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CRYSTALLINS IN LENSES OF GEKKONID LIZARDS (REPTILIA, GEKKONIDAE)

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The ocular lenses of the Gekkonidae differ in their crystallin compositions. The lenses of all species investigated contain variable amounts of α-, β-, γ-, and τ-crystallins, which are commonly found in lenses of various vertebrates. Additionally, in lenses of the distantly related genera *Phelsuma* and *Lepidodactylus* another crystallin has been found which is a 38 kDa monomer and comprises about 20% of the total amount of crystallins. All other gekkonid species investigated, both nocturnal and diurnal, do not possess this crystallin. This distribution is highly surprising. It neither supports a possible correlation between similarity of crystallin composition and similarity of habits, nor a possible correlation between crystallin composition and phylogenetic relationship. Closer examination of the biochemical properties of the 38 kDa 'gecko' crystallin and comparison with other taxon-specific crystallins, especially the ε-crystallin of some other sauropsids, leads to the conclusion that this 38 kDa crystallin may be a so far undescribed novel type of crystallin.

INTRODUCTION

Vertebrate eye lenses consist mainly of numerous lens fiber cells which contain high concentrations of water-soluble proteins called crystallins. Despite the similar functions, i.e., light transmission and focus, of the lenses, they differ in shape, protein content and especially in their crystallin composition. These heterogenous crystallins can be divided into two main groups (reviews: Wistow & Piatigorsky, 1988; de Jong, Hendriks, Mulders & Bloemendal, 1989; Wistow, 1993). The first group comprises the α-, β- and γ-crystallins which are present in almost all vertebrates. The second group of crystallins is taxon-specific and frequently involves crystallins which are related or identical to enzymes of metabolic pathways (Wistow & Piatigorsky, 1987, 1988). Some of these taxon-specific enzyme crystallins have preserved their enzymatic activity, e.g. the ε-crystallin of some birds and crocodiles, which is identical with lactate dehydrogenase B4, and the τ-crystallin of several fishes, reptiles and birds, which is related to α-enolase (Wistow, Mulders & de Jong, 1987; Wistow *et al.,* 1988). Other enzyme crystallins do not show enzymatic activity, e.g. the η-crystallin of elephant shrews, which is identical with aldehyde dehydrogenase (Wistow & Kim, 1991).

Both the correlation between the distribution of taxon-specific crystallins and the phylogenetic relationships between these taxa, and the correlation between the distribution and the similarities in habits are still unclear. On the one hand, the scattered occurrence of the ε-crystallin does not correlate to phylogenetic relationships between the avian orders. On the other hand, this crystallin is also expressed in crocodiles (Stapel *et al.*,1985) which are supposed to be closely related to birds. Among birds, the ε-crystallin is found in diurnal species which occupy habitats with high ambient light intensities (Wistow *et al.,* 1987). So far, the ε-crystallin has not been found in nocturnal birds. In contrast, the expression of the δ-crystallin seems to be more closely connected with phylogenetic relationships (Wistow, Anderson & Piatigorsky, 1990). Despite the very similar feeding habits of chimney swift (*Chaetura pelagica*) and barn swallow (*Hirundo rustica*) only the latter possesses the δ-crystallin. The crystallin composition in the swift resembles that in Anna's hummingbird (*Calypte anna*). Swifts and hummingbirds are closely related but have very different habits.

To clarify the role of either relationship or similarity of habits, it seemed logical to choose a group of animals which combines close phylogenetic relationships with markedly different habits. Among the Reptilia, these conditions are met by the Gekkonidae. This family comprises both nocturnal and diurnal genera. According to the transmutation theory of Walls (1934, 1942), the nocturnal geckos are supposed to descend from primarily diurnal lizard ancestors whose visual cells had been cones with coloured oil droplets. Thus, the rods of 'secondarily' nocturnal geckos, which generally lack oil droplets, had transmuted from cones. Furthermore, Walls (1934, 1942), suggested that some of the gekkonid species which are now diurnal have reverted from nocturnal gecko ancestors. Their visual cells have undergone a second transmutation from rods back to cones. Most of these cones lack oil droplets, but as a substitute for these droplets the lenses of the 'tertiarily' diurnal geckos are yellow, in contrast to the colourless lenses of nocturnal species. Perhaps these decisive changes in habits resulted in both morphological modifications of the visual cells and biochemical modifications of the lenses.

