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Short sequence-paper

O-Crystallin, arginine kinase and ferritin from the octopus lens[1]

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Abstract

Three proteins have been identified in the eye lens of the octopus, *Octopus dofleini*. A 22 kDa protein comprising 3–5% of the soluble protein of the lens is 35–43% identical to a family of phosphatidylethanolamine-binding proteins of vertebrates. Other members of this family include the immunodominant antigen of the filarial parasite, *Onchocerca volvulus*, putative odorant-binding proteins of *Drosophila* and a protein with unknown function of *Caenorhabditis elegans*. We have called this protein *O*-crystallin on the basis of its abundance in the transparent lens. *O*-Crystallin mRNA was detected only in the lens. Two tryptic peptides of another octopus lens protein, less abundant than *O*-crystallin, showed 80% identity to arginine kinase of invertebrates, a relative of creatine kinase of vertebrates. Finally, ferritin cDNA was isolated as an abundant cDNA from the octopus lens library. Northern blots showed that ferritin mRNA is not lens-specific.

Keywords: Crystallin; Octopus; Lens; Ferritin; Phosphatidylethanolamine-binding protein

The eyes of cephalopod molluscs (squid and octopus) and vertebrates have striking similarities in overall appearance [1]. One common structure of interest to our laboratory is their cellular lens. Both cephalopods and vertebrates have used a similar strategy of recruiting pre-existing proteins with ubiquitous functions as lens structural proteins, called crystallins, that are responsible for the refractive properties of this transparent tissue [2–4]. Vertebrate crystallins represent a surprisingly diverse group of soluble proteins often showing little structural similarity to each other. Some (α- and β/γ-crystallins) are present in all vertebrate species while others (the taxon-specific crystallins) are confined to selected species or phylogenetic groups [2,5]. In general, the taxon-specific crystallins are identical or related to metabolic enzymes [6,7]. Cephalopods, like vertebrates, have also utilized enzymes as lens crystallins. For example, *S*-crystallins are related to glutathione *S*-transferase (GST) and may account for 70–90% of the soluble protein of the squid or octopus eye lens [8–11]. Ω-Crystallin is related to aldehyde dehydrogenase (ALDH) and is much more prevalent in the lenses of octopus than squid, suggestive of an incomplete taxon specificity [10,12]. Ω-Crystallin represents up

Abbreviations: PEBP, phosphatidylethanolamine-binding protein

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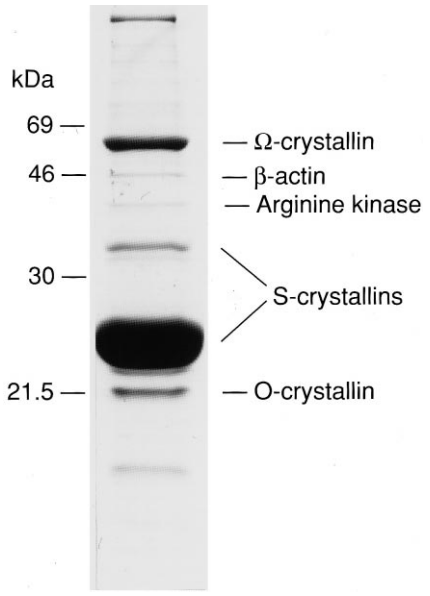

Fig. 1. Coomassie blue stained SDS-14% polyacrylamide gel of water-soluble octopus lens proteins. Approx. 5 μg of proteins were used. Identified proteins are marked on the right. Position of markers is shown on the left.

to approx. 10% of the soluble protein of the octopus lens [13]. Since only *S*-crystallins and Ω-crystallin of the octopus lens are known, we investigated the other prominent proteins expressed in the lens.

A SDS-polyacrylamide gel showing the GST/*S*-crystallins and ALDH/Ω-crystallin is presented in Fig. 1. A number of less prominent bands of protein are also evident. One is β-actin which has been identified earlier [14]. After the *S*- and Ω-crystallins, the third most abundant lens protein on the gel in Fig. 1 has an apparent molecular weight of approx. 22 kDa. Initial sequencing of two tryptic peptides from this gel-purified protein band indicated that it is related to phosphatidylethanolamine-binding protein (PEBP) isolated from the mammalian brain [15]. This polypeptide represents 3–5% of the total soluble protein of the octopus lens and therefore is not as abundant as *S*- or Ω-crystallin. It is not established exactly how much of a given protein is necessary to be considered a crystallin. Among the β/γ-crystallins in vertebrates, or even in the case of individual *S*-crystallin polypeptides in the octopus lens (see Fig. 1), some crystallin members represent only a few percent of the total protein. Thus, since the 22 kDa protein is one of the most prominent proteins in the octopus lens we have assumed that it contributes significantly to the refractive index of the cytoplasm and called it *O*-crystallin.

