

## STRUCTURE OF $\gamma$ -CRYSTALLIN IIIb FROM CALF LENS AT 5 Å RESOLUTION

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### 1. Introduction

Crystallins are water-soluble proteins from eye lens of vertebrates. By electrophoretic mobility they are usually subdivided into three main groups  $\alpha$ ,  $\beta$  and  $\gamma$ .  $\gamma$ -Crystallins have the lowest relative molecular mass of  $\sim 20\,000$  and are separated into several fractions [1,2]. Proteins of these fractions are very similar in amino acid composition and sequence [3,4].  $\gamma$ -Crystallins are actively synthesized at early stages of the organism's development being subjected to only small modifications during the whole life span. One of the remarkable differences of  $\gamma$ -crystallins as compared with  $\alpha$ - and  $\beta$ -crystallins is the large amount of SH groups which are converted into S—S bonds on ageing and in eye cataract. Recent X-ray studies of  $\gamma$ -crystallin fraction II have allowed its structure to be obtained at 5.5 Å [5] and 5.0 Å [6] resolution. The structure of the homologous  $\gamma$ -crystallin fraction IIIb also appears to be of interest in the sense of a possible change of the protein space structure. We had reported preliminary crystallographic data on  $\gamma$ -crystallin IIIb [7] which were confirmed later in [5].

### 2. Materials and methods

$\gamma$ -Crystallin of the IIIb fraction was obtained from the water-soluble part of the eye lens of three-month old calves. The main procedure of isolation, purification, crystallization and growing of large monocrystals was described in [7,8]. Fraction IIIb was separated from fraction IIIa by a preparative isoelectrofocusing method. Large crystals were obtained at slightly changed conditions as compared with the published data. The mother liquor contained 0.4% proteins, 0.2 saturation  $(\text{NH}_4)_2\text{SO}_4$ , 5 mM dithiothreitol, 0.05 M  $\text{Na}_2\text{HPO}_4$  (pH  $\sim 7$ ). The crystals had the dimensions

of  $0.2 \times 0.25 \times 1.0$  mm. They belong to the space group  $P2_12_12_1$  with the unit cell dimensions  $a = 58.7$  Å,  $b = 69.5$  Å and  $c = 116.9$  Å. The unit cell contains 8 molecules with 2 molecules in the asymmetric part.

The protein structure was determined by the method of multiple isomorphous replacement. About 20 compounds were tested when searching for derivatives. The solutions of heavy atom salts were prepared on the basis of 0.3 saturation ammonium sulphate, 0.05 M  $\text{Na}_2\text{HPO}_4$  (pH 7) solution. The derivatives were chosen by difference Patterson centrosymmetrical projections. Four derivatives indicated in table 1 were chosen finally. Na-Mersalyl was a Mann Research Lab. preparation, potassium cyanoaurate and mercurials were kindly synthesized by Dr K. Zikherman. Mercurials were synthesized as in [9]. Data for Patterson projections as well as 3-dimensional diffraction sets were obtained by the precession photomethod. GX6 X-ray rotating anode generator (Elliot, England) operated at 40 kV, 40 mA and precession cameras (Nonius, Holland) were used. In each set 18 layers of reciprocal space were taken in the zone of 4.5 Å resolution. When measuring the intensity of reflections we did not take into account either very weak spots on the film with the density  $< 0.03$  optical units or reflections with the reliability factor  $R_{\text{sym}} > 15\%$ . Most of the ignored spots were weak and very weak. In the zone of 18–5 Å resolution for the native protein 1625 independent reflections were collected. On the average, about 1000 independent reflections consistent with the corresponding reflections of the native protein were collected for each derivative. The mean value of  $R_{\text{sym}}$  in each set was  $\sim 5\%$ . The complete set of data was partially consistent, i.e., for each non-zero reflection of the native protein the derivative reflection did not necessarily have a non-zero amplitude. The coordinates of heavy atoms were refined only for

Table 1  
Heavy atom compounds and phase statistics of  $\gamma$ -crystallin IIIb at 5 Å resolution

Heavy atom compound	Conditions for derivatives	Site number	Phase statistics		
			$R_F$	$R_K$	$R_C$
Na-mersalyl	1 mM, 1 day	6	19.9	9.4	38.2
KAu(CN) <sub>2</sub>	1 mM, 1 day	5	11.8	5.7	46.6
Hg(SCH <sub>2</sub> CH <sub>2</sub> NH <sub>3</sub> Cl) <sub>2</sub>	1 mM, 2 days	5	20.6	9.7	43.1
Hg(SCH <sub>2</sub> COONa) <sub>2</sub>	1 mM, 5 days	6	15.3	7.9	44.0

- (i) All reliability factors  $R$  are given in % and calculated for a fully consistent set of data (see the text)
- (ii) Factor  $R_F = 2|F_P - F_{PH}|/|F_P + F_{PH}|$  where  $F_P$  and  $F_{PH}$  – modules of structure amplitudes of the native protein and the derivative, are calculated from the full data sets
- (iii) Values for  $R_K$  and  $R_C$  have been determined as in [10].

fully consistent data of 584 reflections. In this case the structure amplitudes were different from zero for all derivatives. Mean figure of merit for phase determination was equal to 0.82 for this data set. The numbers of heavy atom sites and other statistics are presented in table 1. The main heavy atom sites were found from Harker sections. The solution of Patterson syntheses for the other sites was found with the use of cross difference and double difference Fourier syntheses. We used the phase refinement program and the program of fast Fourier transform kindly provided by Dr L. Ten Eyck.

