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Comparison of structures of dry and wet hen egg-white lysozyme molecule at 1.8 Å resolution

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A high resolution structure of hen egg-white lysozyme containing 36 ± 1 mol H_2O per mol of protein has been obtained using triclinic (P_1) crystals cross-linked with glutaraldehyde. Analysis of dehydration-induced structural changes has revealed displacement in relative position of domains and numerous small displacements in positions of individual atoms with r.m.s. deviation of main atoms 0.60 Å, and that of all atoms 0.97 Å. An increase in the average packing density of atoms in dry lysozyme by 4–6% seems to be the most probable reason for the loss of its activity and mobility.

Dehydration; Protein structure; Lysozyme; X-ray diffraction

1. INTRODUCTION

The use of X-ray analysis to monitor dehydration-induced structural changes in proteins has been inhibited by the conventional viewpoint that the drying of protein crystals causes a fading of the diffraction pattern and a decrease in the minimum X-ray spacing, making it impossible to analyze the structure with a high resolution. This phenomenon was discovered at the very beginning of X-ray analysis of proteins [1] and repeatedly observed later. However, it does not occur in all protein crystals. Recently we have found that triclinic crystals of hen egg-white lysozyme, even when extensively dehydrated, retain their ability to diffract X-rays at high angles [2,3]. In this work this unique property of the crystals is used to obtain the structure of the lysozyme molecule in a hydrated state and to compare this structure with that in more hydrated forms.

2. MATERIALS AND METHODS

Triclinic (P_1) crystals of hen egg-white lysozyme were grown and cross-linked with glutaraldehyde as described in [4]. Crystals were dehydrated for several days in open quartz capillaries placed in a closed chamber over $CaCl_2$ solutions (98–32% humidity range), or silica gel (about 0.01%). The amount of water in the crystal at any given humidity was determined from its dehydration isotherm [5,6]. The unit cell parameters of the 'dry' crystal (dried over silica gel) were: $a = 26.18$ Å, $b = 30.67$ Å, $c = 32.33$ Å, $\alpha = 84.8°$, $\beta = 115.1°$, $\gamma = 112.3°$. Those of the 'wet' crystal: $a = 27.3$ Å, $b = 31.8$ Å, $c = 34.3$ Å, $\alpha = 88.7°$, $\beta = 108.5°$, $\gamma = 112.0°$.

Diffraction data were collected for 98, 70, 39, 30 and 0.01% humidities using a SYNTAX $P2_1$ 4-circle automatic diffractometer. Intensities were corrected for LP, radiation damage and adsorption [7]. The programs CORELS [8] and PROFFT [9] were used for the refinement of the structure, with the last one being an improved version of the PROLSQ [10].

The structure of the hydrated (230 ± 8 H_2O/protein mol) lysozyme in the mother liquid with water activity corresponding to 98% humidity, was refined starting from the coordinates of the 7LYZ model in the Brookhaven Data Bank. Each structure with a reduced water content was refined in a step-wise procedure using coordinates of more hydrated lysozyme. In refinement of the structure of the 'dry' molecule 6185 independent reflections were taken in a zone between 10 and 1.8 Å. 2781 covalent bond lengths, 178 bond angles, 144 chiral centers and 640 torsion angles were used as steric limitations in the refinement. The final value of the R-factor in the resolution zone between 5 and 1.8 Å reached 0.27; r.m.s. deviation of the bond lengths from the standard values was 0.025 Å. Water molecules were not included in the refinement at this stage (refinement with water molecules included is now in progress).

3. RESULTS AND DISCUSSION

The loss of 80% of all intracrystalline water in the crystal resulted in shrinkage of its unit cell volume by 16%, accompanied with a rotation of the lysozyme molecule as a whole by 1.1° and a shift in position of its center of gravity by 0.15 Å [3].

