ELSEVIER

Strain tensor field in proteins

Takahisa Yamato

Faculty of Technology, Tokyo University of Agriculture and Technology, Koganei, Tokyo, Japan

The tensor fields of pressure strain imposed on protein molecules have been visualized by computer graphics and computational geometry. The pressure-induced deformations of lysozyme and myoglobin were analyzed by the present method, which regards each molecule as a patchwork of microscopic continuous bodies of Delaunay tetrahedra. The strain tensor describes the deformation of each tetrahedron. The illustrated deformations turned out to be complex and inhomogeneous ones in which local expansions and contractions concomitantly occurred. Not only the pressure deformation but also any other type of moderate deformation can be analyzed by this method.

INTRODUCTION

A protein can be regarded as a sophisticated molecular machine made up of various parts: Residues important for protein function are located properly in the active site on the framework of secondary structures. Biological activity of a protein, however, is controlled not only by the residues in the active site but also by the physical properties of the framework. To study mechanical features of a protein, it is useful to analyze structural changes caused by external perturbations. For example, when a high hydrostatic pressure is applied to a protein molecule, it undergoes inhomogeneous deformation according to the diverse flexibility from part to part.[1,2] It should be noted that the values of compressibility in different parts of a protein molecule extend over a wide range.[1] Because a protein molecule has numerous degrees of freedom, pressure-induced deformation is likely to be complex. Therefore, it is necessary to develop relevant computational tools for extracting useful information from the pressure-induced deformation of protein molecules.

Voronoi tessellation is often employed in the analysis of complex condensed matter such as amorphous materials or protein molecules. For example, Richards calculated the packing density of atoms in proteins by means of Voronoi polyhedra.[3] The volume of atoms on the protein surface during a molecular dynamics simulation of a small protein molecule was analyzed with Voronoi polyhedra.[4] Voronoi polyhedra define the territory of each atom and contiguity relationships among atoms uniquely. Using the contiguity relationships, it is possible to define an alternate tessellation of the space occupied by a protein: Delaunay tessellation. As a result of Delaunay tessellation, the space is decomposed into an assembly of Delaunay tetrahedra. Each edge of Delaunay tetrahedra connects a pair of contiguous atoms. Delaunay tetrahedra were employed to analyze a protein structure that is thermally fluctuating.[5] To the author's knowledge, the work reported in Ref. 5 is the first description of the application of Delaunay tetrahedra to the analysis of a protein structure.

Since a tetrahedron is the simplest polyhedron, it is possible to take advantage of its simplicity and to describe the structural change of a protein molecule as a continuum deformation mathematically in terms of linear algebra. Suppose that the structure of a protein is tessellated into an assembly of Delaunay tetrahedra prior to deformation. As the protein undergoes a structural change, each tetrahedron also changes its structure. The structural change of each tetrahedron is described mathematically by a strain tensor. Therefore the deformation of the protein molecule is represented by a strain tensor field. On the other hand, it is almost impossible to calculate the strain tensor field by means of Voronoi polyhedra. Therefore, Delaunay tetrahedra are more useful than Voronoi polyhedra for the structural analysis of protein deformation in this sense.

Strain tensor analysis was originally developed to study the pressure-induced deformation of myoglobin.[1] In the present work, Delaunay tessellation was combined with strain tensor analysis to establish a more sophisticated method of analyzing the structural changes in proteins. Furthermore, a computer graphics tool has been implemented to illustrate the strain tensor field in various parts of a protein molecule. These methods were applied to the analysis of pressure-induced deformation of two globular proteins: lysozyme and myoglobin. Computer graphics images of the strain tensor fields have provided us with comprehensive pictures of complex deformation in these proteins.

METHODS

The calculation and visualization of the tensor fields of a strain imposed on each protein required a set of structures,

Color Plates for this article are on pages 98 and 99.

Address reprint requests to: Assistant Professor Takahisa Yamato, Faculty of Technology, Tokyo University of Agriculture and Technology, Nakacho, 2-24-16, Koganei 184, Tokyo, Japan.
Received 16 January 1996; revised 22 February 1996; accepted 27 February 1996.

