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# The Muscle-derived Lens of a Squid Bioluminescent Organ Is Biochemically Convergent with the Ocular Lens

## EVIDENCE FOR RECRUITMENT OF ALDEHYDE DEHYDROGENASE AS A PREDOMINANT STRUCTURAL PROTEIN*

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**Many of the structural proteins of ocular lenses, commonly referred to as crystallins, are identical to specific enzymes or the result of a recent gene duplication (Piatigorsky, J., and Wistow, G. (1991) *Science* 252, 1078–1079). One such enzyme, aldehyde dehydrogenase (ALDH), has been recruited as a lens crystallin in certain mammals (Wistow, G., and Kim, H. (1991) *J. Mol. Evol.* 32, 262–269) and cephalopods (Tomarev, S., Zinovieva, R., and Piatigorsky, J. (1991) *J. Biol. Chem.* 266, 24226–24231). We report here that a transparent tissue, derived from muscle but functioning as a lens in the light-emitting organ of a squid, *Euprymna scolopes*, shows striking biochemical convergence with the epidermally derived ocular lenses of some mammals and cephalopods. In the light organ lens of *E. scolopes*, an ALDH-like protein is the predominant molecular component. The typical muscle-specific proteins are replaced as the dominant species by a protein composed of 54-kDa subunits. This protein, which we designate as L-crystallin, constitutes approximately 70% of the total soluble protein of the light organ lens. The amino acid sequences of three peptides of L-crystallin (approximately 9% of the total protein) showed 54.5% sequence identity with human cytosolic ALDH. Using polyclonal antiserum made against L-crystallin, we found that it is present in low abundance in other tissues of the squid, including muscle and the ocular lens. This polyclonal antiserum also cross-reacted with the ALDH-like crystallins found in the ocular lenses of certain mammals and cephalopods. L-Crystallin showed no ALDH activity, which is similar to several other enzyme/crystallins, including ALDH/η-crystallin (Wistow, G., and Kim, H. (1991) *J. Mol. Evol.* 32, 262–269). The characteristics of this muscle-derived lens are evidence that a common biochemical basis underlies transparency and that certain proteins may possess properties that promote their selection as lens structural proteins.**

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Biological lenses are often vital components of the eyes and light-emitting organs of animals, where they function to refract environmental and endogenously produced light, respectively (1). One striking example occurs in certain species of squids that not only have the typical cephalopod visual system with an epidermally derived lens (2), but also have a complex bacterial light organ with a prominent muscle-derived lens (3, 4) that focuses light into the environment (Fig. 1). Although these two classes of lenses are often derived from different embryological tissues and have thus evolved independently to perform similar functions, the extent to which the functional convergence is expressed at the biochemical level has remained unexplored.

Numerous studies of the ocular lenses of vertebrates and cephalopods show that they achieve a high refractive index by the abundant expression of only a few protein species, the crystallins (5). Some of these crystallins, although serving as structural proteins in the ocular lens, are identical or closely related to certain metabolic enzymes (6, 7). These enzyme/crystallins, which are diverse (*i.e.* different enzyme/crystallins are present in different animal species), are often the same gene product, such as lactate dehydrogenase B4/ε-crystallin and α-enolase/τ-crystallin in duck lenses (6). Piatigorsky and Wistow (6) have called this phenomenon "gene-sharing" and have offered an alternative view of protein evolution whereby new functions may precede rather than follow gene duplication.

Among the enzyme/crystallins is η-crystallin, a major structural protein found in the ocular lenses of elephant shrews and thought to be encoded by the same gene as cytosolic aldehyde dehydrogenase (ALDH)[1] (EC 1.2.1.3) (8). Similarly, in the ocular lenses of cephalopods, enzymes have been recruited as structural proteins (6), even though the cephalopod and vertebrate eye have evolved independently (9). The major structural proteins present in the ocular lenses of both squid (*Ommastrephes sloanei*) and octopus (*Octopus dofleini*) are S-crystallins, which are related to glutathione *S*-transferase (10, 11). In addition to S-crystallins, the species of octopus that have been examined possess a moderately abundant protein, Ω-crystallin, in the ocular lens (10, 12). By partial protein sequencing Tomarev *et al.* (10) recently determined that Ω-crystallin in the ocular lens of *Octopus vulgaris* is either identical or closely related to ALDH. The ALDH or ALDH-like crystallin present in both the elephant shrew and the octopus is the first major structural protein known to be similar in vertebrate and invertebrate lenses.

