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Characterization of lysozyme crystals with unusually low solvent content. By H. G. Nagendra, C. Sudarsanakumar and M. Vijayan, *Molecular Biophysics Unit, Indian Institute of Science, Bangalore 560 012, India*

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Abstract

Studies on the low-humidity (88%) forms of tetragonal and monoclinic lysozyme, resulting from water-mediated transformations, have provided a wealth of information on the variability in protein hydration, its structural consequences and the water structure associated with proteins, in addition to facilitating the delineation of the rigid and the flexible regions in the protein molecule and the invariant features in its hydration shell. Surprisingly, monoclinic lysozyme continues to diffract even when the environmental humidity is drastically reduced, thus permitting the structural study of the enzyme at different levels of hydration. As part of a study in this direction, three very low humidity forms, two of them occuring at a nominal relative humidity of 38% and the other at 5% relative humidity, have been characterized. These have unprecedented low solvent contents of 16.9, 17.6 and 9.4%, respectively, as determined by the Matthews method.

Introduction

Water-mediated transformations in protein crystals, resulting from changes in levels of hydration consequent to the variation in environmental humidity, provides a useful handle for exploring the variability in protein hydration and its structural consequences (Salunke, Veerapandian & Vijayan, 1984; Salunke, Veerapandian, Kodandapani & Vijayan, 1985; Kodandapani, Suresh & Vijayan, 1990). Studies involving them have also led to the delineation of the rigid and flexible regions of the lysozyme molecule and the invariant features in its hydration shell (Madhusudan & Vijayan, 1991). Among the water-mediated transformations examined so far, that in monoclinic lysozyme is the most remarkable (Salunke *et al.*, 1985). The form at 88% relative humidity (r.h.) diffracts better than the native crystals and has a solvent content (Matthews, 1968) as low as 22% by volume which is perhaps the lowest observed to date in protein crystals. Indeed, X-ray analysis of this form has yielded valuable information on the water structure associated with proteins (Madhusudan, Kodandapani & Vijayan, 1993). It may be noted that the solvent content of 88% r.h. monoclinic lysozyme nearly corresponds to 0.2 g of water per g of protein, the minimum level of hydration shown to be necessary for the onset of mobility and enzyme activity of lysozyme (Rupley, Gratton & Careri, 1983). We present here the X-ray characterization of well ordered monoclinic forms of the enzyme with still lower solvent contents obtained at very low values of relative humidity.

Experimental

The monoclinic crystals of hen egg-white lysozyme were grown employing the well known method described in the literature (Steinrauf, 1959) using a protein sample bought from Sigma Chemicals Co., USA. The r.h. around the crystals was maintained at desired levels by replacing the mother liquor at one end of the thin-walled capillary containing the crystal by the appropriate saturated salt solution or concentrated sulfuric acid (Rockland, 1960; CRC Handbook of Chemistry and Physics, 1980–81). Typically the 1 mm capillary was 7 cm long of which the salt solution or sulfuric acid covered 3 cm. A few additional grains of the solute were left in the solution to ensure that it was indeed saturated. The crystals were allowed to equilibrate for about 24 h after replacing the mother liquor before they were examined using precession photography. The r.h. around the crystal in the capillary was assumed to be the same as that reported in enclosed space above the relevant solution or acid (Rockland, 1960; CRC Handbook of Chemistry and Physics, 1980–81). In addition to those from the known native and 88% r.h. forms, diffraction patterns were recorded from crystals at nominal r.h. values of 79, 57, 47, 38, 20, 12 and 5%. The unit-cell parameters were determined from precession photographs. In a few instances, they were refined on a Siemens area-detector system. Matthews' method was used to estimate the solvent content in the crystals, assuming the partial specific volume to be 0.74.

Results and discussion

The most remarkable, and indeed unexpected, result of the experiments is the extraordinary stability of the crystals which retain moderate order even when concentrated sulfuric acid is used for dehydration. Evidence of discontinuities in structural changes, is provided by changes in the diffraction pattern. These discontinuities are not as pronounced at lower humidities as it is in the transformation at around 90% r.h. (Salunke *et al.*, 1985). However, the diffraction pattern and the unit-cell dimensions at the nominal r.h. of 38% obtained using saturated zinc nitrate solution are distinctly different from those of the 88% r.h. form. This is true of both of the slightly different forms obtained at this r.h. value. Further pronounced changes are observed when the nominal environmental humidity is reduced to 5% using concentrated sulfuric acid with a density of 1.67 g cm^{-3}. The diffraction patterns obtained from the native crystals and the 88, 38 and the 5% r.h. forms are shown in Fig. 1 for comparison. The corresponding unit-cell dimensions and the solvent contents are given in Table 1. Although the quality of the pattern improved when the r.h. is reduced to 88%, further reduction in humidity results in its slow progressive deterioration. The reliability of the Matthews method for estimating solvent content when it is abnormally low is a moot point. In any case the solvent content of the 38 and 5% r.h. forms, which diffract to better than 2.4 Å resolution, are much lower than expected or observed so far. The detailed analysis of these forms, which is in progress using area-detector data, would provide a description of the protein molecule at extremely low levels of hydration. The comparison of these structures with those of the native and 88% r.h. forms is also expected to yield information on structural changes that accompany hydration or dehydration.