655 Avenue of the Americas, New York, NY 10010

0263-7855/96/$15.00
PII S0263-7855(96)00022-7

before and after pressure deformation. Two sets of X-ray coordinates, 2LYM (at 0.1 MPa) and 3LYM (at 100 MPa), were taken from the Protein Data Bank (PDB)[6] for lysozyme.[2] The structures under zero pressure and high pressure (100 MPa) were calculated by a normal mode method for myoglobin. A structure of myoglobin under high pressure was obtained from minimum energy structure[7] as a reference structure under no applied pressure. The volume change of the molecule was approximated as a linear function of normal mode variables. The pressure-induced shifts of the normal mode variables were then calculated as the thermal average of these variables by isothermal–isobaric partition function. These two structures are denoted hereafter as MB0 and MB100, respectively. Only heavy atoms were considered. Delaunay tetrahedra were constructed around buried atoms in each of the two structures. To distinguish buried atoms and exposed atoms, solvent-accessible surface areas (ASAs) were calculated for all atoms. Solvent-accessible surface areas are calculated by the Shrake and Ruply method.[8] Values of van der Waals radii of atoms were taken from Ooi et al.[9] Mesh points on atom spheres were taken by the improved method of Eisenhaber et al.[10] An atom was classified as buried if its ASA vanished. Delaunay tessellation was carried out following the algorithm developed by Tanemura et al.[11] Tetrahedra whose circumradii are greater than 5 Å were neglected. The regions that underwent continuous-body-like deformation were defined by the common tetrahedra between the two structures at the two different pressures. A tetrahedron was regarded as a common tetrahedron if the four atoms at the four vertices are conserved between the two structures.

Moderate deformation of a continuous body is described in terms of continuum mechanics. It is not possible to presume that a protein molecule is a continuum as a whole. It may be feasible, however, to regard a molecule as a patchwork of small continuous bodies. A set of Delaunay tetrahedra, conserved during deformation, is among the most relevant for such small continuous bodies. The deformation of each tetrahedron is described by a microscopic displacement gradient and strain tensor; the tetrahedron can be regarded as a microscopic continuum. A computer graphics tool has been designed to illustrate the strain tensor field. This visualization clearly illustrates the mechanical constructions of protein molecules.

RESULTS AND DISCUSSION

Color Plate 1 shows the pressure-induced deformation in lysozyme. The overall pattern of the strain tensor field shows at least two significant characteristics of the deformation: an inhomogeneous feature and continuous-body-like property. These two attributes are illustrated by colors and directions of the principal axes of the strain tensors. If the mechanical construction of the molecule were homogeneous, a monochromatic axes would appear. The color scheme adopted for the visualization of the principal axes of the strain tensors is one in which eigenvalues increase from blue to red. Axes in warm colors (orange to red) are recognized from place to place in Color Plate 1. This shows that local expansions were permitted, even though the overall volume change was negative. Another interesting feature is understood by comparing the colors and directions of the principal axes close to each other. They are often similar to each other. Such regions where nearby principal axes are similar to each other can be regarded as continuous-body-like regions. The RASMOL computer program[12] was employed to produce the pictures of the protein structure.

As mentioned above, the mechanical constructions of the protein molecules were inhomogeneous: They underwent pressure-induced structural changes, in which local contractions and local expansions occurred concomitantly. A small value of compressibility is possible for an inhomogeneous body in which expansions and contractions cancel each other out. Therefore, it should be noted that small compressibility of an inhomogeneous body does not always mean that the body is rigid. Considering the flexibility, mechanical constructions of the molecules were analyzed. Flexibility of an inhomogeneous body might be described better by the root-mean-square eigenvalues of the microscopic strain tensors rather than the compressibility itself.

Lysozyme is a typical $\alpha + \beta$ protein. The pictures of the strain field in the β sheets and α helices are shown in Color Plate 1A and B, respectively. There is a three-stranded β sheet in lysozyme. Color Plate 1A shows that the distribution of the eigenvalues of the strain tensors in the β sheet is wider on the right side, where turn T4 (residues 47–49) belongs, than on the left side, closer to the connecting region between domain 1 (residues 1–39, 89–129) and domain 2 (residues 40–88). This shows that the β sheet is flexible at the right side and is relatively rigid at the opposite side. Color Plate 1B shows the strain tensor field in the intrahelix regions. The mechanical construction of the helix (residues 108–115) at the right side of Color Plate 1B (indicated by the two arrows) was peculiar in that the structural response against pressure was different from those of the others. The upper pitch is rigid while the lower pitch is flexible. As we can see in Color Plate 1A and B, flexibility depends on the location, even within a secondary structure. The strain tensor field in the interface region of the two domains is shown in Color Plate 1C. Regions having high flexibility are indicated by the two arrows. Understanding of the mechanical constructions of different parts of a molecule is greatly enhanced by the aid of computer graphics and computational geometry.

Myoglobin is a typical α protein. Color Plate 2A and B shows the strain tensor field in intrahelix and interhelix regions, respectively. There are eight helices (A to H) in myoglobin. In Color Plate 2A, the backbone structure of the A helix (residues 3–18) is shown in red and the other parts are shown in yellow. In the A helix, red/blue (nongreen) axes appear more frequently than the other parts. This means that this region is more flexible than the rest. In Color Plate 2B the locations of helices G (residue 100–118) and H (residues 129–149) are indicated by arrows. The region between the two helices is magnified and is shown in Color Plate 2C. Most axes in the region are shown in blue and their directions are perpendicular to the axes of the two helices. The strain tensor fields between these two helices clearly show that the two helices became closer, due to pressure.

