brief communication

Comparison of the dynamics of myoglobin in different crystal forms

George N. Phillips, Jr.
Department of Biochemistry and Cell Biology, Rice University, Houston, Texas 77251

ABSTRACT Crystals have been grown of "sperm whale" myoglobin produced in *Escherichia coli* from a synthetic gene and the structure has been solved to 1.9 Å resolution. Because of a remaining initiator methionine, this protein crystallizes in a different space group from native sperm whale myoglobin. The three-dimensional structure of the synthetic protein is essentially identical to the native sperm whale protein. However, the crystallographic B-factors for parts of the molecule are quite different in the two crystal forms, and provide a measure of the effect of different packing constraints on the flexibility of the protein. The effect of the packing forces is to reduce the mobility of the protein in the regions of contact and thereby introduce differences in mobilities between the two crystal forms. Discrepancies between mobilities calculated from molecular dynamics simulations and crystallography can be reduced by considering the data from both crystal forms.

INTRODUCTION

That the average structure of proteins in crystals and in solution are essentially the same has been well established (see references 1, 2). However, data on whether the dynamics of the protein in the crystal and in solution are the same has been more difficult to obtain. X-Ray crystallography can provide estimates of the mean square deviation from the average position of each atom, but cannot provide time constants or information about the coupling of motions of different parts of the molecule. X-Ray diffuse scattering (3, 4), nuclear magnetic resonance (5, 6), and inelastic neutron (7, 8) and gamma-ray scattering (9) will be useful experimental tools in this regard, but their applications to proteins have yet been fully developed. Another approach is to simulate the dynamics of proteins with computer models (see reference 10). This theoretical tool can be used to make specific predictions about the behavior of proteins, including details of their conformation and dynamic behavior. A major challenge has been to relate these predictions to experimentally measurable quantities.

X-Ray crystallography is being used to provide experimental data not only for protein structure but also dynamics (see reference 11). But whether crystal packing forces can significantly affect the dynamics of the protein has not been well established. Artymiuk et al. (12) have argued that except in very restricted regions, the crystal packing of lysozyme in two different space groups has not affected the crystallographic B-factors and hence the protein dynamics. On the other hand, Sheriff et al. (13) have noted different atomic mobilities for similar hemerythrins under different packing constraints. The structure of sperm whale myoglobin has now been solved in two space groups, $P2_1$ (14) and P6 (15) and a comparison of the atomic mobilities of this protein in the two crystal forms provides additional information on the effect of crystal packing on protein dynamics.

Address correspondence to George N. Phillips, Jr.

MATERIALS AND METHODS

Data for the P6 form of metmyoglobin were collected at 295° K and the structure was solved using molecular replacement as described elsewhere (15). Rigid body refinement and molecular dynamics refinement of the structure were done with the program XPLOR (16). Final refinement was done with program PROFFT (17) with electron density maps and difference maps calculated with program PROTEIN (18). Molecular graphics were generated with PSFRODO (19). Temperature factor data for the $P2_1$ form of metmyoglobin at 300° K were taken from Hartmann et al. (20).

RESULTS AND DISCUSSION

Metmyoglobin from a synthetic gene (21) has been crystallized in space group P6 and the structure has been solved and refined at 1.9 Å resolution. The crystallographic R-factor is 14.8% with appropriate stereochemical constraints (15). The packing of the myoglobin molecules in the P6 unit cell is necessarily different from the $P2_1$ form. In the new crystal form the molecules are packed rather tightly around the threefold crystallographic axes, but large solvent channels exist along the sixfold axes and smaller solvent channels near the twofold axes. Despite the much looser packing of molecules in space group P6 (62% solvent vs. 37% for the $P2_1$ form), the conformations of the protein backbones in the two forms are virtually identical, confirming that crystal packing

forces have had minimal effect on the average structure of the protein.

