Volume 241, number 1,2, 261–264 FEB 06556 December 1988

A novel crystallin from octopus lens

S.-H. Chiou

Institute of Biochemical Sciences, National Taiwan University and Institute of Biological Chemistry, Academia Sinica, PO Box 23-195, Taipei 10764, Taiwan, Republic of China

Received 26 August 1988

Lens crystallins were isolated from cephalopods, octopus and squid. Two protein fractions were obtained from the octopus in contrast to only one crystallin from the squid. The native molecular mass for these purified fractions and their polypeptide compositions were determined by gel filtration, sedimentation analysis, and SDS-gel electrophoresis. Octopod and decapod lenses share one common major squid-type crystallin of 29 kDa, with one additional novel crystallin present only in the octopus lens. This newly-characterized crystallin (termed Ω-crystallin) exists as a tetrameric protein of 230 kDa, consisting of 4 identical subunits of approx. 59 kDa. It is distinct from the previously known crystallins both in amino acid composition and subunit structure. N-terminal sequence analysis indicated that the Ω-crystallin is N-terminally blocked, whereas the major octopus crystallin is identical to the reported squid crystallin with regard to the first 25 residues of protein sequence. Sequence similarity between this major cephalopod crystallin and glutathione *S*-transferase were found, which suggested some enzymatic role of crystallins inside the cephalopod lens.

Octopus lens; Invertebrate crystallin; Glutathione *S*-transferase; Phylogeny; Protein evolution

1. INTRODUCTION

The evolution of image-forming eyes in some molluscs of invertebrates with camera-type single lens similar to most higher classes of vertebrates is very striking. The lens evolved initially as the protein-filling structure for concentrating the incident light from the environment onto the receptor cells of the retina in the eye. From the morphological development and the serological properties of lens proteins (called crystallins) from vertebrates and invertebrates it has been commonly assumed that the evolution of lens and its crystallin components must follow two independent pathways (i.e. convergent evolution) [1]. However morphological and functional similarities sometimes also entail structural similarity on the level of primary sequences of constituent polypeptide chains. Previous studies [2–4] have shown a single type of crystallin (Sq crystallin) present in the squid lens of cephalopod in contrast to the multiple classes of crystallins in various vertebrate lenses. It would be of great interest to compare crystallins of octopus and squid lenses and other vertebrate crystallins in order to provide a coherent picture of crystallin evolution in general. In this report we have identified one novel crystallin (Ω-crystallin), which has not been reported to date, and have established the same identity of the major squid-type crystallin present in both octopod and decapod lenses.

Correspondence address: S.-H. Chiou, Institute of Biochemical Sciences, National Taiwan University and Institute of Biological Chemistry, Academia Sinica, PO Box 23-195, Taipei 10764, Taiwan, Republic of China

2. MATERIALS AND METHODS

The common octopus (*Octopus vulgaris*) and squid (*Sepia esculenta*) lenses were obtained from local fish market. The pooled lenses were decapsulated and homogenized in 10–20 ml of 0.05 M Tris-Na bisulfite buffer, pH 7.5, containing 5 mM EDTA as described before [5]. The supernatant from centrifugation at 27000 × *g* was adjusted to give a concentration of about 20–30 mg/ml and the 5.0 ml aliquot was applied to Fractogel TSK HW-55 (Superfine Grade, Merck). Estimation of Stokes' radii of crystallin fractions was according to Siegel and Monty [6] using the following standard proteins on the TSK

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/88/$3.50

HW-55 gel permeation column: thyroglobulin (85 Å); ferritin (79 Å); human γ-globulin (53 Å); β-lactoglobulin (29 Å) and myoglobin (19 Å). Sedimentation velocity measurements were carried out in Spinco model E ultracentrifuge according to the previous report on bovine crystallins [7].

SDS-polyacrylamide slab gel (SDS-PAGE, 5% stacking/14% resolving gel) was as described [8] with some modifications. Isoelectric focusing in 5% polyacrylamide gel containing 2.8% (v/v) carrier ampholytes of pH 3.5–10 was carried out on a slab gel with the incorporation of 0.1% 2-mercaptoethanol and 8 M urea. Two-dimensional electrophoresis was performed using isoelectric focusing in the first dimension and SDS-PAGE in the second dimension essentially as described before [3].