TABLE 1. Occurrence of 38 kDa crystallin and of yellow colour in lenses of gekkonid species with different activity periods. C-D: crepusculo-diurnal, D: diurnal, D-N: diurno-nocturnal, N: nocturnal.

Species	Activity	Yellow lens	38 kDa-crystallin
Ailuronyx seychellensis	D-N	-	-
Gekko gecko	N	-	-
Hemidactylus frenatus	N	-	-
Lepidodactylus lugubris	D-N	-	+
Lygodactylus picturatus	D	+	-
Oedura castelnaui	N	-	-
Pachydactylus geitje	N	-	-
Pachydactylus maculatus	N	-	-
Paroedura pictus	N	-	-
Phelsuma andamanensis	D	+	+
Phelsuma barbouri	D	+	+
Phelsuma dubia	D	+	+
Phelsuma guentheri	D-N	+	+
Phelsuma madagascariensis	D	+	+
Phelsuma standingi	D	+	+
Sphaerodactylus cinereus	C-D	-	-
Stenodactylus sthenodactylus	N	-	-
Uroplatus henkeli	N	-	-
Uroplatus phantasticus	N	-	-

MATERIALS AND METHODS

ANIMALS

The lenses were obtained from nineteen species of the Gekkonidae (Table 1). *Sphaerodacylus cinereus* belongs to the subfamily Sphaerodactylinae, *Oedura castelnaui* belongs to the subfamily Diplodactylinae, whereas all other species belong to the subfamily Gekkoninae. Only adult animals were used.

PREPARATION OF LENS EXTRACTS

The animals were anaesthetized by chilling to 4°C and then humanely killed by decapitation. The eyes were enucleated and either used immediately or kept frozen at -80°C. The lenses were removed, weighed and carefully homogenized in minimum volumes of various buffer solutions depending on the different experiments. Insoluble fractions were removed by centrifugation at 4°C for 15 min at 15 000 x g. Protein concentrations of the different lens extracts were determined by a modified method of Neuhoff, Zimmer & Mesecke (1979) using bovine serum albumine as standard.

ENZYME ASSAY

Lactate dehydrogenase (LDH) activities of freshly prepared water-soluble proteins were determined in a standard assay mixture containing 100 m*M* bis-Tris-propane buffer pH 7.0, 0.15 m*M* NADH, 1 m*M* pyruvate and an appropriate amount of extract in a total volume of 1 ml. The reactions were started by the addition of pyruvate and monitored spectrophotometrically by following the decrease in absorbance at 340 nm as a function of time. One unit of enzyme activity is defined as the amount of enzyme that caused the oxidation of 1 µMol NADH/min (equivalent to the generation of 1 µMol lactate/min) at pH 7.0 and 25°C. A molar absorption coefficient of 6.2 10^6 cm^2/Mol for NADH was used for calculation.

GEL ELECTROPHORESIS

Samples for sodiumdodecyl sulphate (SDS) electrophoresis gels were prepared in two different ways. For the preparation of detergent-soluble proteins, lenses were homogenized in a solution containing 50 m*M*

Tris-HCl buffer pH 8.8, 3.75% SDS, 20 m*M* dithioerythritol and 10 m*M* dithiothreit. These samples were incubated at 30°C for 16 hours. After centrifugation (10 000 x g, 15 min) the protein concentrations of the supernatants were determined. Aliquots with 5, 10 or 20 mg protein brought to 30% mercaptoethanol and 6% glycerol with bromphenol blue, were separated on 6-20% gradient polyacrylamide gels containing 0.1% SDS and 3.2 *M* urea.

For the preparation of water-soluble proteins only, lens extracts were prepared in 125 m*M* Tris-HCl buffer pH 6.8. After determination of the protein concentration, the samples were denatured in a solution containing 5% SDS, 2% mercaptoethanol and 10% glycerol with bromphenol blue and boiled for 3 - 5 min. Aliquots with either 10 or 20 mg protein were run on 14% polyacrylamide gels containing 0.1% SDS. Protein bands were stained with Coomassie Brilliant Blue R250.

The molecular weights of crystallin subunits were calculated from the mobilities of the following standard marker proteins: phosphorylase b 92.5 kDa, bovine serum albumin 69 kDa, ovalbumin 45 kDa, carbonic anhydrase 29 kDa, soybean trypsin inhibitor 22 kDa, myoglobin from horse skeletal muscle 17.8 kDa, cytochrome c 12.5 kDa, lung trypsin inhibitor 6.5 kDa.