Changes in internal structure of the lysozyme molecule were calculated by fitting the whole structures of hydrated and dry molecules, individual domains or elements of secondary structure. The results, presented in Figs. 1–3 and in Table I, show that the average hydration-induced perturbations in internal structure of the molecule are not large, they do not exceed the dif-

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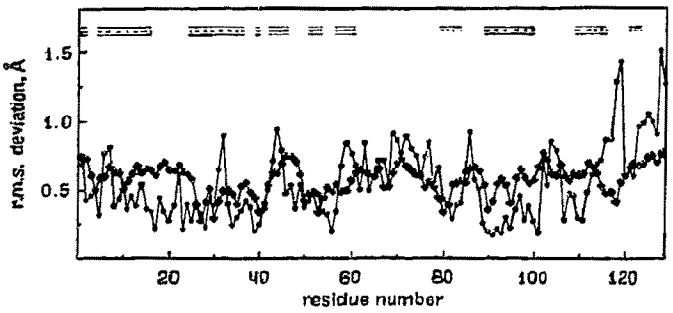

Fig. 1. Distribution of r.m.s. displacements of the main chain atoms in dry lysozyme from their positions in a completely hydrated crystal - circles. For a comparison r.m.s. deviations calculated from B-factor in the dehydrated crystals are presented - squares (residues ranges marked ═══ indicate α-helix, ═══ β-sheet or ═══ 3_{10}-helix).

ferences between the structures of homologous proteins. The dehydration-induced displacements are seen from Fig. 1 to be nearly equal to the overall deviations in position of atoms calculated from B-factor. However, no correlation between the distributions of these parameters over the amino-acid sequence seems to exist.

R.m.s. deviations in position of main-chain atoms of α-helices are almost everywhere lower than average one and the r.m.s. deviations in loops. Comparing the figures in the last two columns of Table I, one can conclude that r.m.s. deviations in position of the atoms originate both from the internal distortion of structural elements and from their displacement as a whole within the molecule. Thus, dehydration induces a motion of domains within the lysozyme molecule. It could be seen in a stereo view of atomic displacements presented in Fig. 2 and may be described as a relative rotation of the domains by 2.26° around the axes shown in the picture (it goes approximately through the positions of C_α atoms of 25-28 and 56-58 residues).

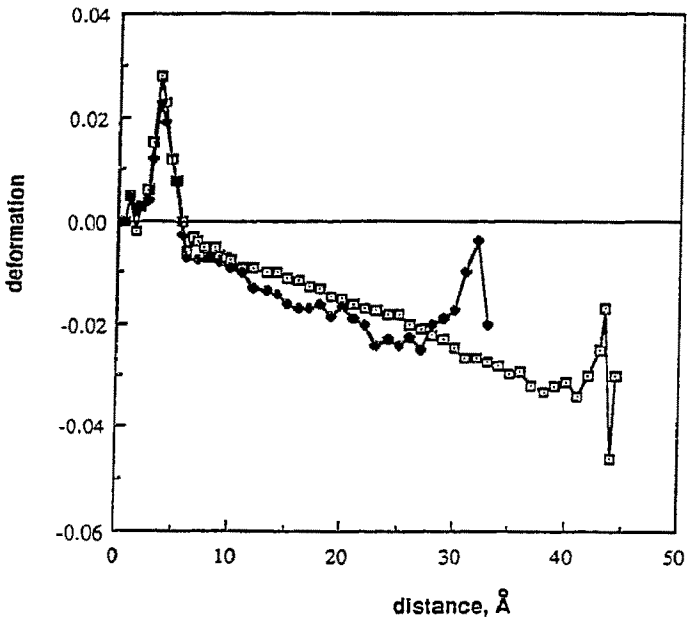

Fig. 3. Average relative dehydration-induced changes of interatomic distances, $[|r_i-r_j|_{dry}-|r_i-r_j|_{wet}]/|r_i-r_j|_{wet}$, in lysozyme molecule plotted versus the interatomic distance value, $|r_i-r_j|_{wet}$ (r_i is radius-vector of i-th atom in dry or wet molecule). Empty squares are for all atoms (except hydrogen) in the whole molecule; filled squares, for those in domain 2 only.

Physically, the most significant difference between 'dry' and 'wet' lysozyme molecules seems to consist in compression of the globule upon drying. The inflated molecular volume calculated according to [11] at 98, 30 and 0.01% humidities is equal to $1.93 \cdot 10^4$ Å^3, $1.84 \cdot 10^4$ Å^3 and $1.82 \cdot 10^4$ Å^3, respectively. Drying compresses the whole molecule and its domains by about 4-6%. The volume deformation found is in good agreement with the energies available in the dehydration process. Assuming purely elastic compressibility upon dehydration and taking for bulk modulus the value $\beta^{-1} = 7 \cdot 10^9$ N/m^2 from ultrasonic measurements [12], we obtain 90

