Reports

Recruitment of Enzymes as Lens Structural Proteins

GRAEME WISTOW AND JORAM PIATIGORSKY

Crystallins, the principal components of the lens, have been regarded simply as soluble, structural proteins. It now appears that the major taxon-specific crystallins of vertebrates and invertebrates are either enzymes or closely related to enzymes. In terms of sequence similarity, size, and other physical characteristics δ-crystallin is closely related to argininosuccinate lyase, τ-crystallin to enolase, and S_{III}-crystallin to glutathione *S*-transferase; moreover, it has recently been demonstrated that ε-crystallin is an active lactate dehydrogenase. Enzymes may have been recruited several times as lens proteins, perhaps because of the developmental history of the tissue or simply because of evolutionary pragmatism (the selection of existing stable structures for a new structural role).

THE LENS IS A REMARKABLY SPECIALized product of evolution (*1*). Between 20 and 60% (depending on species) of its wet weight is composed of crystallins, soluble proteins of a few distinct types, whose abundance necessarily defines them as structural components of the lens (*1, 2*). α-Crystallins belong to the same protein superfamily as small heat-shock proteins and a *Schistosoma mansoni* egg antigen, p40 (*3*), whereas β- and γ-crystallins belong to a single superfamily that may also include the calcium-binding protein S of the bacterium *Myxococcus xanthus* (*4*). These crystallins seem to have arisen by gene duplication and divergence, specializing for the lens environment. In contrast, it has recently been shown that ε-crystallin, a major component of the lens in crocodiles and many birds, is a functional lactate dehydrogenase (LDH), apparently identical to LDH-B4 (*5*). This observation led to the prediction that other taxon-specific crystallins might have similar relations with enzymes (*5*). As described here, this seems to be the case.

The major protein of the embryonic lens in all birds and reptiles is δ-crystallin (*6*). Genomic sequencing has revealed the existence of two closely linked, highly similar genes in the chicken (*7*), although only complementary DNA (cDNA) corresponding to δ1 has been observed (*7, 8*). Low levels of δ-like protein and messenger RNA (mRNA) have been detected in a variety of nonlens tissues in the chick, and nonlens expression of the δ1 promoter has been observed (*9*). Now it is apparent that the δ-crystallins have significant sequence similarity to the urea cycle enzyme argininosuccinate lyase (E.C. 4.3.2.1) (ASL) of both humans and yeast (*10*). Specifically, δ1 and δ2 are 51 and 56% identical to the published yeast ASL (ARG4) sequence at the protein level and 58 and 62% identical to the predicted human sequence. Indeed, the similarity with the human ASL sequence may be even higher. A shift in reading frame toward the 3′ end of the published cDNA sequence yields a predicted protein sequence that is 64 and 69% identical to chicken δ1 and δ2, respectively (Fig. 1A).

ASL, like δ-crystallin, is a tetramer of 50-kD subunits and acid *p*I (*11*). Predictions of secondary structure and hydrophobicity profiles for human and yeast ASL and the two δ-crystallins at the protein level are essentially identical (*12*). The similarity between the chicken δ-crystallins and human ASL does not prove homology but strongly suggests that they belong to the same protein superfamily, which may perhaps include other lyases such as adenylosuccinate lyase, aspartase, and fumarase (*13*). The nonlens expression of δ (*9*) is consistent with the possibility that one or both δ genes could indeed code for a functional enzyme present at low levels in other tissues.

Another major lens protein in some fish, reptiles, birds, and lampreys has been designated τ-crystallin (*14*). A preliminary amino acid sequence for turtle τ-crystallin (*15*) shows strong similarity with the sequences of human and yeast enolases (*16*) (Fig. 1B). Enolase (E.C. 4.2.1.11) is a glycolytic enzyme of 48-kD subunit size, identical to the reported size of turtle τ-crystallin (*14, 16*). Circular dichroic spectroscopy of turtle τ-crystallin has predicted a high content of α-helix (52%) (*14*), in good agreement with the values predicted for the human α-enolase sequence (*12*). Enolase, however, is dimeric whereas isolated turtle τ-crystallin is predominately monomeric (*14, 16*). This difference may be due to the removal of Mg^{2+} essential for enolase dimerization by dialysis during purification of τ-crystallin or to aging in the lens. However, τ-crystallin is clearly closely related to α-enolase. The extremely close match with the human sequence shows that an important protein has been highly conserved during vertebrate evolution.