Another type of lens occurs as a component of the biolu-

[1] The abbreviations used are: ALDH, aldehyde dehydrogenase; HPLC, high performance liquid chromatography.

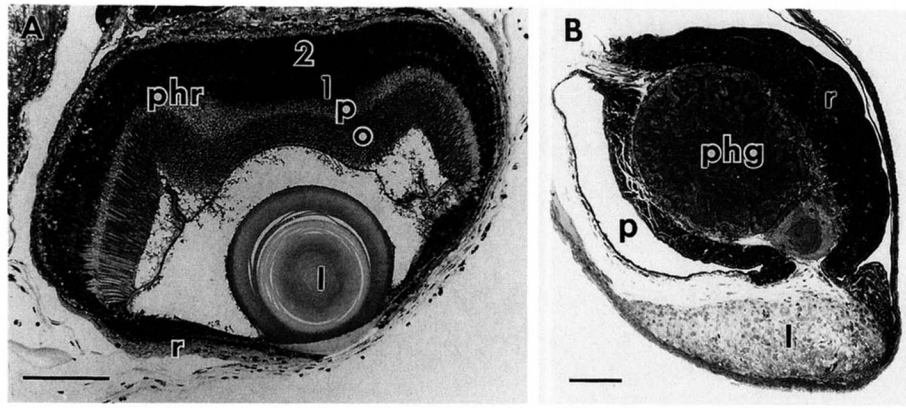


FIG. 1. **Comparison of the eye and light organ of *E. scolopes*.** Because they both interact with light, the eye and the light organ are composed of functionally analogous tissues. In the eye (*A*) light passes through the epidermally derived lens (*l*) and is focused on photoreceptive tissue (*phr*), the retina. The retina of cephalopods forms from an invagination of the epithelium and includes the outer rod segment layer (*o*), a pigment layer (*p*), and a first nuclear layer (*1*) and second nuclear layer (*2*) of photoreceptors. A reflective lining (*r*) both surrounds the outside of the eye as well as forms the iris. In the light organ (*B*), light is produced from the central photogenic tissue (*phg*) by the light-producing bacterial symbionts housed within. Like the retina, this tissue is also formed from invaginations of the epithelium. Surrounding the photogenic tissue is a reflective lining (*r*) and a pigmented layer formed by the ink sac (*p*), both tissues of which are involved in controlling the direction and intensity of light emitted by the organ. The light is directed ventrally and passes through the muscle-derived lens (*l*), which serves to refract the point-source light into the environment (*scale bar* = 100 μm).

minescent organs of several species of squids and fishes. These organs emit light that is either autogenic or produced by bacterial symbionts. Because they interact with light, bioluminescent organs include tissues that are in part functionally analogous to those of the eye (Fig. 1) (1). Thus, eyes and light organs provide striking examples of convergent evolution. *Euprymna scolopes* is a Hawaiian sepiolid squid that maintains luminous bacterial symbionts (13) in a bilobed light organ in the center of its mantle cavity. The squid uses the light emitted by the symbiotic bacteria in its behavior, presumably in antipredatory displays and/or intraspecific communication. The light organ is complex and includes several host tissues that interact to control the quality of bacterial light emission (Fig. 1*B*) (4). The lens is a thick pad of transparent tissue that covers the ventral surface of the light organ. This tissue functions as a convex lens to refract the light from the localized bacterial source over the ventral surface of the squid. Embryological studies and transmission electron microscopy have indicated that the light organ lens of *E. scolopes*, and that of other closely related sepiolid squids, is derived from muscle of the hindgut (3, 4).

We investigated whether the biochemical composition of the light organ lens of *E. scolopes* is more similar to that of muscle tissue, from which it is derived, or to that of ocular lenses, with which it shares an analogous function. Although the light organ lens has evolved independently, we report that its biochemistry is convergent with that of ocular lenses in two important ways: 1) its relatively simple protein composition, and 2) the apparent recruitment of an ALDH-like protein as a major structural lens protein, which we designate L-crystallin.