The number of common tetrahedra and their value of volume-weighted-average compressibility were 3,915 and $3.7 \times 10^{-11} \mathrm{Pa}^{-1}$ for lysozyme, while they were 6,064 and

9.6×10^{-11} Pa^{-1}, respectively, for myoglobin. The structures of the side chains of residues Arg-61, Arg-73, Lys-97, Gln-121, Arg-125, and Arg-128 in the lysozyme molecule were not determined accurately beyond C^{β}; these atoms were tentatively neglected. The calculated value of compressibility of the whole molecule of lysozyme is $4.7 \times 10^{-11} Pa^{-1}$.[2] The difference came from the fact that the common tetrahedra do not cover the whole molecule. The numbers of Delaunay tetrahedra contained in the molecules were 4756 and 4709 in 2LYM and 3LYM, respectively, while the numbers were 6456 and 6457 in MB0 and MB100, respectively. More than half of the tetrahedra were conserved during the pressure-induced deformations. The remaining part defines the region where the rearrangement of atom packing occurred. The rearrangement of atom packing associated with thermal fluctuations is discussed in the literature.[5]

So far, the visual analysis of strain tensor fields was confined to the regions where the spatial arrangement of atoms remained unchanged during deformation. These so-called elastic regions were defined by the common tetrahedra appearing both in the tessellation of one structure and in that of the other. On the other hand, the tetrahedra that do not belong to the common tetrahedra define the regions where the spatial pattern of atom packing was changed. A method capable of describing the deformation in these so-called plastic regions would be useful. Color Plate 3 shows the distortion in the plastic regions of hen egg white lysozyme. It appears that the two different strain fields, shown in blue and in red, are similar. However, care must be taken because the molecule should have undergone large-scale deformation here. For instance, around the position indicated by the arrow in Color Plate 3, the two different strain fields behave somewhat differently. If a pair of segments, one blue and one red, is in close proximity and each has nearly the same orientation and eigenvalue, then either of them can represent the local mechanical property well. It is necessary to develop a quantitative way to find the regions where the two different tensor fields are similar (Color Plate 4). Even though the absolute values of the eigenvectors in Color Plate 4 are relatively large compared to those appearing in Color Plate 1, these vectors also clearly illustrate what happens in a protein.

It should be noted that the present method is not restricted to analysis of the deformation caused by pressure but can be used for any kind of deformation of any type of protein. It would be interesting to apply this method in studying the mechanical properties of proteins belonging to other structural classes. An application of the present method to analysis of thermal expansion of a protein is currently underway.

CONCLUSIONS

A novel technique proposed to visualize the strain tensor fields in proteins has enabled us to understand at a glance what happens to proteins when external perturbations are applied to them. This method is ideally suited for the tensor fields resulting from small perturbations, during which the spatial patterns of atom packing are conserved in protein molecules. As a result of analysis by this technique, two examples of pressure-induced deformation of lysozyme and myoglobin show complex and inhomogeneous structural changes in which local expansions and contractions occurred concomitantly.

ACKNOWLEDGMENTS

Computations were performed at the Computer Center of Institute for Molecular Science. Supported by Grants-in-Aid from the Ministry of Education, Science and Culture, Japan. The author thanks Mr. Izumi for technical assistance.

REFERENCES

1 Yamato, T., Higo, J., Seno, Y., and Gō, N. Proteins 1993, **16,** 327–340
2 Kundrot, C.E. and Richards, F.M. *J. Mol. Biol.* 1987, **193,** 157–170
3 Richards, F.M. *J. Mol. Biol.* 1974, **82,** 1–14
4 Gernstein, M., Tsai, J., and Levitt, M. *J. Mol. Biol.* 1995, **249,** 955–966
5 Yamato, T., Saito, M., and Higo, J. *Chem. Phys. Lett.* 1994, **219,** 155–159
6 Bernstein, F.C., et al. *J. Mol. Biol.* 1977, **112,** 535–542
7 Seno, Y. and Gō, N. *J. Mol. Biol.* 1990, **216,** 95–109
8 Sharke, A. and Ruply, J.A. *J. Mol. Biol.* 1973, **79,** 351–371
9 Ooi, T., Oobatake, M., Nemethy, G., and Scheraga, H.A. *Proc. Natl. Acad. Sci. U.S.A.* 1987, **84,** 3086–3090
10 Eisenhaber, F., et al. *J. Comput. Chem.* 195, **16,** 273–284
11 Tanemura, M., Ogawa, T., and Ogita, N. *J. Comput. Phys.* 1983, **51,** 191–207
12 Sayle, R. *RASMOL.* Glaxo, Hertfordshire U.K., 1994