As they differentiate, the fiber cells of the lens lose all organelles, including mitochondria (*1*). This means that they must rely on cytoplasmic glycolysis as a source of energy (*17*). LDH and enolase are both glycolytic enzymes, but ε- and τ-crystallin are present in quantities [as much as 23% of lens protein (*5, 14*)] that seem to exceed any likely requirements of a purely glycolytic role. Interestingly, high levels of mRNA for LDH and enolase have been detected after the induction of proliferation of rat fibroblasts by epidermal growth factor (*18*), whereas high levels of enolase have also been observed during oogenesis and in tumorigenic cell lines of *Xenopus* (*19*). Both LDH and enolase have been shown to be substrates for *src* tyrosine kinase (*20*), and enolase, to be a substrate for *fes/fps* kinase (*21*). LDH has DNA helix–destabilizing activity and has been found associated with regions of actively transcribing chromatin in vivo (*22*), whereas enolase has been identified as the 48-kD heat-shock protein in yeast and has a region of similarity with yeast *hsp*70 and the product of the *Escherichia coli dnaK* gene (*23*). These observations suggest the possibility that these enzymes have important cellular roles beyond those in metabolism. Perhaps lens differentiation at least partly resembles an early growth phase of other cell types. Some crystallins may be proteins associated with such a phase but synthesized at high levels over a longer period in the lens.

The α-, β-, γ-, δ-, ε-, and τ-crystallins are all components of vertebrate lenses. Some invertebrates also have cellular lenses, usually regarded as the products of convergent evolution (*24*). The major protein component of the squid (*Nototodarus gouldi*) lens is S_{III}-crystallin (70% of the soluble fraction), consisting of dimers of related 27- to 30-kD subunits for which a partial NH_2-terminal amino acid sequence has been published (*25*). Comparison of this sequence with the National Biomedical Research Foundation (NBRF) protein database reveals striking similarity with the NH_2-terminal sequence of rat glutathione *S*-transferase (GST) (E.C. 2.5.1.18) Ya subunit (*26*) (Fig. 1C). In the

Laboratory of Molecular and Developmental Biology, National Eye Institute, National Institutes of Health, Bethesda, MD 20892.