To clone the cDNA encoding *O*-crystallin, we used a PCR approach. A 5′ degenerate primer (5′-CAG/ACAC/TTGGC/TTG/TGTC/TGTC/TAAC/TATC/TCCC/A/TGG-3′) designed from one of the tryptic peptides (NT55, positions 79–88 in the complete amino acid sequence) and a 3′ oligo(dT) primer were used to generate a PCR product with a length of about 600 bp. Sequencing of a cloned PCR fragment confirmed that it encoded part of *O*-crystallin since sequences encoding other tryptic peptides in the 22

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O-CRY   MEAFNVHGLVGKII-DRVPHKQLSIRYGN-TEVQPGMNLTPSMTKHQP-QIKFEA-ETNVYYTLI   61
C.ELE   A   TK EVIPDVLASNP S VV VKFNSGV ANL NV   TQV DT -EV WD - PGAL      101
O.VOL   DS  KE  I PDVV-STA T LVNVS N -LT NL NE   TQV N  TKVSWD - PGAL   V  95
DROS    RRIMKEMEVIPE L- EP REL R K D TIDIEE KTY  TEL F  -RLDWN -DPESF  VL  88
BOV     DLSKWSGP SLQEV- ER QHP QVK  GAEVDEL KV   TQV NR TS TWDGLDPGKL   V  66
          *           *                          *           *
O-CRY   MNDADFPSRSDQKLNEFQHWLVVNIPGSDISRGDVLTDYIGPLPNKGTGYHRYVLMLFKQSKGRM  126
C.ELE   KT P A   KEPTYR WH        N  AK  T SE   AG PPK  L    YLIY   -  I  165
O.VOL    T P A   KNPVFR WH   II  S QNV S T  S    SG R    L    FLVY  PGSIT  159
DROS     ICP A N ENPMYRSRL      V  L  MK QPISE F    P DS IQ  LILVYQ  -DKL  152
BOV     LT P A   K P YR WH F    MK NN  S T  S  V SG P    L    WLVYE -E PL  130

O-CRY   EFRGEKKINNRTSEGRKSYNMMEFARKHFLVEPVYGNFFQSEWDDSVPKIYEQLKM           182
C.ELE    DAEHGRLT TSGDK GGWKAAD VA  K GA  F  L  A Y  Y  ILNK  GA            221
O.VOL   D---------TQHGGN RNFKV D  N  H GN  A     AKHE +                    197
DROS    D -D   MELSNAD HSNFDV K TQ YEMGS  A  I   R  EY  ELMKT YGVSE         210
BOV     KC-D PILS  SGDH GKFKVAS RK YE GA  A TCY A    Y   L    SGK           186
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Fig. 2. Comparison of amino acid sequence of *O*-crystallin with amino acid sequences of the *C. elegans* protein (accession No. AF016423), immunodominant antigen of *O. volvulus* [18], *Drosophila melanogaster* putative odorant-binding protein [17] and bovine PEBP [15]. The sequence of *O*-crystallin is shown in full; for other sequences only differing amino acid residues are shown. All but *O*-crystallin sequences have extra N-terminal sequences. + marks the end of the *O. volvulus* sequence. − indicates gaps that were introduced to maximize similarity. Regions corresponding to putative ligand-binding sites in the human PEBP [25] are boxed. * marks residues defining the ligand-binding site in the model of the bovine PEBP [26].

kDa protein were identified (not shown). The 600 bp PCR fragment was used for screening the octopus lens cDNA library [10]. Three positive plaques (among about 2000) were identified and sequenced. The longest cDNA clone was 802 nt excluding the poly(A) tail. It encoded a protein with a deduced molecular mass of 21.3 kDa. Comparison of the deduced amino acid sequence of *O*-crystallin with the GenBank database revealed a 35–43% identity to a family of PEBP-related proteins [15,16]. This family included a *Caenorhabditis elegans* protein with an unknown function (GenBank accession No. AF016423), *Drosophila* putative odorant-binding proteins [17] and an immunodominant antigen of the filarial parasite, *Onchocerca volvulus* [18], among other members (Fig. 2). All these proteins show about 25–30% identity with yeast TSF1 protein, a suppressor of *cdc25* mutations in *Saccharomyces cerevisiae*; TSF1 is implicated in *ras*/*cdc25* signal transduction [19].