## EXPERIMENTAL PROCEDURES

*Animals/Tissues*—Specimens of *E. scolopes* were collected from shallow sandflats in Oahu, Hawaii and transported alive to our laboratory at the University of Southern California in Los Angeles. Specimens of *O. bimaculatus* were obtained from Gregorio Aquatech, Los Angeles, CA. Specimens of *E. scolopes* were anesthetized by lowering their body temperature to 10 °C. The ocular lenses were first removed and then the animals were ventrally dissected to expose the internal light organ. Light organs were removed whole and either processed for histology or the lens was dissected from the surrounding tissue and biochemically analyzed. Ocular lenses were removed from specimens of *O. bimaculatus* after the animals were anesthetized with a 1:1 mixture of 7.5% $MgCl_2$:seawater. Approximately 6 μg of total soluble protein from the ocular lens of the elephant shrew *Elephantulus edwardi* were generously donated by G. Wistow of the National Eye Institute at the National Institutes of Health.

*Histology*—Following excision, light organs of *E. scolopes* were fixed for 20 h in 2.5% glutaraldehyde in 0.2 M sodium phosphate buffer with 0.14 M NaCl, pH 7.4. Samples were postfixed in 1% osmium tetroxide in the same buffer as the primary fixative. To examine the ocular lens, juvenile specimens of *E. scolopes* were fixed whole for 24 h in 5% formaldehyde in 0.5 M sodium phosphate buffer, pH 7.5. All specimens were then dehydrated through a graded ethanol series, infiltrated in propylene oxide, and embedded as described previously (4). Sections were cut 1.5 μm thick and visualized with Richardson's stain (14).

*Electrophoresis*—Tissues were homogenized in ice-cold phosphate-buffered saline (50 mM $NaPO_4$ with 0.1 M NaCl, pH 7.1) in a hand-held glass tissue homogenizer, and the resulting homogenate was centrifuged at 15,000 × *g* for 15 min at 4 °C. Protein concentrations of supernatant fluids were determined spectrophotometrically (15). Protein samples were analyzed by polyacrylamide gel electrophoresis (12.5% running gel) in the presence of sodium dodecyl sulfate (SDS-polyacrylamide gel electrophoresis) by a modification of the method described by Laemmli (16). Supernatant fluids containing 0.2–6.0 μg of total soluble protein of each tissue as well as of the purified L-crystallin (see below) were loaded and the separated protein subunits visualized by Coomassie Blue staining. Relative abundances of subunits were approximated by gel densitometry.

*High Performance Liquid Chromatography*—Soluble proteins of the light organ lens were extracted in either phosphate-buffered saline (see above) or in 20 mM Tris-HCl, pH 7.9. L-Crystallin was purified to homogeneity by gel filtration at room temperature on a high performance liquid chromatography (HPLC) system (Beckman System Gold) with one of two different types of columns, depending on whether the run was analytical or preparative. For analytical runs, two TSK SW-3000 columns (7.5 × 300 mm) with a guard column (7.5 × 100 mm) connected in series were used, and proteins were eluted with 50 mM $NaPO_4$ with 0.1 M $Na_2SO_4$, pH 7.0, at a flow rate of 1 ml $min^{-1}$. Absorbance of the proteins was monitored at 280 nm. The major peak, corresponding to native L-crystallin, was collected and the protein concentrated in preparation for gel loading. For preparative runs (as when purifying large amounts of L-crystallin for antibody production) a Pharmacia LKB Superose 12 HR 10/30 column was used. Absorbance of proteins was monitored at either 219 or 280 nm. Proteins were eluted with phosphate-buffered saline at a flow rate of 0.5 ml $min^{-1}$.

*Isoelectric Focusing*—The isoelectric point of L-crystallin was determined by isoelectric focusing according to the method of Pharmacia for polyacrylamide isoelectric focusing (Pharmacia LKB Biotechnology, Uppsala, Sweden), using LKB Ampholine PAGplates (pH ranges 5.5–8.5 and 5.0–6.5).

*Protein Microsequencing*—In attempting to sequence L-crystallin with automated Edman degradation, we found that the amino terminus was blocked. To generate several peptides, L-crystallin was purified by HPLC (as described under "High Performance Liquid Chromatography") and subjected to cleavage with either trypsin or *Staphylococcus aureus* V8 extracellular protease. Partial tryptic digestion was done in 50 mM ammonium carbonate buffer, pH 8.3, with 1% (w/w) L-1-tosyl-amido-2-phenylethyl chloromethyl ketone-treated trypsin for 30 min at room temperature. Glu-specific cleavage by staphylococcal protease (2%, w/w) was done in 50 mM ammonium bicarbonate buffer, pH 7.8, overnight at 37 °C. The resultant peptides were separated on a 16% SDS-polyacrylamide gel and electroblotted onto polyvinylidene difluoride membrane (Immobilon-P, Millipore). Peptides present in the electrophoretically derived bands were then subjected to automated sequencing, performed as a service by the UCLA Protein Microsequencing Facility.