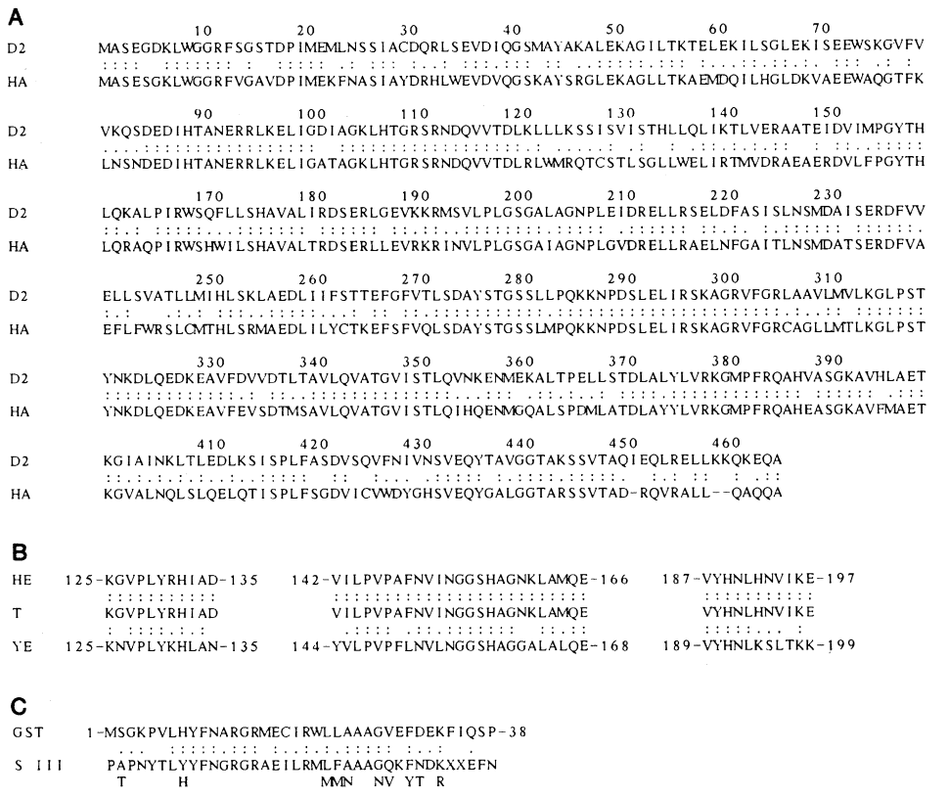

Fig. 1. Similarity in amino acid sequence between lens proteins and enzymes. In all cases a colon indicates identity and a period indicates structurally conservative changes. (**A**) Alignment of the complete predicted amino acid sequence of chicken δ2-crystallin (D2) (*7*) and human argininosuccinate lyase (HA) (*11*). The HA sequence differs from that published by a frame shift between amino acid residues 386 and 450 (as numbered here). This corresponds to an insertion of G after base 1272 of the cDNA sequence and an insertion of C after base 1281 of that sequence. These two bases could base pair in a potential stem structure in the modified sequence. The frame is restored by deletion of G at position 1457 and 1461 of the cDNA sequence. The modified amino acid sequence in this region is similar to those of the δ-crystallins (*7*) and yeast ASL (*11*), whereas the unmodified sequence has no significant match. The identity between the sequences shown is 69%. (**B**) Alignment of preliminary amino acid sequences for turtle τ-crystallin (T) with human (HE) and yeast (YE) enolase sequences (*16*). The residue numbers for the enzymes are shown. This represents about 10% of the total enolase/τ-crystallin sequence. The τ-crystallin peptides sequenced were from three major HPLC peaks of V8 protease digested, purified turtle τ-crystallin (*15*). The match with human α-enolase in these peptides is 100%. No τ-crystallin sequences were observed that did not match α-enolase. (**C**) Alignment of the NH_2-terminal sequence of squid S_{III}-crystallin (S III) (*25*) with the NH_2-terminal sequence of rat glutathione *S*-transferase Ya (GST) (*26*). The squid sequence apparently derives from the similar NH_2-termini of two related subunits. Identity is shown where one of those positions matches the GST sequence. Over the NH_2-terminal 33 residues of the rat GST and squid S_{III}-crystallin the sequences exhibit identity at 18 positions (55%). The sequences shown represent about 15% of the total for each protein. This similarity is greater than that previously proposed with βγ-crystallins (*25*).

rat there are three major GST subunits of 26- to 28-kD size that form dimers, giving good agreement in size with the squid lens protein. The secondary structure of rat GST has been estimated as 40% α helix, 15% β sheet (*26*), compared with 20 to 30% α helix, 10 to 35% β sheet for the squid crystallin (*25*). It therefore seems likely that S_{III}-crystallin is structurally and evolutionarily related to GST, a detoxification enzyme that is also found at high levels (up to 10%) in mammalian liver (*27*).

Thermodynamic stability is required for lens proteins because of their long life in the fiber cells of the lens (*1*). It is possible that during evolution these crystallins have been recruited as lens proteins simply because of their stable structure. The same strategy, involving different proteins, has been used in both vertebrates and invertebrates. Pragmatically, natural selection may have made further use of stable molecules already expressed for various cellular functions in new structural roles in a specialized tissue. This implies that, at least to start with, the same gene coded for both enzyme and structural protein, presumably by the acquisition of new gene promoter elements, before gene duplication, divergence, and specialization occurred.

