

For the  $x$  component of the modulation function this means that if  $u_x^1 = u_x(\mathbf{q}_i \cdot \mathbf{r}_1^0)$ , then  $u_x^2 = -u_x(\mathbf{q}_i \cdot \mathbf{r}_2^0)$ , with  $\mathbf{r}_1^0 = (x, y, 0)$  and  $\mathbf{r}_2^0 = (-x, y, \frac{1}{2})$ .

In the dualistic description the symmetry is split into two parts. The first is  $G_B$ , which gives the symmetry of the basic structure. Atoms 1 and 2 are related by a  $c$  glide,  $c_x$ . The second is  $G_M$ , which gives the symmetry of the modulation pattern. The key point of the dualistic interpretation is that  $G_M$  defines the symmetry of the modulation functions considered as continuous functions in space. In general,

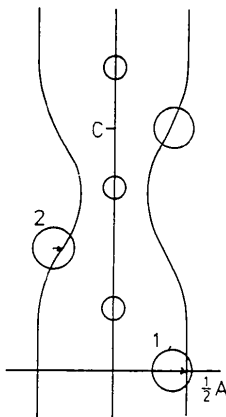


Fig. 2. Projection on the  $AC$  plane (see Fig. 1). The wavy lines describe the modulation functions of atoms 1 and 2, respectively. The amplitude is exaggerated by a factor of five.

this symmetry does not apply to the displacements of the two atoms related by an element of  $G_B$ . Therefore, to test for the presence of a mirror plane in  $G_M$ , we have to compare the values of  $\mathbf{u}^1$  and  $\mathbf{u}^2$  at the corresponding positions. Substitution of the components of  $\mathbf{q}_i$  and of  $\mathbf{r}_1^0 = (x, y, z)$  and  $\mathbf{r}_2^0 = (-x, y, z)$  into the above expressions for  $\mathbf{u}^1$  and  $\mathbf{u}^2$ , immediately shows the presence of a mirror plane as symmetry element of the modulation pattern. In Fig. 2 the relationship between average positions, atomic displacements and the modulation pattern is illustrated. A similar analysis for the other atoms and the other symmetry elements shows that the structure as determined by van Smaalen *et al.* (1986) has the full  $G_M$  symmetry.

In conclusion, we have shown that, contrary to the assertion of Prodan & Boswell (1987), the previously determined modulated structure of  $\text{NbTe}_4$  (van Smaalen *et al.*, 1986) is in full accordance with the dualistic interpretation of the symmetry of this compound.

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**Preliminary X-ray studies of adult  $\delta$ -crystallin: evidence of a space-group transition.** By S. E. MYLVAGANAM, C. SLINGSBY, P. F. LINDLEY\* and T. L. BLUNDELL, *Laboratory of Molecular Biology, Department of Crystallography, Birkbeck College (University of London), Malet St., London WC1E 7HX, England*

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#### Abstract

A new calcium-dependent crystal form of  $\delta$ -crystallin grown at ambient temperatures using vapour-diffusion methods exhibits a temperature-dependent space-group transition. At lower temperatures the crystal form adopts space group  $P2_12_12$  with  $Z=2$  and exhibits a diffraction pattern corresponding to the crystal form reported previously, while at 294 K the resulting space group is  $P2_12_12_1$  in which the  $c$  axis doubles to  $140.2(2) \text{ \AA}$  with  $Z=4$ . A third crystal form which requires a higher concentration of calcium ions shows no such transition and appears to be remarkably stable to X-rays in comparison with the other crystal forms. These crystals diffract to at least  $2.4 \text{ \AA}$  in space group  $P2_12_12_1$  with  $Z=4$  and have the largest cell dimensions of the three crystal forms with  $a = 88.9(1)$ ,  $b = 144.8(1)$  and  $c = 152.5(1) \text{ \AA}$ .

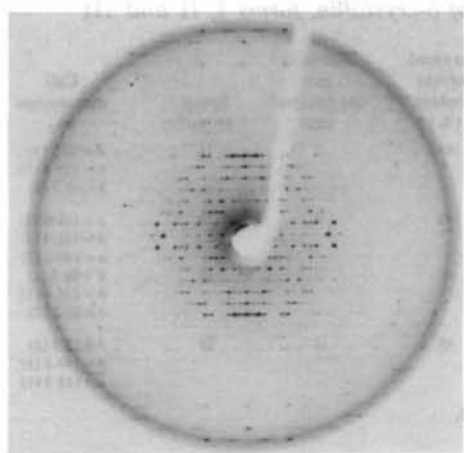
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#### Introduction

