

Compressibility of lysozyme protein crystals by X-ray diffraction

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(Received 26 September 1995; accepted 8 January 1996)

Abstract

Single-crystal high-pressure X-ray diffraction studies on the protein crystals of orthorhombic and tetragonal hen egg-white lysozyme polymorphs were carried out using a Merrill-Bassett diamond-anvil cell, image-plate detector and synchrotron radiation. The orthorhombic crystal has been squeezed to 85.5% of its ambient pressure volume at about 1.0 GPa; the crystal compresses anisotropically, and neither a glass transition or denaturation was observed. The tetragonal polymorph of lysozyme undergoes amorphization at pressures about 0.2 GPa.

1. Introduction

Applications of high pressure in studies of proteins steadily increase over the past two decades (*e.g.* see Steenbock Symposium, 1994; Jannasch, Marquis & Zimmerman, 1987; Krzyżaniak, Barciszewski, Satański & Jurczak, 1994; Weber & Drickamer, 1983; Wong, 1990). Recently high-pressure studies on proteins were surveyed by Frauenfelder *et al.* (1990); the authors pointed out at the possibility offered by high-pressure methods to investigate biological phenomena at the molecular level of isolated proteins, they also stressed the significance of the information which could be provided by structural studies of proteins at high pressures. High-pressure crystallographic studies of proteins are scarce. Kundrot & Richards (1986) designed especially for diffractometric studies of protein crystal a pressure cell withstanding about 200 MPa,† attached to an external pressure generator. They found that the unit-cell volume of tetragonal hen egg-white lysozyme (hereafter tLy) crystals is squeezed by about 1.1% at 100 MPa. Another high-pressure cell for X-ray studies of biopolymers at pressures up to 300 MPa has been designed recently by Kriechbaum, Steinhart, Pressel & Lagner (1994). This study investigates the application of a commercially available Merrill-Bassett (Merrill & Bassett, 1974) diamond-anvil cell for protein crystallography, using image-plate detectors, and examines the compressibilities of the orthorhombic and tetragonal hen egg-white lysozyme protein crystal polymorphs (abbreviated oLy and tLy, respectively).

2. Experimental

An oLy crystal (prism of 0.22 × 0.12 × 0.09 mm) together with a ruby chip and a cotton-wool fibre to prevent movements of the protein crystal (a protein sample cannot be glued to a diamond culet like other types of crystals) were mounted in a pressure chamber (0.4 mm hole in a 0.2 mm tungsten foil) filled with the stabilizing mother liquor applied as hydrostatic fluid. Pressure in the cell was assessed from the compression

Table 1. Pressure dependence of the unit-cell dimensions of hen egg-white lysozyme crystals, orthorhombic form, space group $P2_12_12_1$

The ambient-pressure values are those reported by Artymiuk, Blake, Rice & Wilson (1982).

Pressure (MPa)	<i>a</i> (Å)	<i>b</i> (Å)	<i>c</i> (Å)	<i>V</i> (Å ³)
0.1	59.4	68.7	30.8	125700
450	57.53 (2)	67.51 (2)	29.94 (2)	116300
640	56.92 (2)	66.79 (2)	29.53 (2)	112300
1000	56.33 (3)	65.96 (4)	28.97 (2)	107400

of the oLy crystal, by assuming that the crystal contains 43% solvent by volume (Matthews, 1968), and that compressibility of the water and the macromolecules can be roughly approximated by combining the compressibility of 0.03 normal solution of NaCl in water (Lange's Handbook of Chemistry, 1979; Kalendarz Chemiczny, 1954), with the compressibility of molecular crystals (Katrusiak, 1991a), respectively. This pressure assessment agreed within experimental error with the pressure determined by measuring the ruby-fluorescence shift (Piermarini, Block, Barnett & Forman, 1975) after the last pressure generation. The cell was mounted on a single-axis rotation camera on the X31 synchrotron beamline of EMBL, at DESY, Hamburg. The wavelength used was 0.9200 Å. The detector used to record the diffraction pattern was an EMBL image-plate scanner (Hendrix & Lentfer, 1994) with the circular plate of 210 mm in diameter and pixel size 187 µm. Two 1° rotation images were recorded for each value of pressure in the cell; rotation range of the spindle axis was the same for each pair of images and exposure time was 3 min per image. The crystal-to-detector distance was 200 mm giving a maximum resolution of 1.85 Å at the edge of the plate. The first pair of images was already recorded at the elevated pressure to ensure that the cell was sealed. Three pairs of images were recorded in this way. The images were interpreted by the use of program *DENZO* (Otwinowski, 1993) and successfully autoindexed for the primitive orthorhombic lattice. Each image was then interpreted individually and appropriate parameters refined by least-squares (l.s.) fit of observed and predicted positions and partialities of all reflections. There were about 600 reflections on every image. The parameters refined were: crystal *a*, *b*, *c* unit-cell dimensions, its three orientation angles and coordinates of the direct beam and the scanner distortion parameters. The crystal-to-plate distance was not calibrated precisely, and, as a consequence, absolute values of the unit-cell dimensions obtained for different pressures are not highly accurate; much more precise are the relative differences between them. Results of the l.s. refinements are shown in Table 1. It has been established that the tLy polymorph pressurized above 0.4 GPa gave no diffraction pattern, indicating that the crystals underwent amorphization. Several tLy samples were examined; in each case the samples after amorphization have not