This may still be the case for ε-crystallin, which in terms of sequence and activity appears to be identical to LDH-B4 (*5*). For the other enzymes discussed here the answer is not yet so clear. It has been argued that retention of enzyme activity by a crystallin suggests that gene duplication has not yet taken place (*5*). Purified τ-crystallin does have significant enolase activity, 5% of that expected for the pure rabbit muscle enzyme (*28*). However, for lens extracts containing chicken δ-crystallins and squid crystallins only low levels of ASL and GST activity, equivalent to those found in other tissues, have so far been detected (*28*). This does not rule out the possibility that these crystallins are expressed as active enzymes. Activity may be lost because of modifications due to aging in the lens or because of the presence of inhibitory factors, points that are under investigation. Genomic and cDNA analyses will eventually be required to determine the precise origin of these proteins and the genetic mechanisms leading to their expression as major lens components. Finally, these observations raise the possibility that even normal, low levels of enzymes could have some kind of structural role in lens and in cataract.

REFERENCES AND NOTES

1. H. Bloemendal, Ed., *Molecular and Cellular Biology of the Eye Lens* (Wiley, New York, 1981); H. Maisel, Ed., *The Ocular Lens* (Dekker, New York, 1985).
2. J. Piatigorsky, *Invest. Ophthalmol. Visual Sci.* **28**, 9 (1987); W. W. de Jong and W. Hendricks, *J. Mol. Evol.* **24**, 121 (1986).
3. T. D. Ingolia and E. A. Craig, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 2360 (1982); G. Wistow, *FEBS Lett.* **181**, 1 (1985); V. Nene, D. W. Dunne, K. S. Johnson, D. W. Taylor, J. S. Cordingley, *Mol. Biochem. Parasitol.* **21**, 179 (1986).
4. H. P. C. Driessen, P. Herbrink, H. Bloemendal, W. W. de Jong, *Eur. J. Biochem.* **121**, 83 (1981); T. Blundell *et al.*, *Nature (London)* **289**, 771 (1981); G. Wistow, L. Summers, T. Blundell, *ibid.* **315**, 771 (1985).
5. G. J. Wistow, J. W. M. Mulders, W. W. de Jong, *Nature (London)* **326**, 622 (1987).
6. J. Piatigorsky, *Mol. Cell. Biochem.* **59**, 33 (1984).
7. J. Nickerson *et al.*, *J. Biol. Chem.* **260**, 9100 (1985); M. Ohno *et al.*, *Nucleic Acids Res.* **13**, 1593 (1985); J. M. Nickerson *et al.*, *J. Biol. Chem.* **261**, 552 (1986).
8. E. F. Wawrousek, J. M. Nickerson, J. Piatigorsky, *FEBS Lett.* **205**, 235 (1986).
9. R. M. Clayton, J. C. Jeanny, D. J. Bower, L. H. Errington, *Curr. Top. Dev. Biol.* **20**, 137 (1986); H. Kondoh, S. Hayashi, Y. Takahashi, T. S. Okada, *Cell Differ.* **19**, 151 (1986).
10. I. R. Beacham, B. W. Schweitzer, H. M. Warrick, J. Carbon, *Gene* **29**, 271 (1984); W. E. O'Brien, R. McInnes, K. Kalumuck, M. Adcock, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 7211 (1986). The resemblance between δ-crystallin and argininosuccinate lyase was brought to our attention by R. F. Doolittle who noted the correspondence while entering the published sequence into the NEWAT database (personal communication). The similarity with the product of the yeast ARG4 gene can be detected with the SEQFT option of IDEAS (M. Kanehisa) to search GenBank.
11. S. Ratner, *Adv. Enzymol.* **39**, 1 (1973).
12. Secondary structure predicted by the method of J. Garnier, D. J. Osguthorpe, and B. Robson [*J. Mol. Biol.* **120**, 97 (1978)]; hydrophobicity by the method of Y. Nozaki and C. Tanford [*J. Biol. Chem.* **246**, 2211 (1971)]. Results not shown.
13. R. M. Pinto *et al.*, *J. Biol. Chem.* **258**, 12513 (1983); J. S. Takagi, M. Tokushige, Y. Shimura, M.