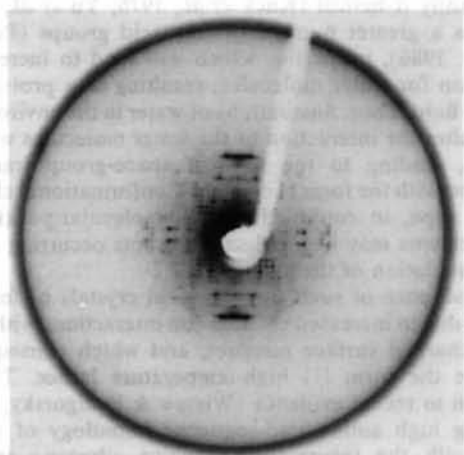
Previous X-ray studies on crystals of the avian eye lens tetrameric protein,  $\delta$ -crystallin (200kd), from adult turkey, showed that variations in the  $c$  dimension (of up to 22%) were dependent on the hydration level of the crystal, and furthermore, that such dramatic changes had little effect on crystal symmetry (Narebor & Slingsby, 1985). We present here further X-ray studies on three crystal forms of  $\delta$ -crystallin grown either in the absence or in the presence of calcium ions.

#### Experimental

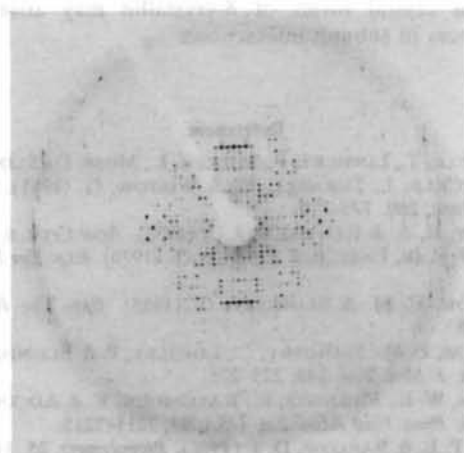
Crystal form I was grown at 277 K using  $0.05 M$  sodium acetate buffer, pH 4.3, and crystallization conditions described by Narebor, Slingsby, Lindley & Blundell (1980). For crystal forms II and III, which were grown at ambient temperature [294(1) K], sodium acetate buffer was



(a)



(b)



(c)

Fig. 1. 12° precession photographs of the  $0kl$  zone of form II crystals of  $\delta$ -crystallin: (a) at 268 K, space group  $P2_12_12$  with  $c = 69.2 \text{ \AA}$ ; (b) at 294 K with the same crystal as in (a), showing the transition to space group  $P2_12_12$ , and doubling of  $c$  to  $140.2 \text{ \AA}$ ; (c) at 294 K only, space group  $P2_12_12$  with  $c = 140.2 \text{ \AA}$ .

replaced by 0.05 M and 0.1 M calcium acetate buffer respectively. The precipitant used in all three cases was 10% (w/v) polyethylene glycol 6000.

12° X-ray precession photographs of all three crystal forms were taken with Cu  $K\alpha$  radiation ( $\lambda = 1.54178 \text{ \AA}$ ) at temperatures of 268 and 294 K (see Figs. 1 and 2).

### Results and discussion

The results of the X-ray diffraction studies, which are summarized in Table 1, show that  $\delta$ -crystallin can be crystallized in three orthorhombic forms having different unit-cell dimensions and asymmetric unit sizes.

The form I crystals, first reported by Narebor *et al.* (1980), have a unit cell which contains two tetramers assuming a water content of 44%. In this case each tetramer lies on a twofold crystallographic axis, indicating that it is composed of at least two pairs of identical subunits. Attempts to obtain suitable diffraction patterns of these crystals at 294 K were unsuccessful mainly because of their sensitivity to higher temperatures.

Replacement of the sodium acetate buffer by calcium acetate in the crystallization of crystal forms II and III provided greater stability of the crystals at ambient temperatures enabling us to proceed with X-ray studies under temperature conditions of 294 (1) K.

The form II crystals at 294 K exhibit a  $c$  cell dimension of  $140.2 (2) \text{ \AA}$ , approximately twice that of form I. In this instance the whole tetramer occupies the asymmetric unit ( $Z = 4$ ), if we assume the water content to be 46%. Since there is no crystallographic symmetry relationship between subunits within the tetramer, we were unable to draw any conclusions on the identity of the subunits.

Crystal form III, grown at higher concentrations of calcium, is remarkably stable to X-rays at 294 K in comparison to forms I and II. Form III has the largest cell volume, and like form II at this temperature, accommodates the whole tetramer in the asymmetric unit, assuming the water content to be 48%. Diffraction patterns of form III (Fig. 2), however, extend to at least  $2.4 \text{ \AA}$ , but show no similarities to those of forms I or II.

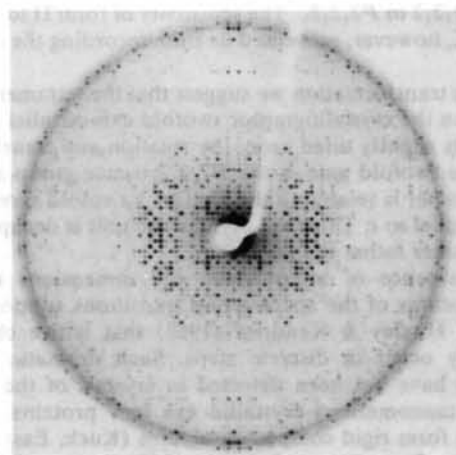


Fig. 2. A 12° precession photograph of the  $0kl$  zone of a form III crystal at 294 K, space group  $P2_12_12$  with  $c = 152.2 \text{ \AA}$ .