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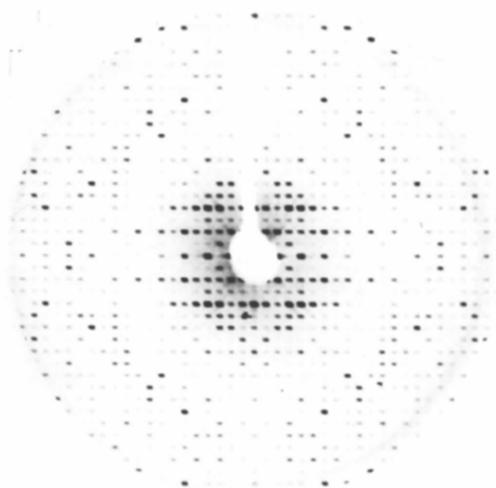
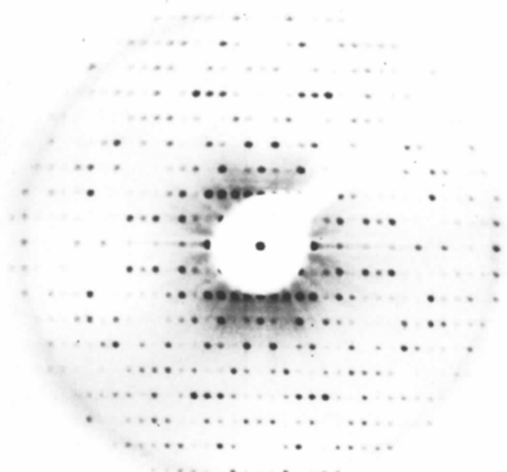

(a)

(b)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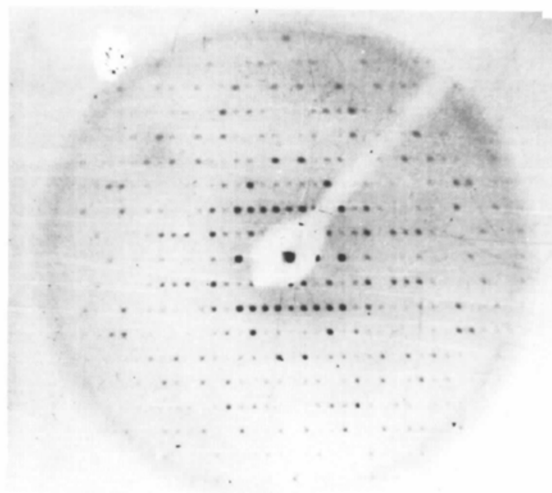
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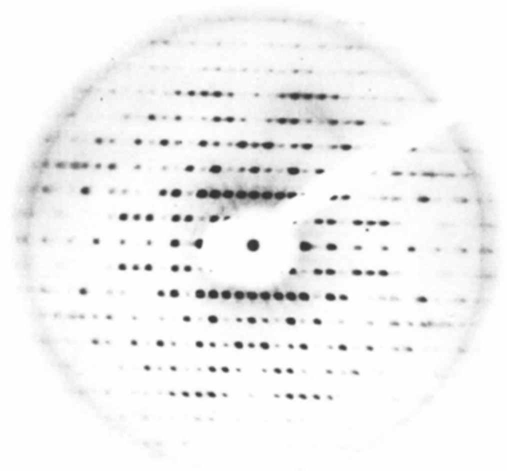
(e)

Fig. 1. 15° $0kl$ precession photographs of (a) native monoclinic lysozyme, and the low-humidity forms obtained at nominal r.h. values of (b) 88%, (c) 38% (form I), (d) 38% (form II) and (e) 5%. In all cases, the crystal was mounted along the b axis and the crystal-to-film distance was 60 mm.

Table 1. *Unit-cell dimensions and solvent contents of monoclinic lysozyme at different levels of hydration*

Form	a (Å)	b (Å)	c (Å)	β (°)	Z	$Ø_S$ (%)
Native	28.0	62.8	60.4	90.9	4	32.4
88%	26.9	59.0	31.3	111.9	2	22.2
Form I	26.6	56.0	31.3	112.2	2	16.9
38%						
Form II	26.3	55.9	31.5	109.9	2	17.6
5%	25.3	54.7	30.7	111.2	2	9.4

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