0.34 Å. On the other hand those between an MEC in group A and another in group B are about 0.7 Å. This difference is caused by two mechanisms. First, local deformations in the intergroup conformational changes are already larger than those in the intragroup conformational changes.[3] Second, relative displacements


b
Amino acid sequence
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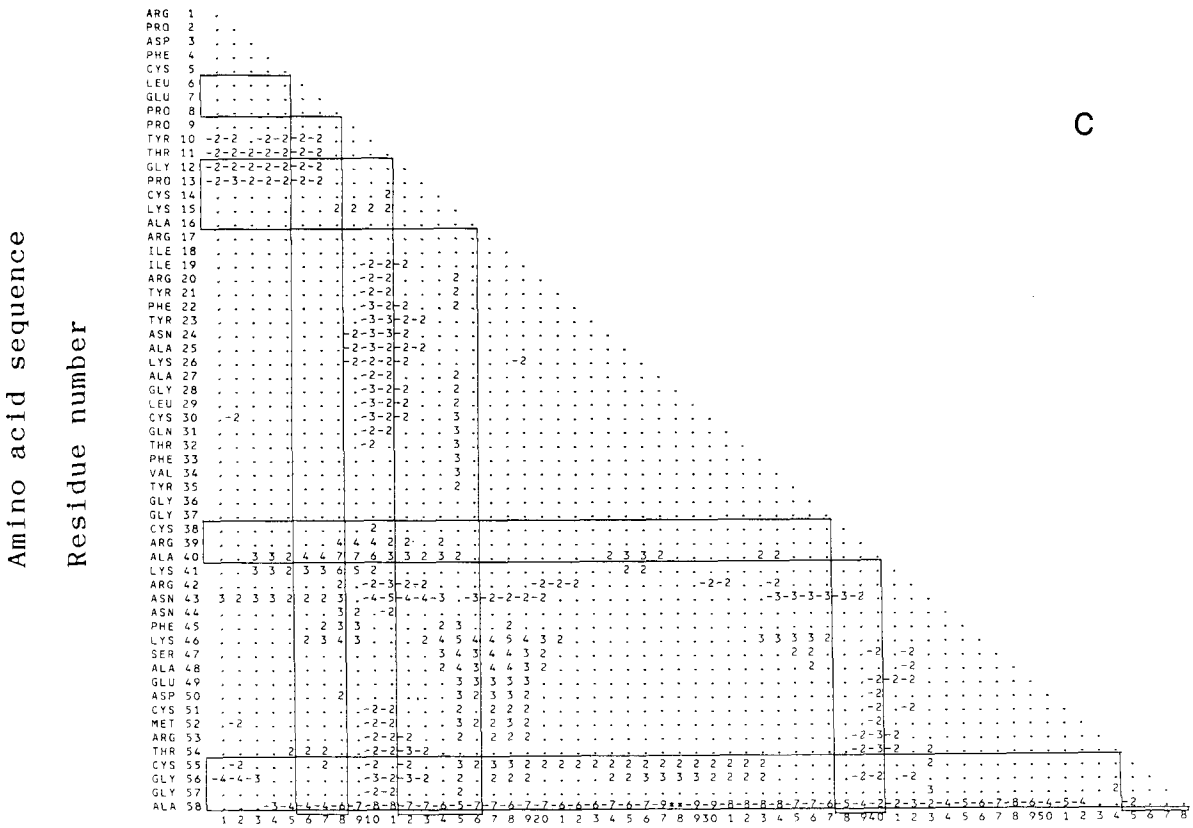
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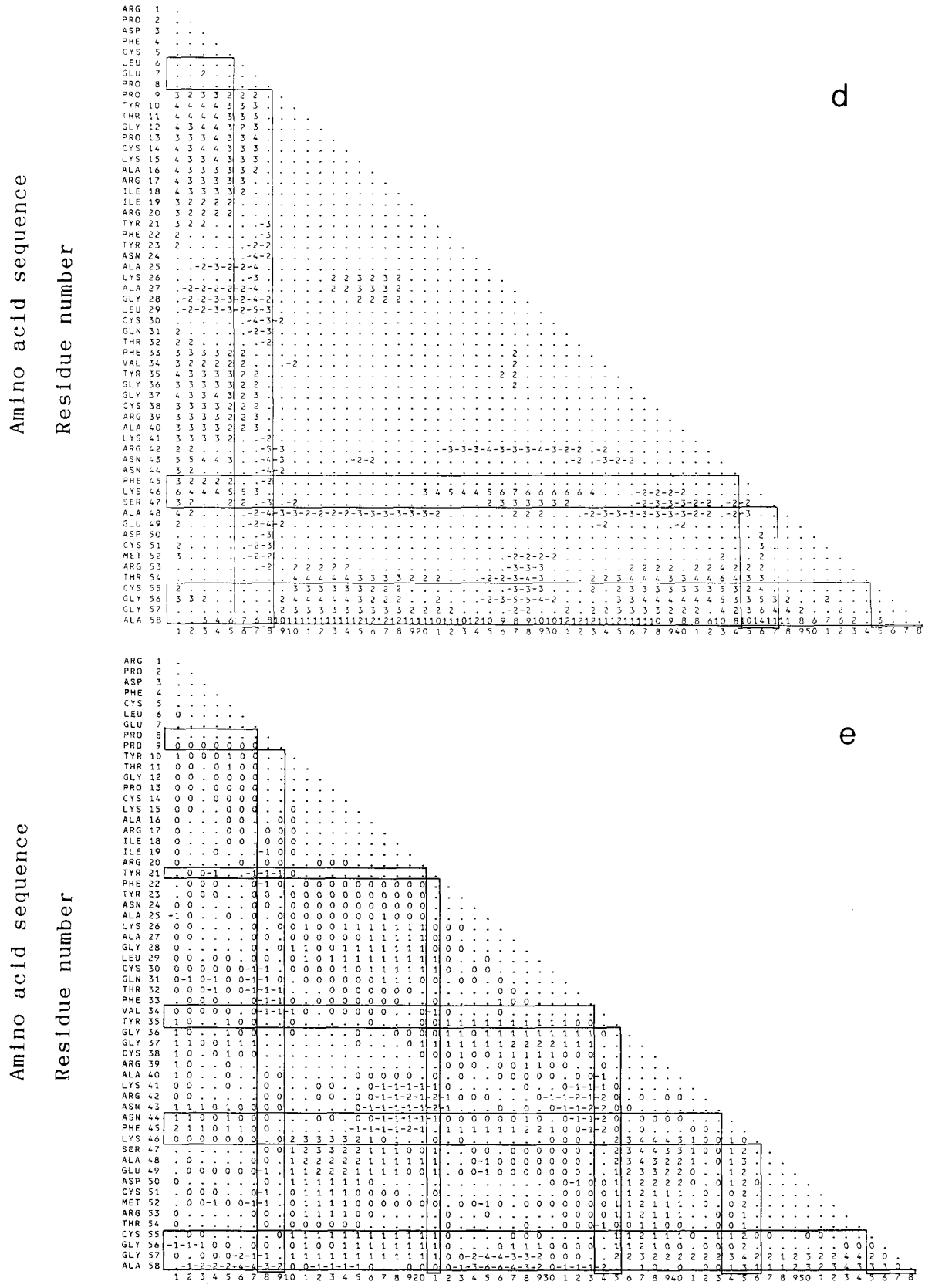
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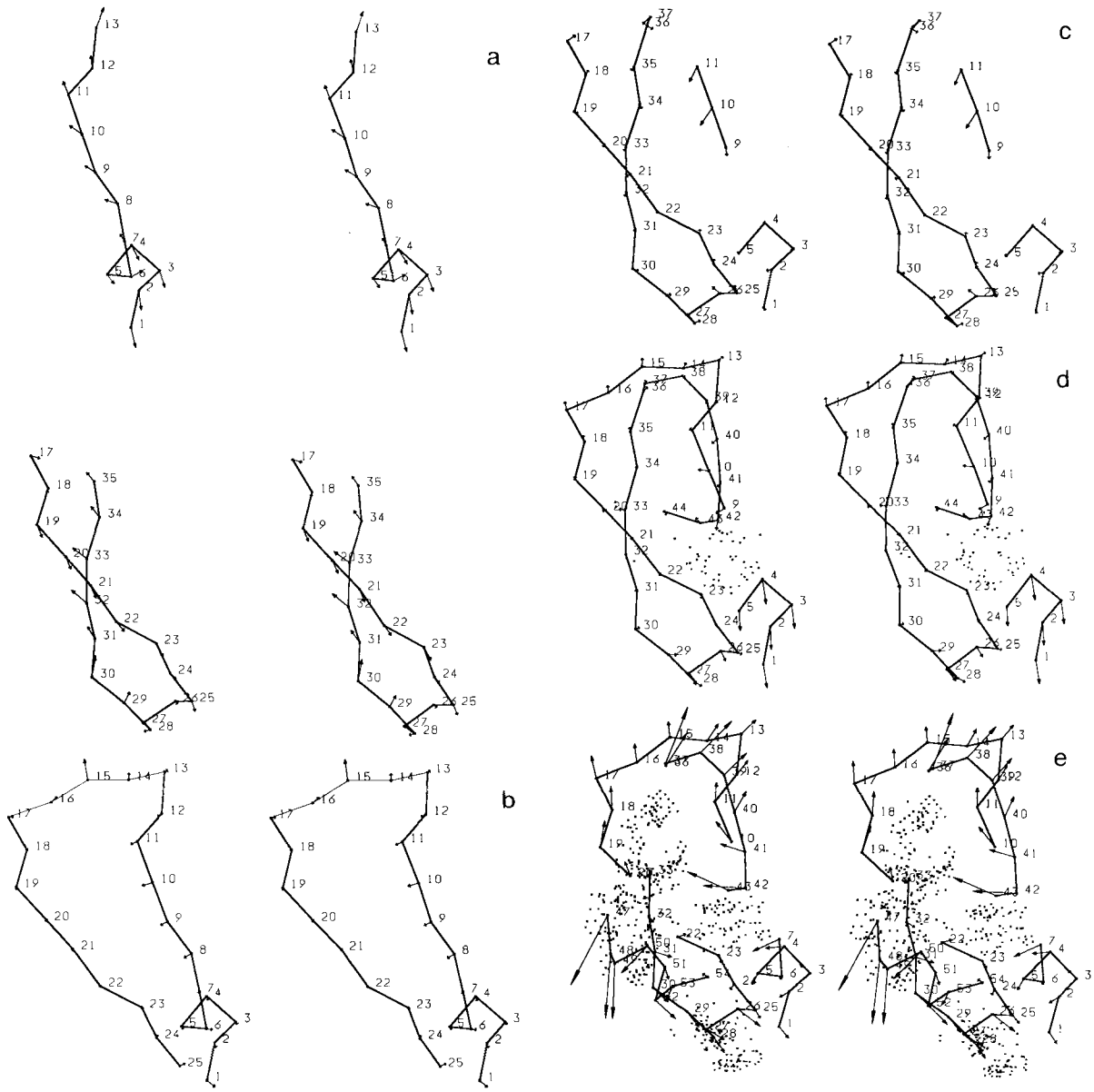