The amino acid compositions were determined with the LKB-4150 amino acid analyzer using a single-column system. The dialyzed and lyophilized protein samples were hydrolyzed at 160°C for 45 min on a dry heating block using 4 N methanesulfonic acid containing 3-(2-aminoethyl)indole (Pierce, USA) for the complete amino acid analysis from a single protein hydrolysate [9]. We have shown the applicability of high temperature (160°C) and shortened time (45 min) in achieving comparable amino acid composition data similar to those obtained by the tedious conventional 110°C/24 h protocol [9]. The half-cystine content and tryptophan were determined with accuracy.

The N-terminal sequences of crystallin fractions from the gel permeation column were carried out by automated Edman degradation with a pulsed-liquid phase sequenator (model 477A, Applied Biosystems). The lyophilized crystallin samples each containing about 1–5 nmol protein were dissolved in 200 ml of 0.1% trifluoroacetic acid (TFA) or 0.1% SDS/0.1% TFA (1:1, v/v) and 10 μl each for sequence determinations.

3. RESULTS AND DISCUSSION

There have been various reports on the characterization of crystallins from different species of vertebrates, with relatively fewer reports on the invertebrate lenses. Understanding the mechanism for the evolution of functionally related proteins from different species remains a general biological problem. Especially intriguing is the evolution of crystallins from the vertebrate and invertebrate lenses. The currently prevalent view of crystallin evolution in the animal kingdom still emphasizes the convergent aspect of evolution regarding the development of these two types of crystallins. The extensive characterization and tracing of various crystallin variants from invertebrate species may complement the study of vertebrate lens crystallins and provide some insight into the phenomenon of species diversification and the accompanying molecular origin of crystallins.

Fig.1 shows elution patterns of lens extracts from *Octopus vulgaris* separated on the TSK gel permeation column. Three peaks were obtained

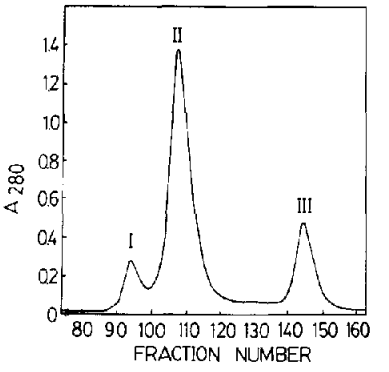

Fig.1. Isolation and fractionation of octopus lens crystallins by gel-permeation chromatography on Fractogel TSK HW-55(S) (2.5 × 115 cm column). The column eluates (3.2 ml/tube per 4.1 min) were monitored for absorbance at 280 nm. Two crystallin fractions (I and II) plus one non-protein fraction (III) were obtained. Peak I (Ω-crystallin) constitutes about 14% with the rest of the lens proteins belonging to peak II.

for this species in contrast to only one major peak for *Sepia esculenta* [5]. The identification of each crystallin fraction from the column was based on the subunit analysis by SDS-gel electrophoresis (fig.2). The native molecular masses of peaks I, II and III were estimated from the elution positions of some standard column-calibration proteins. They were determined to be 190 and 60 kDa for peaks I and II, respectively. Peak III with molecular masses less than 10000 Da was shown to be a mixture of non-protein small molecules. The subunit molecular masses for these fractions were analyzed by SDS-PAGE as shown in fig.2A. Peak I shows a major band of 59 kDa with a minor aggregated sharp band of more than 100 kDa appearing at the top of separation gel. This minor band would disappear upon carboxymethylation of the crystallin sample before electrophoresis as shown in the previous characterization of frog crystallins [10]. Peak II was shown to be a dimeric protein with a subunit of about 29 kDa similar to the squid crystallin characterized before [2–4]. The apparent homogeneity was also corroborated by high-resolution two-dimensional gel analysis as shown in fig.2B. The peak I crystallin displayed a relatively pure component with a p*I* of about 7.7 in 8 M urea and again a subunit of 59 kDa in the SDS-PAGE dimension.