Kanehisa, *Biochem. Biophys. Res. Commun.* **138**, 568 (1986). Comparisons of the ASL and δ-crystallin sequences with those for tetrameric, 50-kD subunit size bacterial aspartases and fumarases show regions of similarity (G. Wistow and J. Piatigorsky, unpublished results).
14. L. A. Williams, L. Ding, J. Horwitz, J. Piatigorsky, *Exp. Eye Res.* **40**, 741 (1985).
15. τ-Crystallin was purified from adult turtle lens by isoelectric focusing, followed by chromatography on a TSK 3000 SW gel permeation column, then on a Synchro Pak AX 300 anion-exchange column [L. A. Williams and J. Horwitz (*14*)]. Protein sequencing was performed by W. S. Lane and D. Andrews, Harvard University Microchemistry Facility, Harvard University, Cambridge, MA 02138. V8-proteolytic fragments of turtle τ-crystallin were separated by high-performance liquid chromatography (HPLC). Some peptides of major yield were then sequenced by an automated AP 470 sequencer. All peptides sequenced showed strong similarity to human α-enolase.
16. A. Giallongo, S. Feo, R. Moore, C. M. Croce, L. C. Showe, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 6741 (1986); C. C. Q. Chin, J. M. Brewer, F. Wold, *J. Biol. Chem.* **256**, 1337 (1981).
17. O. Hockwin and C. Ohrloff, in *Molecular and Cellular Biology of the Eye Lens*, H. Bloemendal, Ed. (Wiley, New York, 1981), pp. 367–413.
18. L. M. Matrisian, G. Rautmann, D. I. Magun, R. Breathnach, *Nucleic Acids Res.* **13**, 711 (1985).
19. J. J. Picard *et al.*, *Exp. Cell Res.* **167**, 157 (1986).
20. J. A. Cooper, N. A. Reiss, R. J. Schwartz, T. Hunter, *Nature (London)* **302**, 218 (1983).
21. I. MacDonald, J. Levy, T. Pawson, *Mol. Cell. Biol.* **5**, 2543 (1985).
22. K. R. Williams, S. Reddigari, G. L. Patel, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 5260 (1985).
23. H. Iida and I. Yahara, *Nature (London)* **315**, 688 (1985).
24. A. Packard, *Biol Rev.* **47**, 241 (1972).
25. R. J. Siezen and D. C. Shaw, *Biochim. Biophys. Acta* **704**, 304 (1982).
26. H.-C. J. Lai, N. Li, M. J. Weiss, C. C. Reddy, C.-P. D. Tu, *J. Biol. Chem.* **259**, 5536 (1984).
27. W. B. Jakoby, *Adv. Enzymol.* **44**, 383 (1978).
28. Relative enolase activity was measured according to the method of E. W. Westhead [*Methods Enzymol.* **9**, 670 (1967)]. ASL activity was measured according to the method of S. Ratner [*ibid.* **17**, 304 (1970); also W. E. O'Brien, unpublished]. GST activity in lens homogenate of commercially available squid was measured according to the procedure of W. A. Habig, M. J. Pabst, and W. B. Jakoby [*J. Biol. Chem.* **249**, 7130 (1974)].
29. We thank L. Williams and J. Horwitz for the turtle τ-crystallin, and W. E. O'Brien, W. W. de Jong, R. F. Doolittle, W. B. Jakoby, and J. Nickerson for helpful discussions, and D. Chicchirichi for manuscript preparation.

22 January 1987; accepted 14 April 1987

Alpha-Decay–Induced Fracturing in Zircon: The Transition from the Crystalline to the Metamict State

BRYAN C. CHAKOUMAKOS, TAKASHI MURAKAMI,*
GREGORY R. LUMPKIN, RODNEY C. EWING

A natural single crystal of zircon, $ZrSiO_4$, from Sri Lanka exhibited zonation due to alpha-decay damage. The zones vary in thickness on a scale from one to hundreds of micrometers. The uranium and thorium concentrations vary from zone to zone such that the alpha-decay dose is between 0.2×10^{16} and 0.8×10^{16} alpha-events per milligram (0.15 to 0.60 displacement per atom). The transition from the crystalline to the aperiodic metamict state occurs over this dose range. Differential expansion of individual layers due to variations in their alpha-decay dose caused a systematic pattern of fractures that do not propagate across aperiodic layers. High-resolution transmission electron microscopy revealed a systematic change in the microstructure from a periodic atomic array to an aperiodic array with increasing alpha-decay dose. At doses greater than 0.8×10^{16} alpha-events per milligram there is no evidence for long-range order. This type of damage will accumulate in actinide-bearing, ceramic nuclear waste forms. The systematic pattern of fractures would occur in crystalline phases that are zoned with respect to actinide radionuclides.