Table 1. Summary of X-ray diffraction patterns of  $\delta$ -crystallin, forms I, II and III

Crystal form	Crystallization buffer	Crystallization temp. (K)	Temperature for X-ray patterns (K)	Assumed solvent content (%)	Subunits per asymmetric unit	Space group*	Cell dimensions (Å)
I Hexagonal plates	0.05 M Na acetate	277	268	44	2	18	$a = 99.9 (1)$ $b = 133.6 (1)$ $c = 68.9 (2)$
II Triangular plates	0.05 M Ca acetate	294	294	46	4	19	$a = 100.6 (2)$ $b = 133.4 (3)$ $c = 140.2 (2)$
			268	45	2	18	$a = 99.9 (1)$ $b = 133.6 (1)$ $c = 69.2 (2)$
III Prisms	0.1 M Ca acetate	294	294	48	4	19	$a = 88.9 (1)$ $b = 144.8 (1)$ $c = 152.5 (1)$

\* 18 =  $P2_12_12$ , 19 =  $P2_12_12_1$ .

For diffraction studies of forms II and III at 268 K we first allowed the crystals to equilibrate overnight at these temperatures before taking diffraction photographs. Form III, however, ceased to diffract at low temperature. At 268 K the diffraction patterns and unit-cell dimensions of form II were seen to correspond to those of form I with slight variations in the  $c$  cell parameter. The latter may be related to the hydration state of the crystal (Narebor *et al.*, 1980; Narebor & Slingsby, 1985).

These two sets of unit cells from identical crystallization conditions were first thought to be indicative of two crystal forms, I and II. However, further X-ray investigations on one crystal of form II at 268 and then 294 K showed that such lattice changes occurred within the same crystal. A precession photograph of crystal form II at 268 K, after equilibration overnight at this temperature, was first taken (Fig. 1a). Then the temperature was raised to 294 K and the crystal allowed to equilibrate for 3 h before a further precession of the  $0kl$  zone was taken (Fig. 1b). The resulting X-ray pattern, limited in resolution to 6.0 Å because of X-ray damage, showed intensities corresponding to the  $0kl$  patterns of form II at both 268 K (Fig. 1a) and 294 K (Fig. 1c). The extra layer lines showed a doubling of the  $c$  parameter to 140.2 (2) Å. Further, systematic absences,  $00l$  with  $l = 2n + 1$ , in addition to those in  $h00$ ,  $h = 2n + 1$ , and  $0k0$ ,  $k = 2n + 1$ , indicated that the crystal had transformed from  $P2_12_12$  to  $P2_12_12_1$ . The sensitivity of form II to X-rays at 294 K, however, prevented us from recording the reverse events.

In the transformation we suggest that the tetramer, positioned on the crystallographic twofold axis parallel to  $c$  in  $P2_12_12$  is slightly tilted away (by rotation and translation) from the twofold axis in the  $P2_12_12_1$  space group so that one tetramer is related to the next by a twofold screw axis also parallel to  $c$ . Thus, the asymmetric unit is occupied by the tetramer rather than a dimer.

The absence of intermediate cell dimensions in our investigations of the space-group transitions supports the view of Huxley & Kendrew (1953) that lattice changes probably occur in discrete steps. Such dramatic lattice changes have not been detected in crystals of the mammalian monomeric  $\gamma$ -crystallin eye lens proteins. These proteins form rigid compact structures (Kuck, East & Yu, 1976; Yu, East, Chang & Kuck, 1977), having little sur-

rounding free or bulk water (Racz, Tompa & Istavan, 1979; Blundell *et al.*, 1981).  $\delta$ -Crystallin, however, is predominantly  $\alpha$ -helical (Kuck *et al.*, 1976; Yu *et al.*, 1977), and has a greater number of free acid groups (Poole & Barlow, 1986), properties which will tend to increase its attraction for water molecules, resulting in a protein that is more fluid. Thus, fluctuations of water in the environment might alter the interaction of the water molecules with the protein, leading to the type of space-group transition observed with the form II crystals. Conformational changes of this type, in conjunction with molecular-packing rearrangements may well reflect the events occurring during accommodation of the avian lens.

The absence of such transitions in crystals of form III may be due to increased calcium-ion interactions with negatively charged surface residues, and which consequently stabilize the form III high-temperature lattice. This, in addition to recent evidence (Wistow & Piatigorsky, 1987), showing high amino-acid-sequence homology of  $\delta$ -crystallin with the temperature-sensitive allosteric enzyme, argininosuccinate lyase, from human liver (O'Brien, McInnes, Kalumuck & Adcock, 1986), indicates that the different crystal forms of  $\delta$ -crystallin may arise from differences in subunit interactions.

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