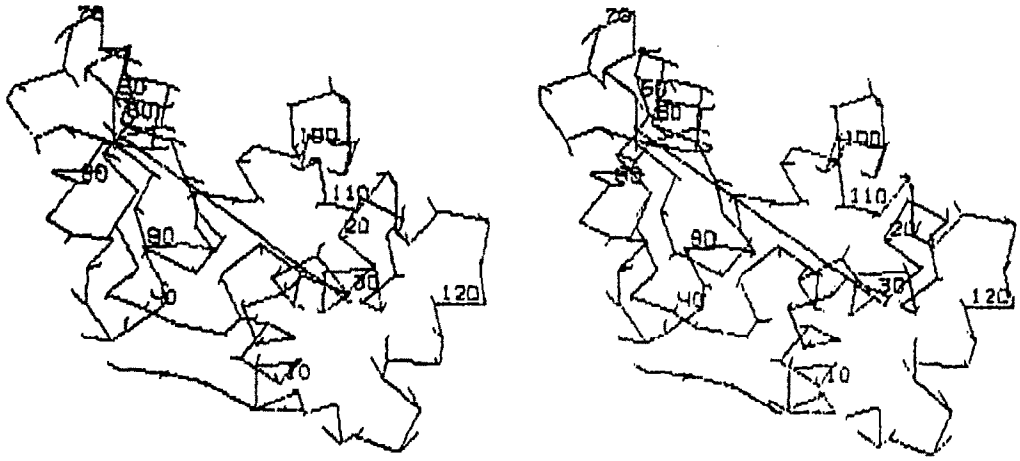

Fig. 2. Stereo pair presenting the displacement of C_α-atoms in lysozyme molecule due to the dehydration on lowering the humidity from 98 to 0.01%.

Table I

R.m.s. differences in positions of atoms in lysozyme molecule within 'dry' and 'wet' triclinic crystals

Structural element	Residues	Number of atoms	R.m.s. deviation, Å	
			Fitting molecules[a]	Fitting elements[b]
Whole[c]	1-129	1000	0.974	-
Domain 1[c]	1-39, 100-129	548	1.008	0.968
Domain 2[c]	40-99	452	0.932	0.857
Whole[d]	1-129	387	0.600	-
Domain 1[d]	1-39, 100-129	207	0.622	0.574
Domain 2[d]	40-99	180	0.575	0.575
α-Helixes[d]	4-15	36	0.492	0.319
	24-36	39	0.441	0.355
	88-99	36	0.317	0.280
	108-115	24	0.505	0.300
3_{10}-helix[d]	79-84	18	0.460	0.305
10	120-124	15	0.718	0.323
Loops[d]	16-23	24	0.396	0.286
	61-78	54	0.731	0.412
	85-87	9	0.734	0.212
	100-107	24	0.563	0.476
β-sheet[d]	39-60	66	0.528	0.415

[a] R.m.s. deviations between dry and wet structures after superimposing the whole molecules are presented.
[b] The same after superimposing the structural elements separately.
[c] Calculation for all atoms except hydrogen.
[d] Calculations for the atoms of the main chain (N, C_α and C).

kJ/mol for energy needed to compress the globule volume by 5%. This is nearly a half of the entire dehydration energy of the crystal, calculated from its isotherm [6].

These small changes in density packing of protein atoms may explain the drastic differences in catalytic activity [13], hydrogen exchange rate [14] and other mobility-related properties of dried proteins as compared to completely hydrated ones, since they are comparable with the volume changes accompanying melting of molecular crystals and with the overall thermal free volume, which makes space for motion of atoms in native proteins (a simple estimate has given about 4% for the last value [15]). Another reason for drastic decrease in enzymatic activity may be dehydration-induced perturbation in the conformation of side chain groups involved in the active site cleft. A detailed analysis of changes in position of these and other side chains and strongly bound water molecules is now in progress and will be published elsewhere.

It is worth noting that dehydration-induced compression of the molecule seen from Fig. 3 is not uniform, (relative changes of distances in a uniformly deformed body would be constant), presumably reflecting inhomogeneity in elastic properties of the lysozyme structure. Another peculiarity of the plots in Fig. 3 needing to be explained, is a peak displaying the stretching in interatomic distances between the nearest neighboring atoms. Since very small atom displacements (less than 0.1 Å) are responsible for the peak, we cannot exclude this peak to be an artifact of the refinement procedure used. However, it might be physically relevant because repacking of the protein atoms upon dehydration appears to be accompanied by distortions in their surrounding.

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