*Immunoreactivity*—To produce a polyclonal antiserum against native L-crystallin, 1.5 mg of the protein were purified using HPLC, as described under "High Performance Liquid Chromatography," and stored in 500-$\mu$l aliquots of 1.0 mg ml$^{-1}$ at −80 °C until used for rabbit immunization (500 $\mu$g for initial injection and 250 $\mu$g for boost injections). Rabbit immunizations and antibody preparation were performed by BAbCo (Berkeley Antibody Company), Berkeley, CA. Western immunoblots were performed according to Towbin *et al.* (17). SDS-polyacrylamide gels were blotted onto nitrocellulose, blocked with 3% milk powder, and probed with the polyclonal rabbit antiserum (1:1000) raised against L-crystallin. Bound antibodies were visualized by a second incubation with goat anti-rabbit conjugated to horseradish peroxidase. In addition, "dot blot" immunoblots were performed to test the antiserum against native proteins. Samples of 1–5 $\mu$g of soluble protein of ocular lenses and light organ lens extracts were pipetted onto nitrocellulose and processed under the same conditions as the Western immunoblot.

*Enzyme Activity*—The light organ lens and the digestive gland from an adult specimen of *E. scolopes*, which had been frozen alive and stored at −80 °C, were assayed for ALDH activity. The digestive gland, which was used as a positive control, is a functional analog to the mammalian liver, an organ that typically exhibits high ALDH activity (18). Tissues were homogenized in ice-cold 50 mM sodium phosphate buffer, pH 7.0, containing 1 mM dithiothreitol and the following protease inhibitors: pepstatin A, leupeptin and aprotinin (1 $\mu$g ml$^{-1}$ each), and 1 mM phenylmethylsulfonyl fluoride. The resulting homogenates were centrifuged at 18,000 × *g* for 20 min, and the supernatants were assayed spectrophotometrically for protein concentration and enzyme activity using described methods (15, 19). Because there may be inhibitors of ALDH activity present in a total soluble lens extract, L-crystallin was purified by gel filtration and tested for enzyme activity. Activity was measured at 25 °C at 340 nm in an assay buffer that contained (final concentrations) 0.02–1.0 mg ml$^{-1}$ soluble protein, 0.1 M sodium pyrophosphate buffer, pH 8.8, 0.2 mM 4-methyl pyrazole, 1 mM $NAD^+$, 1 mM EDTA, and 10 $\mu$M acetaldehyde in a final volume of 2.5 ml. In addition, we tested the light organ lens extract with other substrates, cofactors, and ionic species that are required for activity by some isozymes of ALDH. These included the following: the substrates, propionaldehyde and decyl aldehyde; the cofactor, $NADP^+$; and $MgCl_2$, KCl, and NaCl. One unit of enzyme activity was defined as that amount producing 1 $\mu$mol of NADH min$^{-1}$.

## RESULTS AND DISCUSSION

Our investigation shows that the muscle-derived lens of the light organ of *E. scolopes* is biochemically convergent with ocular lenses in both its relatively simple protein composition and its recruitment of an ALDH-like protein as a major structural protein. The presence of typical muscle-specific proteins is extremely low, and the protein profile of the light organ lens appears more similar to that of the ocular lens than to that of related muscle tissue (Fig. 2*A*). Similar to the ocular lens of the octopus, we found that a single predominant protein, which we designate as L-crystallin, is present in the light organ lens of *E. scolopes*. Gel electrophoresis in the presence of SDS revealed that this predominant soluble protein is composed of 54-kDa subunits (Fig. 2*A*). A protein of the same subunit molecular mass is present in low abundance in both the ocular lens and muscle of this squid, suggesting that L-crystallin may be the result of enhanced expression of a common protein found in several squid tissues. Densitometric analyses of these gels showed that L-crystallin comprises approximately 70% of the total water-soluble protein of the light organ lens, whereas the muscle-specific proteins, such as actin and myosin, are in reduced concentrations. Interestingly, in the ocular lens of the squid, *Notadarius gouldi*, S-crytallin, which is the major protein component of the lens, has been reported to comprise 70% of the soluble protein (7).

