POLYPEPTIDE CHAIN PATHWAY IN γ-CRYSTALLIN IIIb FROM CALF LENS AT 3 Å RESOLUTION

Yu. N. CHIRGADZE, Yu. V. SERGEEV, N. P. FOMENKOVA and V. D. ORESHIN
Institute of Protein Research, USSR Academy of Sciences, 142292 Poustchino, Moscow Region, USSR

Received 4 June 1981

1. Introduction

Crystallins are water-soluble proteins from eye lens of vertebrates [1,2]. γ-Crystallins are a group of homologous proteins with a molecular mass (M_r) of ~20 000 and are very similar in amino acid composition and sequence [3–5]. In contrast to other crystallins, γ-crystallins contain a large amount of free SH-groups. At ageing and in eye cataract these groups within crystallin lens are converted into S–S bonds, which shows the importance of γ-crystallins in the process of normal functioning of eye lens. The structure of γ-crystallin fraction II have been recently obtained at ~2.6 Å resolution [6]. The three-dimensional structure of γ-crystallin fraction IIIb has been studied at ~5 Å resolution [7,8], now the main results for 3.0 Å resolution are reported.

2. Materials and methods

γ-Crystallin of the IIIb fraction was obtained from the water-soluble part of eye lens of 3-month-old calves. The procedure of growing of the protein single-crystals has been described [8]. The crystals belong to the space group $P2_12_12_1$ with the unit cell dimensions 58.7 × 68.5 × 116.9 Å. The asymmetric part of the unit cell contains 2 protein molecules.

The protein structure was determined by the method of multiple isomorphous replacement. Three-dimensional data sets were taken with precession photographs. An Elliott GX6 X-ray rotating anode generator, Nonius precession cameras and a Syntex AD-1 autodensitometer were used [8]. For each data set 26 layers of reciprocal space were taken at ~2.9 Å resolution. Very weak spots on the film with an integrated density <0.03 optical units were discarded. Proceeding from this criterion ~1/3rd of the total amount of reflections with weak and very weak intensities was ignored. Thus, 7220 independent reflections were collected for the native protein crystal in the range from 20–3.0 Å resolution. Five isomorphous derivatives were used (table 1). The preparation procedure has been described [8]. In comparison with the low resolution analysis one new derivative based on

Table 1
Phase refinement statistics of γ-crystallin IIIb at 3 Å resolution

Isomorphous derivative	No. heavy-atom sites	N	F_{PH}	F_H	E	R_C	R_K
$Hg(SCH_2COONa)$	8	5590	352	83	60	49.8	11.4
$Hg(SCH_2CH_2NH_2Cl)_2$	6	5298	365	95	71	59.9	14.1
$Hg(SCH_2CH_2OH)_2$	6	6199	331	52	51	73.0	11.0
Na-mersalyl	10	4464	384	89	83	60.4	15.0
$K\ Au(CN)_2$	4	5478	347	51	45	65.3	9.1

N, no. reflections used in refinement; F_{PH}, mean derivative amplitude; F_H, root-mean-square heavy-atom scattering; E, root-mean-square lack of isomorphism; R_C, R_K, reliability factors [9]

Published by Elsevier/North-Holland Biomedical Press
00145793/81/0000–0000/S02.50 © 1981 Federation of European Biochemical Societies

Table 2
Mean figure of merit m and reflection number N for γ-crystallin IIIb over 20–30 Å

	Interval of resolution (Å)					
	5.5	4.5	3.9	3.4	3.0	20–3.0
m	0.85	0.79	0.72	0.66	0.59	0.72
N	1428	1070	1366	1550	1363	6777

$Hg(SCH_2CH_2OH)_2$ has been included, old sites of heavy atoms have been refined and new low occupancy sites have been found. Anomalous scattering diffraction data were used for the first, second and fourth derivatives as numbered in table 1. Mean anomalous differences were ~10%, while the mean isomorphous differences were equal to ~30–40% of the intensity of the protein crystal. Because of this, spots on the film with $R_{sym} > 10\%$ were ignored when anomalous differences were measured. The largest anomalous contribution was observed with the first derivative. Harker sections of anomalous difference Patterson syntheses for this derivative contained all the main peaks of corresponding isomorphous syntheses. The correct type of the enantiomorphic structure was determined with the use of this derivative. Some details of the phase refinement statistics are presented in table 1. Phases of 6777 independent reflections for the 3.0 Å data set were obtained with a mean figure of merit of 0.72 (see table 2). This set of data was used to calculate