There are, however, differences in the mobilities of certain regions of the molecule as a result of the different lattice contacts in the crystal. These differences are seen in the crystallographic B-factors (Fig. 1). In the $P2_1$ crystal form, the A-helix (residues 18–22) and the EF corner (residues 118–125) of the molecule are relatively more mobile and the C-helix CD corner D-helix loop (residues 35–55) is much more rigid than in the P6 form. These effects on the flexibility of the molecule can be related to the packing contacts in each of the lattices (Fig. 1). In comparing the areas where the mobilities differ, tight crystal packing is associated with lower B-factors and hence less flexibility. This result contrasts with a similar analysis of lysozyme, where a comparison of the structure in orthorhombic and tetragonal space groups

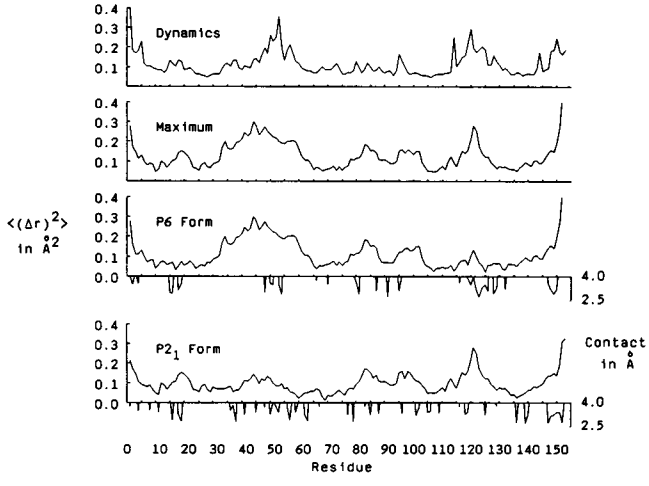

FIGURE 1 Comparison of the fluctuations of myoglobin backbone atoms from two different crystal structures and a molecular dynamics simulation. The ordinate, $\langle(\Delta r)^2\rangle$, is the mean square unidirectional displacement ($B/8*\Pi^2$: averaged over the C_α, N, and C atoms for the crystallographic data and $1/3\ [\langle(\Delta x)^2 + (\Delta y)^2 + (\Delta z)^2\rangle]$ averaged over the same atoms in spans of 25 ps for the dynamics simulation). B-factor data for the $P2_1$ form are from the 300° K curve of Hartmann et al. (18) and data for the P6 form from the structure reported by Phillips et al. (submitted for publication). The molecular dynamics data are from a vacuum simulation by Levy et al. (20). The best agreement between theory and experiment comes from taking the maximum atomic mobility observed in either crystal space group to produce an "unconstrained" profile (labeled as "Maximum"). Crystal packing contacts are shown below the P6 and $P2_1$ fluctuation curves. Contacts were determined by calculating distances between all atoms of the residues on the abscissa and any atom in any neighboring symmetry-related molecule. Contacts for the $P2_1$ form were calculated from the highly refined coordinate data of Johnson et al. (26). The contacts are also plotted as contact distance vs. residue number for both crystal forms. The scale for the crystal contacts goes from 4.0 Å down to 2.5 Å in increments of 0.5 Å. The upper limit on the distance considered to be a contact was 4 Å.

revealed minor differences in B-factors (12). Although some features of the curves remain unchanged, our results indicate that lattice packing constraints can indeed influence the dynamic behavior of proteins.

Results from molecular dynamics simulations can be compared with this new picture of myoglobin dynamics derived from x-ray crystallography. In their 300 ps simulation of myoglobin, Levy et al. (22) noted the discrepancy between the calculated extent of mean square fluctuations and the experimental mobilities. Part of this discrepancy can be accounted for by the demonstrated underestimation of crystallographic B-factors for highly mobile parts of the protein (23). But these authors also noted a large unexplained discrepancy between observed and calculated displacements for the part of the protein including residues 40–60, which includes part of the C-helix, the CD corner, and the D-helix. The discrepancies between observed and calculated mobilities can now also be explained in terms of constraints brought about by packing forces. There is a strong correspondence between regions of reduced mobility and crystal contacts (Fig. 1). In the region comprising residues 40–60 there are extensive contacts and less mobility in the $P2_1$ form. Conversely, in the region 110–130 there are more contacts and less mobility in the P6 form. There still appears to be a weak underlying trend which is insensitive to the packing constraints, but the actual mean square fluctuations can be affected by a multiplicative factor of two to three.