WESTERN BLOTTING

Water-soluble lens proteins of some gekkonid species were separated by sodiumdodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) (14% polyacrylamide), and then electrophoretically transferred to an Immobilon PVDF membrane. Blots were saturated in a solution of 10 m*M* TRIS, 150 m*M* NaCl (TBS) pH 7.2, 1% Tween 20 for at least 1 hr at room temperature and then incubated with anti-duck ε-crystallin antiserum for 4 hr at room temperature. The antiserum was kindly provided by Prof. W. W. de Jong, Nijmegen. After washing with TBS, 0.05% Tween 20 the blots were incubated with peroxidase-conjugated anti-rabbit IgG antiserum for two hours at room temperature. After washing, 4-chloro-naphthol was used for colour development.

GEL FILTRATION CHROMATOGRAPHY

Extracts of water-soluble lens proteins in elution buffer were subjected to fast protein liquid chromatography (FPLC). Samples of 200 ml containing between 1.8 and 2.5 mg protein were applied to a column of Sephacryl 100. The proteins were eluted with 100 m*M* Tris buffer, pH 7.0, at a flow rate of 60 ml/h at room temperature. Protein was monitored by the absorbance at 280 nm. Native molecular weights were estimated by using chromatography of the following standard proteins on the same column: aldolase 160 kDa, bovine serum albumin 67 kDa, ovalbumin 45 kDa, chymotrypsinogen 25 kDa and cytochrome c 12.4 kDa.

Peak fractions of samples were pooled and concentrated 30- to 90-fold over Centricon 10 filters. Aliquots with about 10 mg protein were supplied to a 14% polyacrylamide gel.

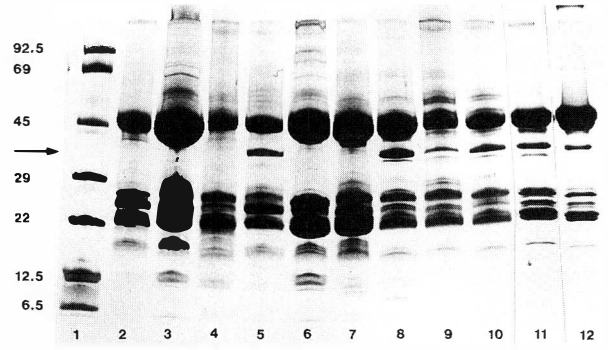

FIG. 1. Protein composition of crude lens extracts. SDS-PAGE (6-20% polyacrylamide) of detergent-soluble proteins (20 µg per lane). Lanes 2 to 7: nocturnal species, lanes 8-12: diurnal species. 2: *H. frenatus*, 3: *G. gecko*, 4: *P. maculatus*, 5: *L. lugubris*, 6: *P. pictus*, 7: *A. seychellensis*, 8: *P. dubia*, 9: *P. barbouri*, 10: P. *andamanensis*, 11: *P. standingi*, 12: *P. madagascariensis*. Lane 1: marker proteins. The molecular weights are given in kDa (left). The arrow indicates the 38 kDa crystallin.

RESULTS

PROTEIN COMPOSITION OF CRUDE LENS EXTRACTS

The isolated lenses of most diurnal gekkonid species are yellow with the exception of the lens of *Sphaerodactylus cinereus*, whereas the lenses of nocturnal geckos are colourless (Table 1). The lenses are generally of a soft consistency. With about 25-30% (e.g. *S. sthenodactylus*: 25%; *L. lugubris*: 27%; *O. castelnaui*: 29%) protein content in relation to lens wet weight; the gecko lenses belong to the more watery lenses, comparable to those of birds (e.g., eider duck *Somateria mollissima*: 26%).

The gel electrophoretic separations of detergent-soluble lens proteins of 19 different gekkonid species reveal remarkable differences in their compositions: A polypeptide of about 38 kDa occurs in considerable amounts in the diurnal genus *Phelsuma* and in the diurno-nocturnal species *L. lugubris* (Fig. 1). The 38 kDa polypeptide is found neither in other nocturnal nor in other diurnal species (Table 1). Thus, it does not correlate with the yellow colour of lenses from diurnal geckos.