Fig. 2. Stereodrawings illustrating displacements of centers of gravity of main chain atoms in individual residues. **a**: From A320 to A180. Corresponding conformations of locally isomorphic segments 1~ 13 (upper) and 17~ 35 (lower) in the two MECs are superposed individually, and resulting displacement vectors are drawn. They are magnified 10 times for easy perception. **b**: From A320 to A240. Conformations of segment 1~ 25 in these MECs are superposed and resulting displacement vectors are shown. Vectors are magnified 5 times. Locally isomorphic segment 1~ 13 and isomorphic segment 17~ 25 are drawn in thick lines, and the locally deformed segment between them is drawn in a thin line. **c**: From A320 to A280. Conformations of isomorphic segment 1~ 5 and locally isomorphic segment 17~ 37 in these MECs are superposed and the displacements in these segments and an isomorphic segment 9~ 11 are shown by vectors with 5 times magnification. **d**: From A200 to A180. Conformations of locally isomorphic segments 9~ 44 in these MECs are superposed, and the displacement vectors in this segment and another isomorphic segment 1~ 5 are drawn with 5 times magnification. Each of the PACs (pairs of atoms in contact) of which interatomic distances change by more than 0.5 Å in local deformations between the two segments is represented by a dot placed at the mid-point between a pair of atoms. **e**: From B440 to A240. Conformations of five locally isomorphic segments, 1~ 7, 10~ 20, 22~ 33, 36~ 43, and 47~ 54, in these MECs are superposed, and the resulting displacement vectors (with 5 times magnification) are drawn. PACs whose interatomic distances change by more than 0.5 Å in local deformations among these segments are represented by the same way as in (**d**).

among locally isomorphic segments are also larger in the intergroup conformational changes.