A "hybrid" experimental mobility curve has been generated by scaling the B-factor data from the two crystal forms and taking the maximum of the two values at each amino acid position. This curve could be considered to be more free of packing constraints than either of the individual curves, and, indeed, matches the block-averaged mean square fluctuations of Levy et al. better than either of the crystallographically determined curves (Fig. 1). The coefficients of correlation have been calculated to quantitatively compare the curves with a value of 1.0 indicating perfect correlation and 0.0 indicating no correlation. The coefficient between the $P2_1$ crystal data and the dynamics curve is 0.47, between the P6 crystal data and the dynamics curve is 0.46, and between the "maximum" function and the dynamics curve is 0.57. Thus, the "maximum" function fits the molecular dynamics data significantly better than either set of crystal data. Furthermore, these results provide evidence that molecular dynamics calculations were correct in predicting high mobility for residues 40–60, even though these residues were relatively immobile in the $P2_1$ crystal form.

The fact that the mobilities of the CD-loop region are higher over an even wider range of residues than the molecular dynamics simulation predicts may be because of the limited time duration of the simulation. There may

be large scale motions of this loop that take longer than 300 ps to develop. Additional evidence for large scale fluctuations in this region come from the crystallographic studies of myoglobin as a function of temperature (24). The CD corner was observed to undergo a more dramatic compaction than the rest of the molecule as the temperature was lowered, implying that this region is more loosely packed at higher temperatures. Large scale movements of this loop may in fact play a role in ligand binding (25–27).

Thus, for myoglobin, there is a significant effect of the crystal packing constraints on the mobility of the protein. The effect can be large and spread over 10–20 amino acid residues. The observation that the packing causes significant reductions but not increases in mobilities further supports the notion that large-scale protein motions are heavily damped and largely diffusive, rather than harmonic (4). This result also suggests that direct comparisons of simulated dynamics of individual protein molecules with molecules in the crystal may not always be valid.

I thank Barry Springer and Stephen Sligar for providing the bacterially expressed myoglobin.

This work was supported by NSF grant DMB-8716507, NIH grant AR32764, and the Robert A. Welch Foundation C-1142.

Received for publication 17 July 1989 and in final form 13 October 1989.