In all species investigated, apart from *Lygodactylus*, protein patterns are similar in the ranges of 69 to 45 kDa and 29 to 12.5 kDa. The major protein δ-crystallin shows up as a relatively broad band with a molecular weight of about 50 kDa (Fig. 1). If the gel is not overloaded, the broad δ-bands of all species except *L. lugubris* are resolved into two bands, whereas the δ-crystallin of the latter always migrates as a single band (Fig. 2). The lens protein composition of *L. picturatus* characteristically has a relatively broad band in the range of about 15 kDa and only a very small one in the range of 20-22 kDa (Figs. 2, 3A). The latter is due to a reduced expression of α-crystallin.

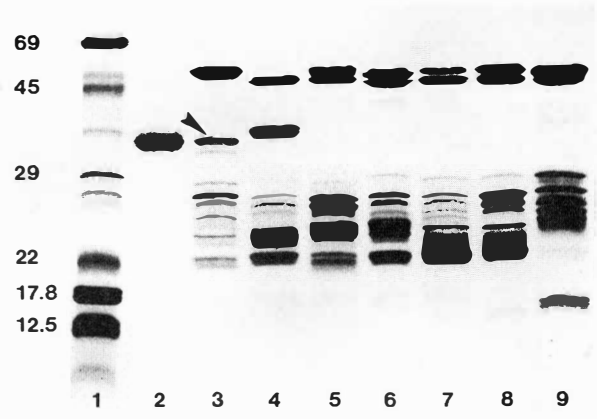

FIG. 2. Protein composition of crude lens extracts of sauropsid species and comparison with LDH. SDS-PAGE (14% polyacrylamide) of water-soluble proteins (10 µg per lane). Lane 1: marker proteins, molecular weights in kDa, lane 2: LDH from pig heart muscle, lane 3: eider duck *S. mollissima*, lane 4: *L. lugubris*, lane 5: *O. castelnaui*, lane 6: *S. sthenodactylus*, lane 7: *U. henkeli*, lane 8: *U. phantasticus*, lane 9: *L. picturatus*. The arrowhead indicates the avian ε-crystallin.

The 38 kDa protein comprises about 20 to 22% of the total amount of water-soluble lens proteins and is, therefore, regarded as one of the major crystallins (Fig. 2, 3A). Concerning the protein pattern of detergent-soluble lens proteins, the amount of this crystallin seems to be smaller (10-12%) (Fig. 1). This difference is due to the use of detergents during the extraction procedure, as these samples contain not only the cytosolic proteins but also the membrane proteins of the numerous lens fibres.

The 38 kDa crystallin seems not to be restricted to the Gekkonidae, because it also occurs in the diurnal lizard *Egernia cunninghami* (Scincidae) (unpublished observation). There are no appreciable differences in the mobility of the denatured 38 kDa crystallins between the genera *Phelsuma*, *Lepidodactylus* and *Egernia*.

Regarding the molecular weight of this special crystallin and its scattered occurrence in the Gekkonidae, it resembles on the one hand the ε-crystallin, identical with LDH, of some avian and reptilian genera, and on the other hand the ρ-crystallin of the amphibian genus *Rana*. Thus, a comparison of the lens crystallin compositions in some of those vertebrates seems useful. The lens protein patterns of *Rana temporaria* and *P. dubia* or *L. lugubris* are quite different (Röll, 1990). The ρ-crystallin has a higher mobility on the gel than the 38 kDa crystallin, pointing to an apparent molecular weight of 36 kDa. The protein pattern of the eider duck *S. mollissima,* is similar to that of *L. lugubris* (Fig. 2). Both the special gekkonid lens protein, the ε-crystallin of *S. mollissima* and the enzyme LDH have, in their denatured form, molecular weights of about 38 kDa (gekkonid crystallin: 38.4 ± 0.9 kDa, n=15; ε-crystallin: 38.1 ± 0.5 kDa, n=3; LDH: 38 ± 0.7 kDa, n=9). So it seems possible that this gekkonid and the avian ε-crystallin are identical or at least related.