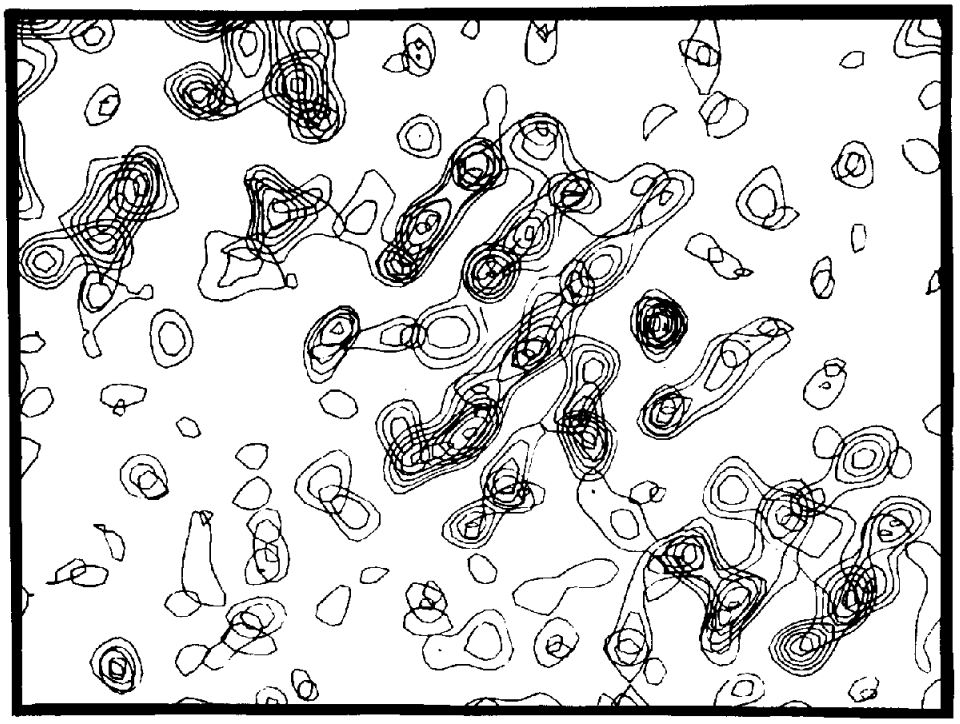

Fig.1. A fragment of the electron density map of γ-crystallin IIIb at 3 Å resolution. A part of the xy-projection with z = (15 17)/80 is shown. There are 4 strands of the molecule C-domain pleated sheet located at the center of the figure.

the 'best' Fourier synthesis of electron density maps. Detailed analysis of the maps was carried out with the use of the optical comparator (Richard's box) and Kendrew wire atomic models in a 1 Å = 2 cm scale.

3. Results

As was seen in the electron density map both the molecules of the asymmetric part of the unit cell were clearly observed. Two domains are also well defined in each molecule. Between the domains there are several points of contact in the form of electron density clusters which appear to be assigned to the amino acid residue side group. Several distinct elongated regions in each domain were assigned to segments of the pleated sheet, as it is clearly seen in the fragment of a few layers of electron density map (fig.1). The main chain pathway of both the molecules in the asymmetric part of the unit cell has been traced well enough and seems to be identical. These two molecules form approximately head-to-tail dimers, being turned into the infinitely long associates in all the crystal space. The arrangement of these molecules obey the 2-fold screw non-crystallographic axis described by the following equation: $y = 0.971\,x - 0.247$; $z = 0.123$. The tentative assignment of the C-end of the molecule to Tyr 165 was made by the presence of the corresponding electron density [4]. Examination of chain folding shows that the N- and C-domains of the molecules are strikingly similar. Each domain has the same type of chain folding with the topology of two simple Greek keys, as is seen in the stereo diagram of the main chain based on the α-carbon atom positions (fig.2). The structure of the domain can be described as a gable roof each side of which is a right-handed twisted pleated sheet consisting of 4 antiparallel segments. Four bent loops are also a characteristic element of the domain structure. The total amount of the antiparallel pleated sheet ordered structure in the molecule is ~40%.

In a whole molecule both domains are related to each other by the 2-fold rotation pseudoaxis. The semi-domains inside each domain, in turn, are also connected by the 2-fold rotation axis. The angle between the domain axes of symmetry is equal to ~45°. Hence, the molecule of γ-crystallin IIIb consists of 4 similar repeating structural elements. As a structural element one can take any 1/2 of the domain structure, for instance, the β-sheet or the three-dimensional fragment of the 'Greek key'.