### 3. Results

The obtained coordinates of heavy atoms were used to perform the three-dimensional Fourier synthesis of the crystal electron density at 5 Å resolution. Synthesis weighted by figure of merit ( $M$ ) was first obtained for fully consistent data. On this synthesis, the contour of protein molecules clearly detaches against the background of interphase liquor. Each of two molecules of the unit cell were also well separated from each other. The Fourier synthesis weighted by  $m$  for partially consistent data with the average  $m$  value of 0.70 (1474 reflections) was also calculated. This synthesis turned out to be a little more distinct.

Analysis of the electron density showed the isolation of 4 layers of molecules along the  $c$  axis of the unit cell. The molecules of neighbouring layers are turned almost perpendicularly in the layer. This testifies to the presence of a 4-fold screw pseudoaxis found on

X-ray patterns of the  $h0l$  zone [7]. Within the layer the molecules are arranged approximately along the diagonal of the unit cell [110]. The two molecules being located in the asymmetric part of the cell form a head-to-tail dimer and are connected by a non-crystallographic 2-fold screw axis. For the first layer it passes approximately along the direction [110] at the level of  $z = 1/8$ . The corresponding absences were observed on the X-ray pattern of the  $hhl$  zone. Thus, in the crystal the protein molecules form indefinite long associates which are separated from each other in the layer by the interphase liquor with the interval of 8–20 Å.



Fig.1. Photograph of the model of  $\gamma$ -crystallin IIIb molecule from calf eye lens at 5 Å resolution. This is a model of one of the two molecules located in the asymmetric part of the unit cell. The strand of electron density between two globules closes with the 'back' convex side of the molecule.

The  $\gamma$ -crystallin IIIb molecule itself represents an ellipsoid with the overall dimensions of  $\sim 50 \times 29 \times 25$  Å (fig.1). Two protein molecules being in the asymmetric part of the unit cell are similar. Small differences between two molecules ought to be due to deviations of the electron density at the given resolution. Three main heavy atom sites were found to be located on the external convex side of each protein molecule. Within the molecule two globules about equal in volume could be easily seen (fig.1). The globules are connected one with another by a strand of electron density. This strand is located near the external convex side of the molecule at a distance of  $\sim 1/3$ rd of its height (25 Å).

#### 4. Discussion

The comparison between the structure of  $\gamma$ -crystallin IIIb and that of  $\gamma$ -crystallin II seems to be interesting. The amino acid sequence of the peptide chain is known only for  $\gamma$ -crystallin II [3]. For  $\gamma$ -crystallin fraction III there are at present only preliminary incomplete data [4]. In the short presentation of paper [4] it is not mentioned whether the partial sequence reported is for fraction IIIa or IIIb and there are no data on fragment 37–55 of the chain. In  $\gamma$ -crystallin II there are 6 cysteine residues in positions 15, 18, 23, 32, 74 and 105 [3]. In  $\gamma$ -crystallin IIIb there are 5 cysteine residues [2]. They are located in the known part of the sequence in the same positions, the sixth position 23 is occupied by a histidine residue [4]. According to the data available [4], one can expect for  $\gamma$ -crystallin IIIb  $\sim 15\%$  of amino acid replacements in the chain in comparison with the protein of fraction II. Some of them seem to be very important, for instance, 26 Pro (II) is replaced by Glx (III), 84 Phe (II) is replaced by Ala (III), 104 Pro (II) is replaced by Asp (III) and others [4].

It would be natural to expect similarity of the space structure of these homologous proteins. In fact, on the one hand, there is similarity of not only overall shape and dimensions, but also of the character of the layer packing of molecules in the crystal [5,6]. Each molecule consists of 2 globules-domains of approximately equal volumes which are interconnected by one strand. In both cases the compounds with heavy atoms are localized practically on one side of the

molecule. However, at this stage of the structure analysis we cannot for certain make a conclusion about differences of many details of the two protein molecules. The small clear difference of  $\gamma$ -crystallin IIIb is as follows: the two globules appear to form a somewhat greater contact surface in comparison with  $\gamma$ -crystallin II. This observation agrees with the fact that the large amount of amino acids are replaced in the middle of the polypeptide chain of protein fraction III in comparison with protein fraction II. At present it is difficult to say whether the difference of space structure of the proteins can be explained by the differences of the amino acid sequence or whether it reflects the features of the structural behaviour of  $\gamma$ -crystallin molecules in the crystal. High resolution data could give an answer to this question.

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