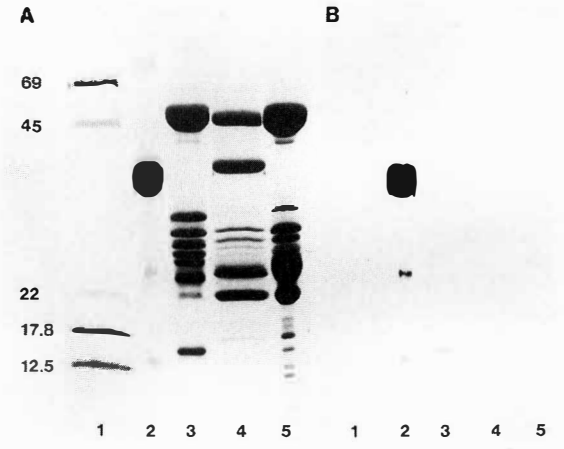

FIG. 3. SDS-PAGE (14% polyacrylamide) (A) and immunoblot (B) of gekkonid water-soluble lens proteins. A Crystallin composition of three gekkonid species and comparison with LDH (20 µg each lane). Lane 1: marker proteins, molecular weights in kDa, lane 2: LDH, lane 3: *L. picturatus*, lane 4: *L. lugubris*, lane 5: *S. sthenodactylus*. B Blotted proteins of the same extracts in the same sequence as in A. Proteins are stained with anti-duck ε-crystallin antiserum.

LDH-ACTIVITY IN GEKKONID LENSES

The ε-crystallin shows high LDH-activity (Wistow *et al.*, 1987; Chiou, Lee & Chang, 1990). Thus, it was examined whether gekkonid lens extracts also develop enzymatic activity. Three species were chosen for the LDH-activity assay: the diurno-nocturnal *L. lugubris* possessing the 38 kDa crystallin in comparison to the diurnal *L. picturatus* and the nocturnal *S. sthenodactylus*, both without this crystallin. The LDH-activities of these different samples are very similar (Table 2). The activity of the sample with the 38 kDa crystallin, presumed to be related to LDH, is not higher than the activities of the other samples. Thus, these activities must be due solely to the cytosolic LDH in the lens cells.

IMMUNOBLOTTING WITH ANTI-DUCK ε-CRYSTALLIN ANTISERUM

Water-soluble proteins of gekkonid lenses and pig heart muscle LDH, electrophoretically separated and blotted onto a PVDF-membrane, were incubated with an antiserum against the duck ε-crystallin (Fig. 3A, B).

TABLE 2. LDH-activity in crude lens extracts of three gekkonid species.

Species	mU/mg lens wet weight	mU/mg total protein
L. lugubris	28.4	104
L. picturatus	17.7	112
S. sthenodactylus	15.1	59

This antiserum does not react with any protein band of the gekkonid lens extracts subjected to the immunoblotting procedure (Fig. 3B, lanes 3-5), but it clearly shows a positive reaction with both the purified pig LDH (Fig. 3B, lane 2) and the ε-crystallin of the lens extract of the eider duck (not shown). Consequently, the 38 kDa and the ε-crystallin are not immunologically related.

GEL FILTRATION CHROMATOGRAPHY

The elution profiles of lens extracts from *L. lugubris* and *S. sthenodactylus* show five peaks each (Fig. 4A). Four peaks of the *L. lugubris* profile correspond in their elution volumes to four peaks of the *S. sthenodactylus* profile. The gel electrophoretic analysis of the protein patterns of these peak fractions reveals that the peaks at nearly identical elution volumes are composed of nearly identical crystallin subunits (Fig. 4B). The first peaks of the two profiles (S1, L1) contain α-crystallin, which is composed of two types of subunits (21 and 23 kDa). The major protein of S2 and L2 is the δ-crystallin, which has subunits of about 50 kDa. S2 and L2 additionally contain a small amount of α-crystallin subunits of the preceding peak fractions. Peak fractions S3 - only a shoulder of the broad peak S4 - and L3 are composed of crystallin subunits of 50 kDa which resemble the τ-crystallin of birds and reptiles. Peak fractions S5 and L5 reveal monomeric γ-crystallins of 22 kDa.

The peak fractions S4 and L4 differ in both their elution volumes and their protein compositions. S4 contains four subunits of 25-30 kDa, probably of β-crystallin, and additionally a 50 kDa protein. In contrast, L4 contains mainly the monomeric 38 kDa crystallin. The additional small bands of low molecular weights (27-30 kDa) could either be parts of ß-crystallin subunits or fragments of the main protein in this fraction. In lenses of *L. lugubris* the 38 kDa crystallin seems to partly replace the ß-crystallin subunits (Fig. 4B). Aliquots of the peak fractions L4 do not show enzymatic activity in the LDH activity assay.