The tissue distribution of mRNAs encoding octopus *O*-crystallin was investigated by Northern blot hybridization. *O*-Crystallin cDNA hybridized exclusively to lens RNA approx. 1 kb in length (Fig. 3). No hybridization was detected with RNA samples from the other tissues. In this respect *O*-crystallin is similar to Ω-crystallin which is lens-specific [12] and *S*-crystallin which is lens- and cornea-specific [11,20]. Vertebrate PEBP mRNA was detected in poly-$(A)^+$RNA derived from many tissues analyzed by Northern blot hybridization, with the highest abundance being in mouse and rat testis [21]. Since we used total RNA rather than poly$(A)^+$RNA in our experiments, we cannot exclude the possibility that *O*-crystallin is expressed in other tissues at a low level. Although *O*-crystallin is relatively abundant in the octopus lens, it appears to be absent in the squid lens as judged by SDS-polyacrylamide gel electrophoresis and Northern blot hybridization (not shown). Therefore, *O*-crystallin may be considered a taxon-specific crystallin in cephalopods.

It is noteworthy that vertebrate lenses also contain a significant amount of lipid-binding protein with a molecular mass of about 15 kDa belonging to the family of lipid/retinoic acid-binding proteins [22,23]. This vertebrate lipid-binding protein, called LP2, is structurally different from *O*-crystallin and was proposed to be involved in differentiation, protection

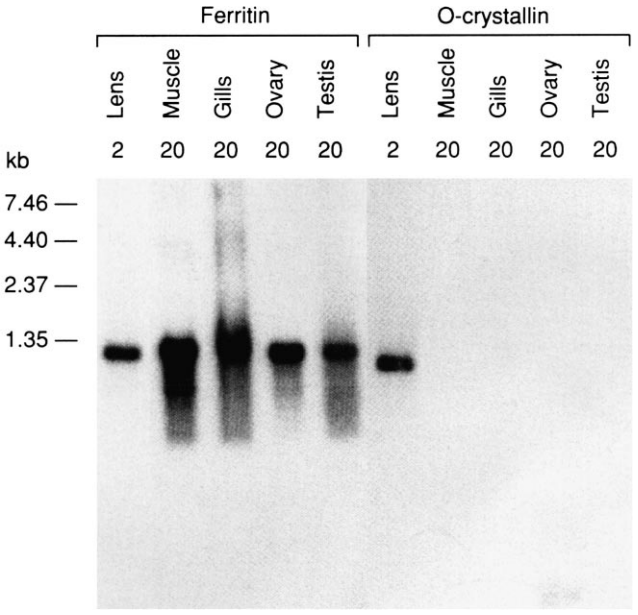

Fig. 3. Tissue distribution of mRNAs for octopus ferritin and *O*-crystallin. 2 or 20 μg of total RNA were used as indicated above each lane. Positions of the marker RNAs are shown on the left.

and/or nutrition of lens cells [22,23]. It has been suggested that proteins belonging to the PEBP family are involved in multiple cellular processes including lipid, opioid and odorant binding and host-parasite interaction [24]. Three-dimensional structures of human and bovine PEBP were resolved recently [25,26]. Several residues and regions in the PEBP molecule have been implicated in ligand binding (Fig. 2) and are conserved in *O*-crystallin. It has been proposed that PEBP may be a membrane-binding protein that interacts with other proteins and modulates catalytic activity of enzymes [26]. It has also been suggested that PEBP might be involved in membrane signal transduction [25]. Virtually nothing is known about the functions of octopus *O*-crystallin. We propose in view of its relative abundance in the octopus lens that it has a role in refraction in this species. This, of course, does not exclude its having another, non-crystallin role in the octopus lens.