ZIRCON, $ZrSiO_4$, OCCURS IN NATURE with total uranium and thorium concentrations that are usually in the range from 0 to 4000 ppm (*1*) but in rare cases up to 6% by weight $UO_2 + ThO_2$ (*2*). Alpha-decay doses over hundreds of millions of years can be as high as 10^{17} alpha-events per milligram, which is equivalent to 7 displacements per atom (dpa). Over the narrow dose range from 10^{15} to 10^{16} alpha-events per milligram (Fig. 1), crystalline zircon is converted to the metamict state, a structure that appears to be amorphous when analyzed by x-ray and electron diffraction. The transition causes pronounced changes in physical properties [for example, a decrease in density (17%) (*3*), a decrease in birefringence until the material is isotropic (*3*), a decrease in the elastic moduli (up to 69%) (*4*), and a decrease in Poisson's ratio (7%) (*4*)]. Plutonium-doped synthetic zircons show approximately the same density change (16%) at doses of 10^{16} alpha-events per milligram (*5*). With increasing alpha-decay dose, x-ray diffraction maxima decrease in intensity, become asymmetric, and shift to lower values of 2θ (because of a unit cell volume expansion of 5%, where θ is the angle between the diffracting plane and the x-ray beam) until the material becomes amorphous to x-ray diffraction (*3, 6*). Individual samples that spanned portions of the transition-dose zone have been studied by transmission electron microscopy, but to our knowledge no high-resolution electron microscopy study has spanned the full range of doses over which the transition occurs (*7, 8*).

The changes in the atomic structure and accompanying changes in physical properties caused by alpha-decay damage are of significance for several reasons. (i) Zircon is a major mineral used in dating igneous and metamorphic rocks by U-Th-Pb methods. Disturbed U-Th-Pb systematics have been ascribed to alpha-decay damage (*9*). (ii) The zircon structure type is an actinide-bearing phase in multiphase ceramic nuclear waste forms (*10*), and natural zircon has been used to evaluate elemental loss from waste form phases under repository conditions (*11*). The change in the atomic structure during the transition is fundamental to the evaluation of the long-term performance of crystalline, polyphase, ceramic nuclear waste forms (*12, 13*). In this report we present data on a zoned zircon from Sri Lanka (Ceylon) in which the variation in the UO_2 and ThO_2 contents from zone to zone is such that the range of dose varies from 0.2×10^{16} to 0.8×10^{16} alpha-events per milligram (equivalent to 0.15 to 0.60 dpa) and thus covers the most important range of dose over which the radiation-induced crystalline-to-metamict transition occurs (Fig. 1).

The single crystal was collected from the gem gravels of the Ratnapura district in Sri Lanka (*14*). The age of the zircons is 570 ± 20 million years (*3*). Although the provenance of these alluvial zircons is problematic, their large size, internal growth zoning, and lack of metamorphic deformation suggest that they are magmatic; their likely source rocks are granitoid plutons or pegmatites. The prismatic crystal (sample 4601 University of New Mexico Research Collection) was sectioned lengthwise to expose an unbanded core capped by coarse (001) layers that are 5 to 400 μm thick. These layers are surrounded by finer (101), (100), and minor (301) layers that are 1 to 5 μm thick. A small phantom crystal within the unlayered core displays prominent (100), (001), (101), and (301) crystal forms. The birefringence of individual layers varies as a function of uranium and thorium contents (*15*) as illustrated by variations in the interference colors (Fig. 2).

Department of Geology, University of New Mexico, Albuquerque, NM 87131.

*On leave from the Japan Atomic Energy Research Institute, Department of Environmental Safety Research, Tokai, Ibaraki 319-11, Japan.