In Figure 1e the map is shown for changes in interresidue distances in the conformational change from B440 to A240. In this conformational change five locally isomorphic segments exist: 1~ 7, involving the 3_{10}-helix; 10~ 20, involving a part of the β-sheet; 22~ 33, a lower part of the β-sheet; 36~ 43,

beginning at the C-terminal end of the β-sheet; and 47~ 54, a main part of the α-helix. The shape of these segments changes slightly. In these segments most of the changes in interresidue distances are less than 0.2 Å and some of them are in the range between 0.2 and 0.5 Å. Only one interresidue distance changes by more than 0.5 Å in these five segments. On the other hand large changes in interresidue distances are observed between the locally isomorphic segments. Especially, relative locations and/or orientations change greatly in the following pairs of the segments: 10~ 20 vs. 22~ 33, 10~ 20 vs. 47~ 54, 36~ 43 vs. 22~ 33, and 36~ 43 vs. 47~ 54. Changes in interresidue distances more than 1.5 Å are observed between 10~ 20 and 47~ 54 and between 36~ 43 and 47~ 54. These displacements are mode 3.

In this intergroup conformational change transitions among multiple local conformations of local parts occur collectively in a wide region, which extends across the whole molecule in such a way that it divides the molecule into upper and lower halves. The above four pairs of locally isomorphic segments are those with one in the upper and the other in the lower halves of the molecule. In Figure 2e the change of topology of atom packing in the above region where collective conformational changes occur is represented in the same way as in Figure 2d. This region lies between each of the four pairs of the locally isomorphic segments. The large relative displacements between the segments are attributed to the plastic deformations which occur in this region. The relative displacement between the two isomorphic segments in the upper half of the molecule (segments 10~ 20 and 36~ 43) is small and does not have any intervening plastic region between them. In this sense they form a locally isomorphic domain. The three isomorphic segments in the lower half of the molecule (segments 1~ 7, 22~ 23, and 47~ 54) form another locally isomorphic domain in a similar way.

SUMMARY AND DISCUSSION

The local deformations discussed in the previous papers in this series[3,4] affect the whole structure of the molecule in various ways. In case 1, the effects are confined within each locally deformed region and the whole shape does not change appreciably (Figs. 1b and 2b). In case 2, locally isomorphic segments are deformed elastically (Figs. 1a and 2a). In case 3, large changes occur in relative locations and orientations by the presence of a plastically deformed extended region between isomorphic segments (Figs. 1c–e and 2c–e).

Two categories of nonlocal deformations exist. The first is elastic deformation of parts of the molecule in a locally isomorphic domain. Locally isomorphic segments in such a locally isomorphic domain are deformed elastically under stress caused by local deformations. They also change their relative locations and orientations without a change of topology of atom packing in the domain. Nonlocal deformation in the second category is a relative displacement between locally isomorphic domains. Deformations in this category are plastic ones.

In the light of the results obtained in this study we discuss the characters of fluctuations of the first and second levels which were observed in the Monte Carlo simulation.[2]

Among MECs in group A, both two categories of nonlocal deformations are observed. The magnitude of change in interresidue distances in those deformations is in the range from 0.2 to 0.4 Å. This range is similar to that of the thermal fluctuations of those distances calculated within the harmonic limit. Local deformations occur near the surface and a main part of the molecule is always involved in a locally isomorphic domain. Therefore, deformation of the whole shape of the molecule in this level is determined mainly by the elastic properties. Nonlocal deformations in the second category are observed between such a small segment as the 3_{10}-helix and the remaining part of the molecule. However, these are restricted near the surface of the molecule.

The nonlocal deformation observed in the conformational change from B440 to A240 characterizes the fluctuations of the second level. Both two categories of nonlocal deformations are also observed in this case. The magnitude of relative displacement between the two locally isomorphic domains is beyond the harmonic limit. Thus, deformations of the whole shape of the molecule are determined mainly by the plastic properties. Local deformations which occurred between the two locally isomorphic domains make the whole structure of the molecule much softer than an elastic body such as assumed in the harmonic limit.

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