DISCUSSION

The gel electrophoretic separations of gekkonid lens crystallins reveal a considerable difference in compositions. It concerns a major 38 kDa crystallin occurring in the lenses of all species investigated of the genus *Phelsuma* and in the lens of *L. lugubris*, where it comprises 20-22% of the total amount of crystallin (Fig. 2, Table 1). This distribution is highly surprising. It supports neither a possible correlation between similar crystallin compositions and similar or nearly identical habits nor a conceivable correlation between similar crystallin compositions and the degree of relationship.

Concerning the first possibility, the virtually complete lack of the 38 kDa crystallin in *L. picturatus* is remarkable. Both the genus *Phelsuma* and the genus *Lygodactylus* share numerous common characteristics: generally, they are strictly arboreal and insectivorous, active during the day ('tertiarily' diurnal), and constantly exposed to bright sunlight. Additionally, both genera possess yellow lenses. Because of these nearly identical habits it could be assumed that similar requirements on the function of the lenses result in very similar lens protein compositions.

P. guentheri is the only extant member of the genus *Phelsuma* which is not exclusively diurnal. The morphology of the eye of *P. guentheri* is virtually identical to that of fully diurnal *Phelsuma* species. *P. guentheri* is mainly active during late afternoon up to the evening (5 pm - 8 pm); in the course of night its activity decreases and ceases at about 2 am (Langebaek, 1979). Because of the very close relationships an identical crystallin pattern is not surprising.

The sphaerodactyline *S. cinereus* does not possess the 38 kDa crystallin though being mainly diurnal (Table 1). However, this is not unexpected because the members of this genus generally live in the herbaceous layer, where they have secretive if diurnal habits. Thus, it is seldom exposed to bright sunlight.

If the crystallin composition could be viewed as a measure of the degree of relationship, the 38 kDa crystallin is expected to occur in the lenses of the genera *Phelsuma*, *Ailuronyx* and *Lygodactylus*. These are closely related and are included in the monophyletic Afro-Madagascan group (Joger, 1985). But both *Lygodactylus* and *Ailuronyx* lack the 38 kDa crystallin (Figs. 1, 2). The lens protein pattern of *A. seychellensis* resembles that of the nocturnal species. Indeed, this gecko is mainly, but not exclusively, active at night.

The unexpected presence of the 38 kDa crystallin in *L. lugubris* can be explained neither by similar habits nor by a close phylogenetic relationship to *Phelsuma*. The 38 kDa crystallins of *Lepidodactylus* and *Phelsuma* show identical mobilities on SDS-gels. Though this does not prove their identity, there is so far no evidence to the contrary.

Several vertebrate enzyme-crystallins with molecular weights of their subunits in the range of 35-38 kDa have been described. These are the ζ-crystallin of guinea pig, degu rock cavy, camel and llama, the λ-crystallin of rabbits and hares, the μ-crystallin of some marsupials and the ρ-crystallin of the genus *Rana*.

The ζ-crystallin is a homotetramer (Huang, Russell, Stone & Zigler 1987), while the λ-crystallin is a dimer or a tetramer (Mulders *et al.*, 1988). The ρ-crystallin of the genus *Rana* is an oligomer (Tomarev, Zinovieva, Dolgilevich, Luchin, Krayev, Skryabin & Gause 1984) or, more likely, a monomer (Bindels *et al.*, 1983; Fujii *et al.*, 1990). In any case, the ρ-crystallin of *Rana temporaria* has a higher mobility on my gels than the 38 kDa crystallin of geckos, pointing to an apparent molecular weight of 36 kDa. As the μ-crystallin of marsupials, the quaternary structure of which seems to be unknown, was not even observed in all marsupial species investigated (Wistow & Kim, 1991), it is highly unlikely that this crystallin should occur in gekkonid lenses.