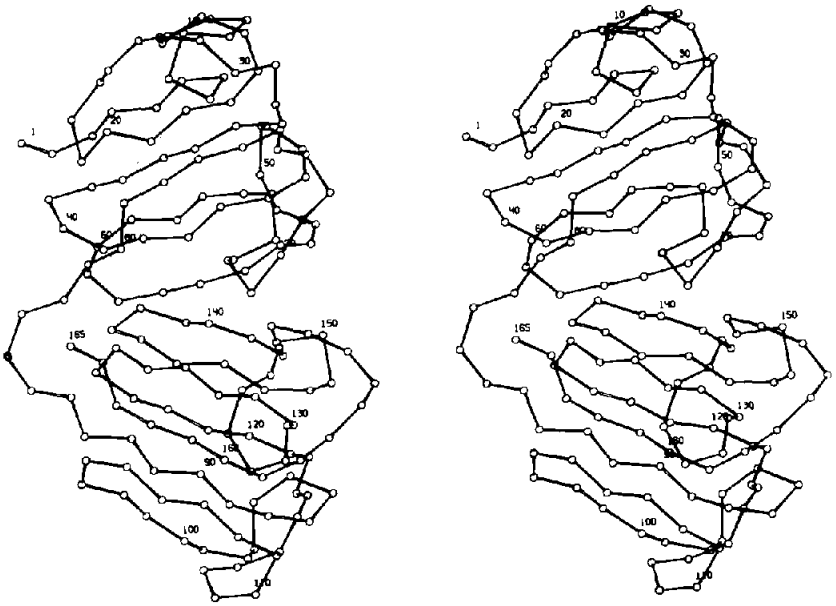

Fig.2. Stereoscopic view of the main chain of γ-crystallin IIIb based on the α-carbon atom positions.

4. Discussion

γ-Crystallin IIIb is a two-domain β-protein. The pathway of the chain in each domain is determined by the topology of two 'simple Greek keys'. The pathway of the chain in γ-crystallin IIIb is, apparently, similar to that of homologous γ-crystallin II [6]. The homology of amino acid sequences of these two proteins is ~85%, as can be derived from the partial sequence of γ-crystallin IIIb [4]. Most of the replacements are found to be in the middle part of the polypeptide chain. Hence it follows that possible structural differences in homologous proteins can be in the region of the interdomain link. The topography of the SH-groups will also be somewhat different, since the number of cysteine residues varies from 5 in γ-crystallin IIIb to 6 in γ-crystallin II. These and other structural features of 2 γ-crystallins could be discussed after localization of side groups at the next stage of structure analysis.

One can assume that both the two domains of the molecule are necessarily related to the function of γ-crystallins. This is in agreement with the fact that the water-soluble part of human eye lens contains only fractions of γ-crystallins with $M_r \sim 20\,000$ while the water-insoluble albuminoid part of the lens contains a fraction with $M_r \sim 10\,000$ [10]. At present we have not found any essential differences between the structure of the two domains in γ-crystallin IIIb. We can only report that the C-terminal domain, as compared with the N-terminal one, has a somewhat larger cavity on the 'botom' side of the domain.

Acknowledgements

The authors are grateful to Dr M. Garber and L. Reshetnikova for growing the protein crystals, to Dr K. Zikherman for synthesis of heavy atom compounds, to Dr N. Nevskaya and S. Nikonov for help in data processing, to Drs L. Ten Eyck and V. Lunin for the phase refinement program and Mr M. Kozlov for computer graphics. The authors express their thanks to Professors O. Ptitsyn and N. Andreeva for valuable discussions.

References

[1] Björk, I. (1961) Exp. Eye Res. 1, 145–154.
[2] Björk, I. (1964) Exp. Eye Res. 3, 254–261.
[3] Croft, L. F. (1972) Biochem. J. 128, 961–970.
[4] Croft, L. R. and Slingsby, C. (1973) in: The Human Lens – In Relation to Cataract, Ciba Found. Symp. 19, pp. 212–213, Elsevier/Excerpta Medica, Amsterdam, New York.
[5] Slingsby, C. and Croft, L. R. (1978) Exp. Eye Res. 26, 291–304.
[6] Blundell, T. L., Lindley, P., Miller, L., Moss, D., Slingsby, C., Tickle, I., Turnell, B. and Wistow, G. (1981) Nature 289, 771–777.
[7] Chirgadze, Yu. N., Nikonov, S. V., Garber, M. B. and Reshetnikova, L. S. (1977) J. Mol. Biol. 110, 619–624.
[8] Chirgadze, Yu. N., Oreshin, V. D., Sergeev, Yu. N., Nikonov, S. V. and Lunin, V. Yu. (1980) FEBS Lett. 118, 296–298.
[9] Dickerson, R. E. and Weinzierl, J. E. (1968) Acta Cryst. B24, 997–1003.
[10] Spector, A. and Roy, D. (1978) Proc. Natl. Acad. Sci. USA 75, 3244–3248.