We also sequenced two tryptic peptides obtained from another, less abundant protein with an apparent molecular mass of about 40 kDa from the octopus lens (Fig. 1). This protein was easily identifiable in the spectrum of the octopus water-soluble proteins (see Fig. 1). Analysis of the peptide sequences indicated that this 40 kDa polypeptide is related to argi-

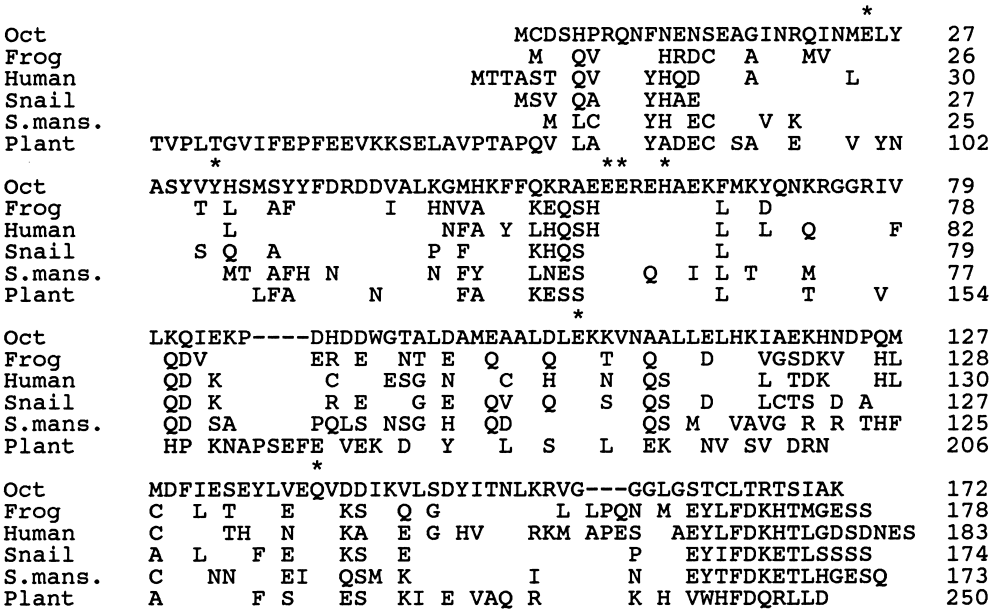

Fig. 4. Comparison of amino acid sequences of octopus ferritin with ferritins from the frog *Rana catesbiana* [37], human H-chain [34], the gastropod mollusc *L. stagnalis* [31], *S. mansoni* [32], and soybean [33]. * mark the residues implicated in metal binding [35].

nine kinase of several invertebrates [27,28] and ATP:guanidino kinase of *Schistosoma mansoni* [29]. These proteins are related to creatine kinase of vertebrates. 80% identity in amino acid sequence was observed with arginine kinase isolated from muscles of the gastropod, *Nordotis madaka* [28]. Arginine and creatine kinases belong to a conserved family of ATP:guanidino phosphotransferases, whose members play an important role in energy metabolism. Arginine kinase is widely distributed among invertebrates. In vertebrates, only creatine kinase activities have been detected. In humans, there is a marked increase in the expression of the BB isoform of creatine kinase in the epithelial cells of the lens with the onset of sexual maturation [30]. It was proposed that the localization of creatine kinase in the epithelial cells reflects its involvement in high transport ATPase activity [30]. In the octopus lens, the arginine kinase-related protein might also be involved in intracellular transport.

Finally, to identify other cDNA clones corresponding to abundant mRNAs, we screened the octopus lens cDNA library with total cDNAs synthesized using lens poly(A)$^+$RNA as templates and characterized the clones giving the strongest signals. Most of the clones encoded octopus *S*-crystallins [12]. We also identified a group of clones that encoded ferritin. The longest cDNA insert was 1103 nt long without counting the poly(A) tail. Ferritin cDNA was present in all tissues analyzed by Northern blot hybridization, giving a single band with a size around 1.2 kb (Fig. 3). Octopus ferritin showed 67% identity to ferritin isolated from the gastropod mollusc *Lymnaea stagnalis* [31] and 55–60% identity to ferritins of *S. mansoni* [32], soybean [33] and H-chain ferritins from vertebrates [34] (Fig. 4). All of the residues implicated in metal binding of human heavy chain ferritin are conserved in the octopus ferritin. Ferritin is an iron storage protein that is essential for protection of cells against oxidative stress. In vertebrates, cytoplasmic ferritin is composed of a heavy and light subunits [35] and plays an important role in normal lens function. Recent data demonstrated that a mutation in the iron-responsive element of the human L-ferritin leads to early bilateral cataract [36]. It is not clear at present whether octopus ferritin has any special role in the lens besides storage of iron atoms.

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