Previous studies of cephalopod crystallins [11–13] emphasizing the comparison of im-

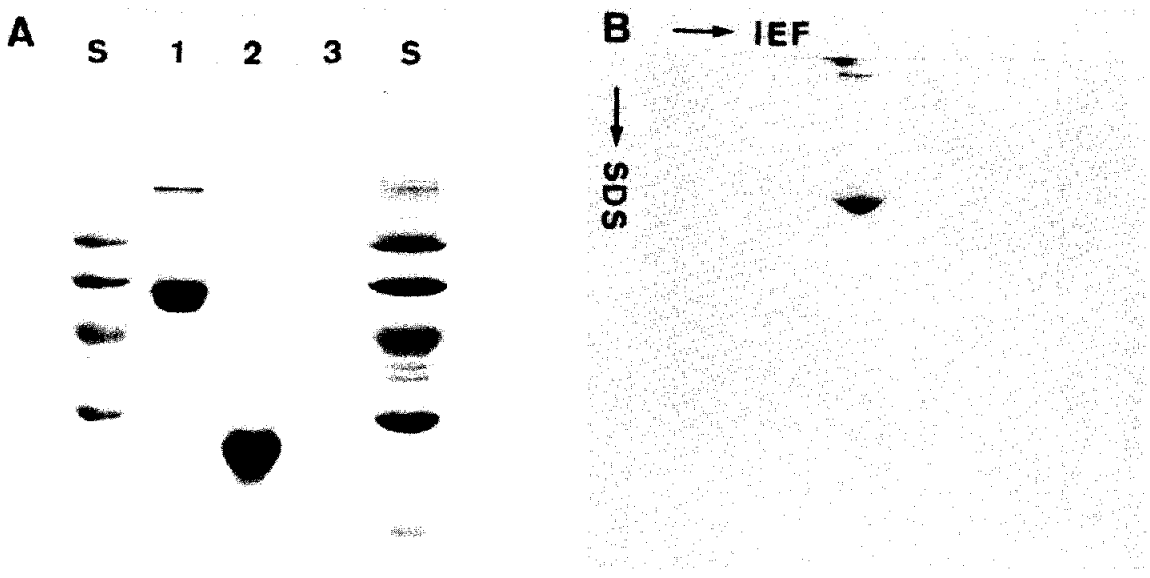

Fig.2. (A) Gel electrophoresis of fractionated octopus crystallins under denaturing conditions (SDS-PAGE) in the presence of 5 mM dithiothreitol. Lanes S, standard proteins used as molecular mass markers (in kDa): transferrin (80), bovine serum albumin (66), ovalbumin (45), carbonic anhydrase (30) and soybean trypsin inhibitor (20). Lanes 1–3 are the 3 fractions of fig.1 corresponding to peaks I, II and III, respectively. Note that molecular masses estimated for peaks I and II were 59 and 29 kDa, respectively, with the peak III showing no protein bands. The gel was stained with Coomassie blue. (B) Two-dimensional gel analysis of Ω-crystallin (peak I of fig.1). About 10 μg of crystallin was layered on a 5% polyacrylamide gel for isoelectric focusing (IEF) in the first dimension (basic end is on the left), followed by SDS-PAGE in the second dimension (the high molecular mass end is at the top). The p*I* was estimated from p*I* calibration kit. The p*I* of Ω-crystallin in 8 M urea was determined to be about 7.7.

munological properties between lens extracts of vertebrate and invertebrate lenses were limited by the difficulty in obtaining purified crystallins for biochemical study. The number of crystallin components reported in different cephalopod species also varied. Thus far the major squid crystallin is the only cephalopod crystallin with defined physicochemical properties [2–4]. The minor crystallin (about 14% of total lens extract as estimated from protein content) is definitely a new crystallin which has not been reported before. Here the name Ω-crystallin is given to this novel protein in accordance with the nomenclature system by means of Greek letters. Some of its structural parameters are tabulated in table 1 for comparison with squid-type crystallin. Their amino acid compositions are also shown in table 2.