REFERENCES

1. Wuthrich, K. 1989. Protein structure determination in solution by nuclear magnetic resonance spectroscopy. *Science (Wash. DC).* 243:45–50.
2. Makinen, M. W., and A. L. Fink. 1977. Reactivity and cryoenzymology of enzymes in the crystalline state. *Annu. Rev. Biophys. Bioeng.* 6:301–343.
3. Boylan, D, and G. N. Phillips, Jr. 1986. Tropomyosin: Characterization of anisotropic motions and coupled displacements in crystals. *Biophys. J.* 49:76–78.
4. Caspar, D. L. D., J. Clarage, M. Salunke, and M. Clarage. 1988. Liquid-like movements in crystalline insulin. *Nature (Lond.).* 332:659–662.
5. Campbell, I. D., C. M. Dobson, and R. J. P. Williams. 1978. Structures and energetics of proteins and their active sites. *Adv. Chem. Phys.* 39:55–107.
6. London, R. E. 1980. Intramolecular dynamics of proteins and peptides as monitored by nuclear magnetic resonance. *Magn. Res. Biol.* 1:1–69.
7. Middendorf, H. D. 1984. Biophysical applications of quasi-elastic and inelastic neutron scattering. *Annu. Rev. Biophys. Bioeng.* 13:425–451.
8. Cusack, S., J. Smith, J. Finney, B. Tidor, and M. Karplus. 1988. Inelastic neutron scattering analysis of picosecond internal protein dynamics. *J. Mol. Biol.* 202:903–908.
9. Neinhaus, G. U., J. Heinzl, E. Huenges, and F. Parak. 1989. Protein crystal dynamics studied by time-resolved analysis of x-ray diffuse scattering. *Nature (Lond.).* 338:665–666.
10. Karplus, M., and J. A. McCammon. 1983. Dynamics of proteins: elements and function. *Annu. Rev. Biochem.* 53:263–300.
11. Petsko, G. A., and D. Ringe. 1984. Fluctuations in protein structure from X-ray diffraction. *Annu. Rev. Biophys. Bioeng.* 13:331–371.
12. Artymiuk, P. J., C. C. F. Blake, D. E. P. Grace, S. J. Oatley, D. C. Phillips, and M. J. E. Sternberg. 1979. Crystallographic studies of the dynamic properties of lysozyme. *Nature (Lond.).* 280:563–568.
13. Sheriff, S., W. A. Hendrickson, R. E. Stenkamp, L. C. Seiker, and L. H. Jensen. 1985. Influence of solvent accessibility and intermolecular contacts on atomic mobilities in hemerythrins. *Proc. Natl. Acad. Sci. USA.* 82:1104–1107.
14. Kendrew, J. C., H. C. Watson, B. E. Strandberg, R. G. Hart, D. R. Davies, D. C. Phillips, and V. C. Shore. 1960. Structure of myoglobin: a three-dimensional Fourier synthesis at 2 Å resolution. *Nature (Lond.).* 185:422–427.
15. Phillips, G. N. Jr., R. M. Arduini, B. A. Springer, S. G. Sligar. 1990. Crystal structure of myoglobin from a synthetic gene. *Proteins* In press.
16. Brünger, A. T., J. Kuriyan, and M. Karplus. 1987. Crystallographic R factor refinement by molecular dynamics. *Science (Wash. DC).* 235:458–460.
17. Finzel, B. C. 1987. Incorporation of fast Fourier transforms to speed restrained least-squares refinement of protein structures. *J. Appl. Crystallogr.* 20:53–55.
18. Remington, S., G. Wiegand, and R. Huber. 1982. Crystallographic refinement and atomic models of two different forms of citrate synthase at 2.7 Å and 1.7 Å resolution. *J. Mol. Biol.* 158:111–152.
19. Pflugrath, J. W., M. A. Saper, and F. A. Quiocho. 1984. Methods and Applications in Crystallographic Computing. Hall, S. and Ashida, T., editors. Clarendon Press, Oxford. 404–407.
20. Hartmann, H., F. Parak, W. Steigemann, G. A. Petsko, D. Ringe Ponzi, and H. Frauenfelder. 1982. Conformational substates in a protein: structure and dynamics of metmyoglobin at 80 K. *Proc. Natl. Acad. Sci. USA.* 79:4967–4971.
21. Springer, B. A., and S. G. Sligar. 1987. High-level expression of sperm whale myoglobin in *Escherichia coli. Proc. Natl. Acad. Sci. USA.* 84:8961–8965.
22. Levy, R. M., R. P. Sheridan, J. W. Keepers, G. S. Dubey. S. Swaminathan, and M. Karplus. 1985. Molecular dynamics of myoglobin at 298° K. *Biophys. J.* 48:509–518.
23. Kuriyan, J., G. A. Petsko, R. M. Levy, and M. Karplus. 1986. Effect of anisotrophy and anharmonicity on protein crystallographic refinement. *J. Mol. Biol.* 190:227–254.
24. Frauenfelder, H., H. Hartmann, M. Karplus, I. D. Kuntz, Jr., J. Kuriyan, F. Parak, G. A. Petsko, D. Ringe, R. F. Tilton, M. L. Connolly, and N. Max. 1987. Thermal expansion of a protein. *Biochemistry.* 26:254–261.
25. Kuriyan, J., S. Wilz, M. Karplus, and G. A. Petsko. 1986. X-ray structure and refinement of carbon-monoxy (FE II)-myoglobin at 1.5 Å resolution. *J. Mol. Biol.* 192:133–154.
26. Johnson, K. A., J. S. Olson, and G. N. Phillips, Jr. 1989. Structure of myoglobin-ethylisocyanide: histidine as a swinging door for ligand entry. *J. Mol. Biol.* 207:459–463.
27. Lambright, D. G., S. Balasubramanian, and S. G. Boxer. 1989. Ligand and proton exchange dynamics in recombinant human myoglobin mutants. *J. Mol. Biol.* 207:289–299.