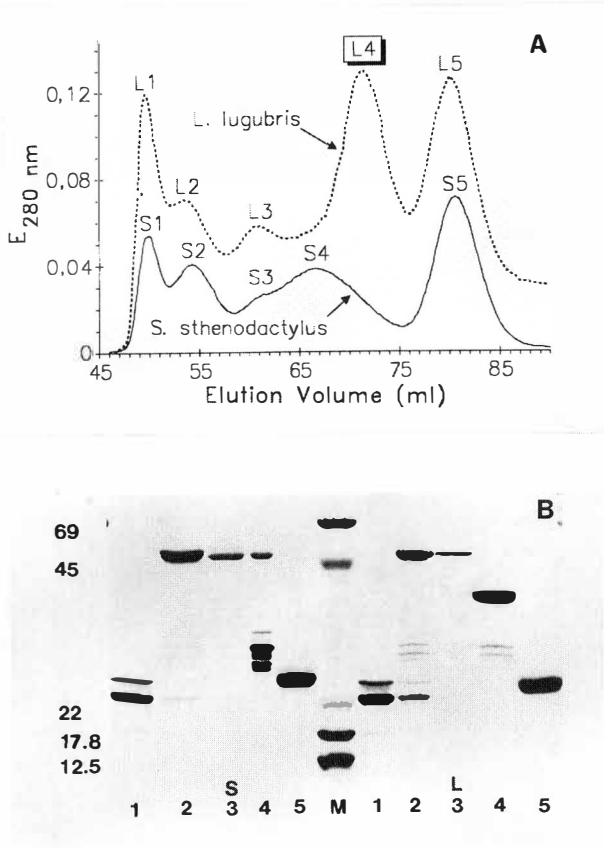

FIG. 4. A. Elution profiles of water-soluble lens proteins of *L. lugubris* and *S. sthenodactylus*. B. Analysis of peak fractions by SDS-PAGE (14% polyacrylamide). Numbering of peak fractions as in A. L4 contains the 38 kDa crystallin. Molecular weights of marker proteins (M) are given in kDa.

The monomeric 38 kDa crystallin of some geckos seems rather to be more similar to the ε-crystallin. The scattered presence of the latter in sauropsid taxa, birds and crocodiles, supports an evolutionary relationship between the ε and the 38 kDa crystallin. The ε-crystallin is identical with LDH-B4. Chiou, Chang & Lin (1988) found a molecular weight of 150 kDa and suggested a tetrameric structure. The isoenzymes of vertebrate LDH are tetrameric proteins with native molecular weights of 140 kDa; only LDH of invertebrates are found to be tetrameric or dimeric (Urich, 1990). In any case, the ε-crystallin possesses high enzymatic activity. The τ-crystallin of reptiles and fishes is closely related to the glycolytic enzyme α-enolase. The normal enzyme, however, is a dimeric protein (Chin, Brewer & Wold 1981), whereas τ-crystallin, isolated from turtle and lamprey, is predominantly monomeric (Stapel & de Jong, 1983; Wistow & Piatigorsky, 1987). Despite being apparently a monomer, the τ-crystallin exhibits reduced enolase activity (Wistow *et al.*, 1988). Thus, lens extracts of *L. lugubris* possessing the 38 kDa crystallin were tested for LDH activity in comparison with extracts of *L. picturatus* and *S. sthenodactylus*. The enzymatic activities of these samples reach only low values in the range of 60 to 110 mU per mg of total protein (Table 2). These activities are due to the LDH activities of the normal lens metabolism, particularly of anaerobic glycolysis. Hockwin & Orloff (1981) found a LDH-activity of 7.5 mU per mg protein of whole bovine lenses. In contrast to this low enzymatic activity, the isolated duck ε-crystallin possesses an extremely high activity of 100 U per mg (Wistow *et al.*, 1987). Even higher values of LDH-activity of isolated duck and swan ε-crystallin, 510/590 and 550 U per mg protein, respectively, have been reported by Chiou, Chang & Lai (1989) and Chiou *et al.* (1990). The isolated 38 kDa crystallin shows no enzymatic activity at all. This is, besides the different quarternary structure, an additional difference between the 38 kDa crystallin and the ε-crystallin.

The lack of enzymatic activity alone does not necessarily imply the lack of structural similarity of subunits. The 38 kDa crystallin might have lost its enzymatic activity because of only small modifications in the protein structure concerning the residues required for LDH activity. In this case the immunological relationship between the 38 kDa crystallin and the ε-crystallin should be retained. As the anti-duck ε-crystallin antiserum does not react with the 38 kDa crystallin (Fig. 3B), the dissimilarity of the gekkonid lens protein and the ε-crystallin is clearly revealed.

On account of the differences between the 38 kDa crystallin and ε-crystallin, it is supposed that the gekkonid crystallin may be a new, undescribed crystallin but only the unravelling of its amino acid sequence can be a real proof for this assumption.

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