We purified L-crystallin to homogeneity by gel filtration and studied its biochemical properties. Several criteria suggested that it is similar to ALDH and the same protein as the enzyme/crystallin in the ocular lenses of certain species of cephalopods and mammals. The apparent subunit molecular mass of L-crystallin is similar to that of ALDH, which is typically expressed as a homotetramer of 54–60-kDa subunits (20). In addition, L-crystallin has the same subunit molecular mass as Ω-crystallin in the ocular lens of *O. bimaculatus* (Fig. 2*A*). Estimation of the molecular mass of native protein by gel filtration indicates that L-crystallin is either trimeric or tetrameric (Fig. 2*B*).

We further characterized L-crystallin and found that its isoelectric point is 5.2, which is similar to those of the common isozymes of mammalian ALDH (21). In attempting to sequence L-crystallin, we found that, like many forms of ALDH (22), the protein was amino-terminally blocked.

Partial protein sequencing of L-crystallin peptides (three peptides, approximately 9% of the total protein) revealed an overall 54.5% sequence identity with human cytosolic ALDH (Fig. 3) (23). A partial trypsin digest resulted in only two major peptides, one of which was amino-terminally blocked. The other tryptic peptide, designated Lcryst-T44 (44 kDa in size), provided a 25-amino-acid sequence that aligns with a portion of human cytosolic ALDH that is adjacent (on the amino-terminal side) to the active site region. (We refer to Johansson *et al.* (24) for functional interpretations of corresponding regions of the ALDH sequence.) Cleavage with staphylococcal protease resulted in three to four major peptides. Two peptides, 30 and 10 kDa in size, were partially sequenced. Comparison of these two sequences with the National Biomedical Research Foundation protein database revealed that ALDH provided the closest sequence matches. A 13-amino-acid sequence from the 30-kDa peptide, designated Lcryst-V30, overlaps with the first seven amino acids of the 44-kDa tryptic peptide. However, the sequence of the first six amino acids of the 30-kDa peptide shows 83.3% (5 of 6) sequence identity with a highly conserved region of ALDH. The sequence from the 10-kDa peptide, designated Lcryst-V10, shows 68.8% sequence identity (11 of 16) with residues 400–415 of human ctytosolic ALDH; although the function of this segment of the ALDH molecule is unknown, it is immediately adjacent (on the carboxyl-terminal side) to a region thought to play a role in subunit interactions. Five tryptic peptides of Ω-crystallin from *O. dofleini*, which have been sequenced by Tomarev *et al.* (10), show 58% sequence identity with vertebrate cytoplasmic ALDH; our peptide sequences align with different portions of the ALDH molecule, except for the first six amino acids of Lcryst-V10, which are identical to the corresponding sequence from the Ω-crystallin tryptic peptide that aligns with the same region of ALDH (residues 400–405).

ALDH catalyzes the oxidation of an aldehyde to an acid using $NAD(P)^+$ as an electron acceptor. We were unable to detect any ALDH activity in either purified L-crystallin or in the light organ lens extract (less than 0.001 unit mg protein$^{-1}$).