An N-terminal sequence analysis of both crystallins in the octopus lens by Edman degradation was carried out in the microsequencing sequenator. The major squid-type crystallin of octopus lens was shown to possess an essentially identical N-terminal segment of 25 residues to that of squid crystallin [4,5], further strengthening the suggestion that the octopod and decapod lenses shared a common major crystallin. Ω-Crystallin was found to be N-terminally blocked, which hampered further structural analysis of this

Table 1

Structural parameters of octopus crystallins

Properties	Ω-Crystallin	Squid-type crystallin
1. Stokes' radius in Å	61 ± 3	28 ± 2
2. Sedimentation coefficient in Svedbergs (S)	9.5 ± 0.3	5.0 ± 0.2
3. Native molecular mass (kDa)	232 ± 14	56 ± 4
4. Subunit molecular mass (kDa)	59	29
5. N-terminal residue	blocked	proline

The methods used for determination of these parameters are described in section 2. Native molecular mass was determined by combining Stokes' radius and sedimentation coefficient [6]

Table 2

Amino acid compositions of octopus crystallins

Amino acids	Ω-Crystallin (Fr. I)	Squid-type crystallin (Fr. II)
1/2Cys	1.9	1.8
Asx	10.1	13.7
Thr	5.9	2.9
Ser	5.7	5.5
Glx	10.9	8.5
Pro	3.7	3.9
Gly	9.4	6.7
Ala	9.5	4.5
Val	4.9	1.5
Met	1.8	12.2
Ile	5.5	3.8
Leu	7.0	6.2
Tyr	3.1	7.6
Phe	4.1	6.8
His	2.5	1.8
Lys	8.4	4.5
Arg	4.8	6.6
Trp	1.0	1.4

Data are expressed as mol%. Each value represents the mean of duplicate determinations. The hydrolysis condition is 160°C and 45 min using 4 N-methanesulfonic acid. The composition of Ω-crystallin is very similar to that of lamprey 48 kDa crystallin [15]

crystallin. Assessment of the relatedness of amino acid compositions [14] between Ω-crystallin and the existing vertebrate crystallins indicated that Ω-crystallin is closely similar to lamprey 48 kDa crystallin [15] regarding their compositions, which constitutes an interesting topic for future study.

It is noteworthy that the computer-based sequence comparison of the 25-residue N-terminal segment of squid crystallin with those sequences in protein data bases [16,17] has indicated sequence identity of about 56% (14 out of 25 residues) between this crystallin and the enzyme glutathione *S*-transferase of rat liver. We have also carried out the enzymatic assay on squid crystallin with negative results. However if the fresh lens homogenate of squid or octopus was used, a moderate activity of glutathione *S*-transferase was detected (unpublished). It remains to be established whether this major cephalopod crystallin plays any role in the detoxification of lens system.

Acknowledgement: This work was supported in part by Academia Sinica and the National Science Council, Taipei, Taiwan, Republic of China.

REFERENCES

[1] Packard, A. (1972) Biol. Rev. 47, 241–304.
[2] Chiou, S.-H. and Bunn, H.F. (1981) Invest. Ophthalmol. Vis. Sci. Suppl.20, 138.
[3] Chiou, S.-H. (1984) J. Biochem. (Tokyo) 95, 75–82.
[4] Siezen, R.J. and Shaw, D.C. (1982) Biochim. Biophys. Acta 704, 304–320.
[5] Chiou, S.-H., Chang, W.-C., Pan, F.-M., Chang, T. and Lo, T.-B. (1987) J. Biochem. (Tokyo) 101, 751–759.
[6] Siegel, L.M. and Monty, K.J. (1966) Biochim. Biophys. Acta 112, 346–362.
[7] Chiou, S.-H., Azari, P. and Himmel, M.E. (1988) J. Protein Chem. 7, 67–80.
[8] Laemmli, U.K. (1970) Nature 227, 680–685.
[9] Chiou, S.-H. and Wang, K.-T. (1988) J. Chromatogr. 448, 404–410.
[10] Chiou, S.-H. (1987) Int. J. Peptide Protein Res. 30, 108–116.
[11] Bon, W.F., Dohrn, A. and Batink, H. (1967) Biochim. Biophys. Acta 140, 312–318.
[12] Swanborn, P.L. (1971) Exp. Eye Res. 11, 111–115.
[13] Brahma, S.K. and Lancieri, M. (1979) Exp. Eye Res. 29, 671–678.
[14] Metzger, H., Shapiro, M.B., Mosimann, J.E. and Vinton, J.E. (1968) Nature 219, 1166–1168.
[15] Stapel, S.O. and De Jong, W.W. (1983) FEBS Lett. 162, 305–309.
[16] Wistow, G.J. and Piatigorsky, J. (1987) Science 236, 1554–1556.
[17] Chiou, S.-H. (1988) J. Prot. Chem. 7, 527–534.