† 1 Pa = 9.869 · 10⁻⁶ atm, 1 MPa = 9.869 atm, 1 GPa = 9869 atm, 0.1 MPa = 1 bar.

changed their appearance: they remained clear, and showed no cracks or any other signs of damage. The tLy samples transferred from the high-pressure cell into capillary for examination at ambient conditions showed no diffraction. On a sample pressurized to about 0.15 GPa, a diffraction pattern of weak and broad reflections, indicating that the amorphization of tLy may already start at this pressure. The diamond-anvil cells are not ideally suited for the work at the 0.1–500 MPa range, and this study is continued by using a gas-pressure system.

3. Results and discussion

The relative changes in the unit-cell dimensions are shown in Fig. 1. No significant changes in reflection intensities were noted to 1.0 GPa, which suggests that the oLy crystals are stable in this pressure range. The oLy unit cell is compressed anisotropically and the pressure dependence of a is clearly non-linear: in the lower range of pressures a is compressed stronger than b or c , while above about 0.5 GPa a becomes harder than the other two unit-cell dimensions. The protein crystals are 'soft' because of void spaces, flexibility of the macromolecules, various types of disorder and substantial contents of water. It is known that by increasing pressure it is possible to reduce or eliminate disorder in molecular crystals and to significantly reduce the amplitudes of thermal vibrations of atoms and molecules (Katrusiak, 1990, 1991*a,b*). It is plausible that the non-linearity in compressibility of a is due to the voids in the oLy structure being squeezed in the lower range of pressures, and ordering of the disordered fragments. The volume compressibility of oLy, $\beta_v = 1/V_0(\delta V/\delta P)$, is 0.17 GPa^{-1} between 0.1 MPa and 0.4 GPa, and 0.15 GPa^{-1} to 1.0 GPa.

The present study of tLy indicates that these crystals become amorphous below 0.4 GPa. The volume compressibility of the tLy crystal calculated from the unit-cell

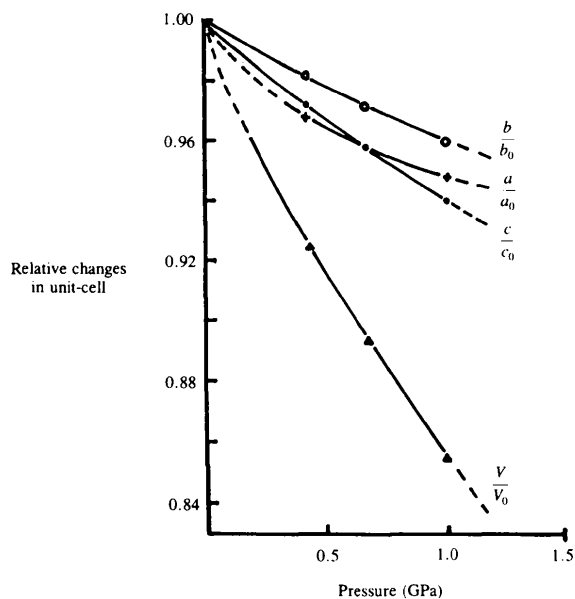


Fig. 1. Relative changes of the oLy unit-cell dimensions with pressure.