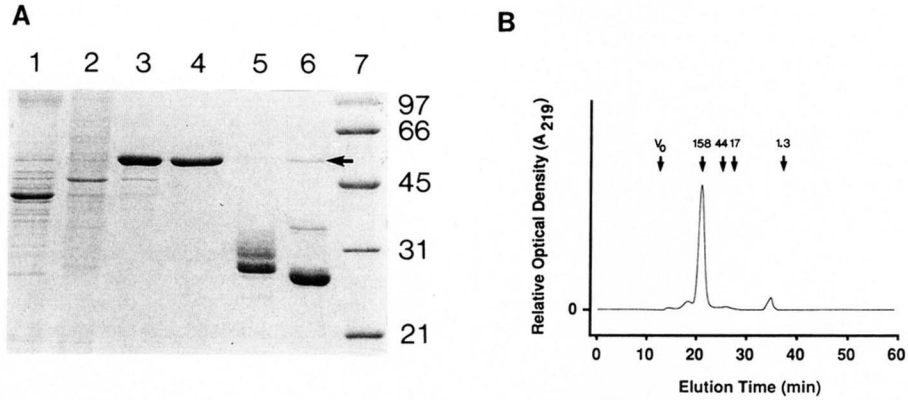


FIG. 2. **Characterization of the light organ lens proteins of *E. scolopes*.** *A*, SDS-polyacrylamide gel electrophoresis of the soluble proteins of various tissues of the squid *E. scolopes* (*lanes 1–5*), and of the ocular lens of *O. bimaculatus* (*lane 6*). Supernatant fluids containing 2 μg of total soluble protein of each tissue sample were loaded as follows: *lane 1*, mantle muscle; *lane 2*, visceral muscle of the hindgut; *lane 3*, light organ lens; *lane 4*, purified L-crystallin from the light organ lens; *lane 5*, ocular lens; *lane 6*, *O. bimaculatus* ocular lens; *lane 7*, standards. The molecular masses of standards are shown in kilodaltons. The *arrow* indicates the position of Ω-crystallin, an ALDH-like protein present in the ocular lens of the octopus (*lane 6*) (10). *B*, a typical gel filtration profile of the soluble proteins of the light organ lens results in a single major peak that contains L-crystallin. Soluble proteins of the light organ lens were extracted in 20 mM Tris, pH 7.9, and separated by HPLC using a Pharmacia LKB Superose 12 HR 10/30 column. The molecular masses of gel filtration standards are shown in kilodaltons at the top of the chromatogram ($V_0$, void volume).

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Lcryst-V28       KQNLK(R)YSMKLNG
ALDH  (259-271)  *S*** * VTLE*G*

Lcryst-T44       YSMKLNGKXPIVIFDDTXLEYAVQQ
ALDH  (265-289)  VTLE*G**S*CIVLA*AD*DN**EF

Lcryst-V10       IFGPVQLI(I/M)KF(S/N)DLND
ALDH  (400-415)  ******Q*  M  **  K  S*D*
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FIG. 3. **Alignment of the amino acid sequences of peptides of L-crystallin (see text) with the deduced amino acid sequence of human cytosolic ALDH (23).** The last seven amino acids of Lcryst-V28 overlap with the first seven amino acids of Lcryst-T44. *Asterisks* mark identical amino acid residues. *Numbers* in *parentheses* show the positions of corresponding regions in the ALDH sequence. The identity of residues that were ambiguous are placed in *parentheses*. *X* = unidentified residue.

ALDH activity was easily detectable in a soluble extract of *E. scolopes* digestive gland (0.013 unit mg protein$^{-1}$). Typical specific activities for purified mammalian ALDHs range from 0.2 to 1.2 units mg protein$^{-1}$ (25); whereas, specific activities for crude homogenates of bovine and human liver, measured under assay conditions similar to those reported here, are 0.006 and 0.019, respectively (26, 27). Thus, L-crystallin may be specialized for a structural role and may not possess enzyme activity. Similarly, some enzyme crystallins of ocular lenses show little to no activity, even though they may be the same gene product as the active enzyme (6). In addition, η-crystallin in the lens of the elephant shrew exhibits no enzyme activity, yet it is thought to be encoded by the same gene as the cytoplasmic ALDH isozyme (8). Because lens crystallins presumably are recruited as structural proteins, enzymatic capabilities may not be maintained by the protein in its new environment (6).

Polyclonal rabbit antiserum, raised against L-crystallin, cross-reacted with a minor protein present in the muscle and in the ocular lens of the squid, *E. scolopes*, and with Ω-crystallin from the ocular lens of *O. bimaculatus* (Fig. 4). Wistow and Kim (8) found that, in addition to functioning as a crystallin in the elephant shrew lens, ALDH is present as a moderately abundant protein in the lenses of several other mammalian species, including cows. We found cross-reactivity with a protein from bovine lenses that is composed of 54-kDa subunits and is most likely ALDH (data not shown). Furthermore, we tested the antiserum for cross-reactivity with η-crystallin from the ocular lens of the elephant shrew, *E. edwardi*. η-Crystallin represents in *E. edwardi* almost 25% of the total soluble protein of the lens (8). Although we found no cross-reactivity in Western immunoblots, using the "dot blot" technique we found strong cross-reactivity with native total soluble protein from the ocular lenses of this mammalian species (data not shown). Because the polyclonal antibody was raised against native L-crystallin, it is not unexpected that the antibody might cross-react with the native form of η-crystallin, and not with the denatured form of the protein.

As a result of the sequence similarity between L-crystallin and ALDH, and the immunological cross-reactivity between L-crystallin and the ALDH-like lens crystallins, η-crystallin and Ω-crystallin, we conclude that the major structural protein of the light organ lens is either identical or closely related to the enzyme ALDH. The light organ lens crystallin is apparently an example of the recruitment of a preexisting protein as a structural protein during the evolution of a lens-type tissue. In addition, our data show that an immunologically related protein is present in relatively low abundance in other squid tissues, including visceral muscle, from which the light organ lens is derived, and the ocular lens.

Ω-Crystallin has not been reported previously as present in the ocular lenses of squids. The 54-kDa protein present in the ocular lens of *E. scolopes*, assuming it is homologous with Ω-crystallin from the octopus, is not as abundant as it is in *O. bimaculatus* (Fig. 4). It is possible that the expression of the ALDH-like protein in the ocular lens of *E. scolopes* is analogous to the moderately abundant presence of non-crystallin ALDH in the lenses of many mammalian species. Due to its relatively low abundance, it is not clear if ALDH in these ocular lenses is functioning solely as an enzyme, or if it plays any structural role.

Wistow and Kim (8) suggested that enzyme/crystallins may be recruited from a pool of enzymes that are moderately abundant, and whose genes are widely expressed in lenses, because these enzymes have important developmental or functional roles. This idea suggests that there have been perhaps at least two major steps involved in recruitment of an enzyme/crystallin. For example, an enzyme may first be recruited into the lens for a particular function that is peculiar to the lens because of its transparent nature, such as protection against

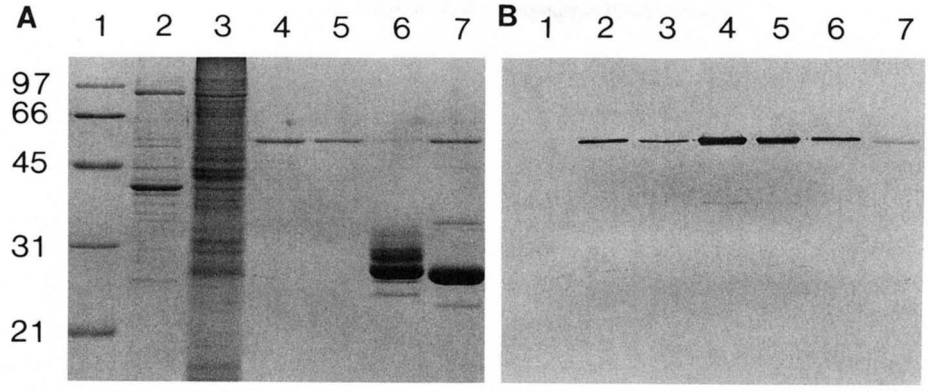


FIG. 4. **Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of soluble proteins of various cephalopod tissues (*A*) and Western immunoblot of these samples reacted against anti-L-crystallin (*B*).** The gel and transblot were loaded with the same concentration of soluble protein in each lane as follows: *lane 1*, standards; *lanes 2–6*, various tissues of *E. scolopes* specimen (total amount of soluble protein loaded): *lane 2*, mantle muscle (3 μg); *lane 3*, visceral muscle (5 μg); *lane 4*, light organ lens (0.2 μg); *lane 5*, purified L-crystallin from the light organ lens (0.2 μg); *lane 6*, ocular lens (6 μg); *lane 7*, ocular lens of *O. bimaculatus* (3 μg). The molecular masses of standards are shown in kilodaltons.

oxidative damage. Secondarily, certain of these proteins were then selected for yet another function, that of a major structural protein that contributes to the refractive properties of the lens. Because many different enzyme/crystallins have been recruited apparently to fulfill the same role, Wistow and Kim (8) suggested that the process of recruitment was probably at least "partially neutral." However, the apparent recruitment of ALDH in three independently evolved lens tissues, *i.e.* the ocular lenses of certain mammals and cephalopods and the light organ lens of a squid, presents the question: What property of this particular enzyme promotes its selection as a lens structural protein? ALDH is present, if not abundant, in the ocular lenses of a wide variety of vertebrate species (8) as well as in the independently evolved ocular lenses of certain cephalopods (10). Further, an additional isozyme of ALDH that has a high specific enzyme activity is present in the cornea of some mammalian species, including humans (21, 28). Holmes *et al.* (21) have suggested that the ALDH in these transparent tissues protects against peroxidative damage. Possibly, ALDH was first recruited for such a purpose, and then secondarily converted in some species to serve a largely structural role. However, as is the case with all other enzyme/crystallins discovered, why ALDH was selected as a structural protein is unknown.