dimensions measured at 101.3 MPa by Kundrot & Richards (1986) of 0.071 GPa^{-1} is two times smaller than the presently measured compressibilities of oLy, and comparable with the compressibilities of much harder hydrogen-bonded molecular crystals. For example, β_v of pentaerythritol is 0.062 GPa^{-1} (Katrusiak, 1995) and of 2-methyl-1,3-cyclohexanedione 0.096 GPa^{-1} (Katrusiak, 1991*b*). The small β_v value of tLy may be connected with a pressure-induced composition change leading to amorphization. Pressure-induced amorphization has been observed in several materials (*e.g.* Luo & Ruoff, 1993; Hazen, Finger, Hemley & Mao, 1989); however, to our knowledge this is the first evidence of a significant difference in the pressure of amorphization between two polymorphs. Observation of denaturation of Ly in solution at pressures above 0.5 GPa (Heremans & Wong, 1985; Chen & Heremans, 1990) indicates, that proteins in certain crystal structures may be more resistant to denaturation than in solutions.

References

- Artymiuk, P. J., Blake, C. C. F., Rice, D. W. & Wilson, K. S. (1982). *Acta Cryst.* **B38**, 778–783.
- Chen, G. & Heremans, K. (1990). *High Pressure Res.* **5**, 749–751.
- Frauenfelder, H., Alberding, N. A., Ansari, A., Braunstein, D., Cowen, B. R., Hong, M. K., Iben, I. E. T., Johnson, J. B., Luck, S., Marden, M. C., Mourant, J. R., Ormos, P., Reinisch, L., Scholl, R., Schulte, A., Syamsunder, E., Sorensen, L. B., Steinbach, P. J., Xie, A., Young, R. D. & Yue, K. T. (1990). *J. Phys. Chem.* **94**, 1024–1037.
- Hazen, R. M., Finger, L. W., Hemley, R. J. & Mao, H. K. (1989). *Solid State Commun.* **72**, 507–511.
- Hendrix, J. & Lentfer, A. (1994). Unpublished data.
- Heremans, K. & Wong, P. T. T. (1985). *Chem. Phys. Lett.* **118**, 101–104.
- Jannasch, H. W., Marquis, R. E. & Zimmerman, A. M. (1987). *Editors. Current Perspectives in High Pressure Biology*. New York: Academic Press.
- Kalendarz Chemiczny (1954). *Państwowe Wydawnictwa Techniczne*. Vol. I, p. 207.
- Katrusiak, A. (1990). *Acta Cryst.* **B46**, 246–256.
- Katrusiak, A. (1991*a*). *Cryst. Struct. Techn.* **26**, 523–531.
- Katrusiak, A. (1991*b*). *High Pressure Res.* **6**, 155–167.
- Katrusiak, A. (1995). *Acta Cryst.* **B51**, 873–879.
- Kriebbaum, M., Steinhart, M., Pressel, K. & Laggner, P. (1994). *23rd Steenbock Symposium on High Pressure Effects in Molecular Biophysics and Enzymology*, University of Wisconsin, Madison, USA.
- Krzyżaniak, A., Barciszewski, J., Szański, P. & Jurczak, J. (1994). *Int. J. Biol. Macromol.* **16**, 153–158.
- Kundrot, C. E. & Richards, F. M. (1986). *J. Appl. Cryst.* **19**, 208–213.
- Lange's Handbook of Chemistry (1979). *XIIth edition*, edited by J. A. Dean, p. 10–118. New York: McGraw Hill.
- Luo, H. & Ruoff, A. L. (1993). *Phys. Rev. B*, **48**, 569–572.
- Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
- Merrill, L. & Bassett, W. A. (1974). *Rev. Sci. Instrum.* **45**, 290–294.
- Otwiniński, Z. (1993). *An Oscillation Data Processing Suite for Macromolecular Crystallography*. Yale University, New Haven, CT, USA.
- Piermarini, G. J., Block, S., Barnett, J. D. & Forman, R. A. (1975). *J. Appl. Phys.* **46**, 2774–2780.
- Steenbock Symposium (1994). *High Pressure Effects in Molecular Biophysics and Enzymology, Collected Abstracts*, University of Wisconsin-Madison, USA.
- Weber, G. & Drickamer, H. G. (1983). *Quart. Rev. Biophys.* **16**, 89–112.
- Wong, P. T. T. (1990). *High Pressure Res.* **5**, 752–754.