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REFERENCES

1. McFall-Ngai, M., and Toller, W. (1991) in *Biochemistry and Molecular Biology of Fishes* (Hochachka, P., and Mommsen, T., eds) Vol. 1, pp. 77–107, Elsevier, Amsterdam
2. Arnold, J. (1967) *J. Ultrastruct. Res.* **17,** 527–543
3. Herring, P. J., Clarke, M. R., von Boletzky, S., and Ryan, K. P. (1981) *J. Mar. Biol. Assoc. U. K.* **61,** 901–916
4. McFall-Ngai, M., and Montgomery, M. K. (1990) *Biol. Bull.* **179,** 332–339
5. Bloemendal, H. (1981) *Molecular and Cellular Biology of the Eye Lens*, Wiley, New York
6. Piatigorsky, J., and Wistow, G. (1991) *Science* **252,** 1078–1079
7. Wistow, G., and Piatigorsky, J. (1987) *Science* **236,** 1554–1556
8. Wistow, G., and Kim, H. (1991) *J. Mol. Evol.* **32,** 262–269
9. Packard, A. (1972) *Biol. Rev.* **47,** 241–307
10. Tomarev, S., Zinovieva, R., and Piatigorsky, J. (1991) *J. Biol. Chem.* **266,** 24226–24231
11. Tomarev, S. I., and Zinovieva, R. D. (1988) *Nature* **336,** 86–88
12. Chiou, S. H. (1988) *FEBS Lett.* **241,** 261–264
13. McFall-Ngai, M. J., and Ruby, E. (1991) *Science* **254,** 1491–1494
14. Richardson, K. C., Jarret, L., and Finke, E. H. (1960) *Stain Technol.* **35,** 313–323
15. Whittaker, J. R., and Granum, P. E. (1980) *Anal. Biochem.* **109,** 156–159
16. Laemmli, U. K. (1970) *Nature* **227,** 680–685
17. Towbin, H., Staehelin, T., and Gordon, J. (1979) *Proc. Natl. Acad. Sci. U. S. A.* **76,** 4350–4353
18. Deitrich, R. A. (1966) *Biochem. Pharmacol.* **15,** 1911–1922
19. Ambroziak, W., Kosley, L. L., and Pietruszko, R. (1989) *Biochemistry* **28,** 5367–5373
20. Weiner, H. (1979) in *Biochemistry and Pharmacology of Ethanol* (Majchrowicz, E., and Noble, E. P., eds) Vol. 1, pp. 107–124, Plenum Press, New York
21. Holmes, R. S., Cheung, B., and Vandeberg, J. L. (1989) *Comp. Biochem. Physiol.* **93B,** 271–277
22. Guan, K. L., Pak, Y. K., Tu, G. C., Cao, Q. N., and Weiner, H. (1988) *Alcohol Clin. Exp. Res.* **12,** 713–719
23. Hempel, J., von Bahr-Lindström, H., and Jornvall, H. (1984) *Eur. J. Biochem.* **141,** 21–35
24. Johansson, J., von Bahr-Lindström, H., Jeck, R., Woenckhaus, C., and Jörnvall, H. (1988) *Eur. J. Biochem.* **172,** 527–533
25. Ramsey, A. J., Hill, J. P., and Dickinson, F. M. (1989) *Comp. Biochem. Physiol.* **93B,** 77–83
26. Leicht, W., Heinz, F., and Freimuller, B. (1978) *Eur. J. Biochem.* **83,** 189–196
27. Greenfield, N. J., and Pietruszko, R. (1977) *Biochim. Biophys. Acta* **483,** 35–45
28. Cuthbertson, R. A., Tomarev, S. I., and Piatigorsky, J. (1992) *Proc. Natl. Acad. Sci. U. S. A.* **89,** 4004–4008