Lamprey 48-kDa lens protein represents a novel class of crystallins

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SDS-PAGE revealed a major M_r 48000 polypeptide of pI around 8 in the water-soluble fraction of lamprey lenses. It occurs as a monomeric protein, and its amino acid composition and tryptic peptides show no resemblances to α-, β-, γ- or δ-crystallin. Immunoblotting with antiserum against the 48-kDa protein revealed an immunologically related polypeptide of similar M_r in reptiles, several birds and a fish, but showed no cross-reactivity with any other water-soluble lens component. The 48-kDa protein is not detected in many birds and fishes, and in the investigated mammals and amphibians.

Crystallin *Lens protein* *Lamprey* *Protein evolution* *Immunoblotting*

1. INTRODUCTION

The vertebrate eye lens contains a number of organ-specific water-soluble proteins, the crystallins, which are classically divided into different groups, designated as α-, β-, γ- and δ-crystallins [1]. Especially the δ-crystallins have caught the investigators eye, not only by the circumstance that they show a number of features concerning regulation of protein synthesis, protein structure and evolutionary development which sets them apart from the other crystallins [2–4], but also by the fact that they appear only in the lenses of the sauropsidan species (birds and reptiles), although cloned chicken δ-crystallin cDNA sequences seem to hybridize weakly to genomic DNA from other phylogenetic groups, insects included [5]. δ-Crystallin is a tetrameric protein, ranging in M_r from 150000 to 200000 and is composed of subunits with M_r between 45000 and 50000 [6–8]. This clearly distinguishes δ-crystallin from the other crystallins, which have monomeric M_r-values between 20000 and 34000 [1]. We therefore were surprised to find, in the course of a comparative electrophoretic analysis of vertebrate lens extracts in SDS-gels, a major M_r 48000 polypeptide in the eye lenses of sea lamprey, and it seemed worthwhile to isolate and characterize this component in order to establish its possible relationship to sauropsidan δ-crystallin.

2. MATERIALS AND METHODS

Sea lampreys (*Petromyzon marinus*) were caught in the river Maas, and after dissection the eye lenses were stored at −20°C. Lenses of 12-week old chickens and of other species were obtained and treated as in [4]. Due to their extreme hardness, the lamprey lenses had to be homogenized by means of a Polytron apparatus (Kinematica GMBH, Luzern) in a small volume of 1% ammonium bicarbonate, pH 7.9. After centrifugation for 30 min at 10000 × *g*, the supernatant was applied to a column (125 × 3.5 cm) of Ultrogel AcA-34 (LKB) and eluted at room temperature with the solvent mentioned. After gel filtration, fractions containing protein material were pooled an lyophilized. Slab gel electrophoresis was performed in 13% polyacrylamide gels containing 0.1% sodium dodecylsulfate as in [9]. Two-dimensional gel electrophoresis was performed as in [10]. Peptide mapping and amino acid analysis of pro-

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teins and their tryptic peptides were performed as in [4]. To obtain antisera against the sea lamprey protein and chicken δ-crystallin, immunization was carried out with protein samples which, after gel filtration, had been purified by preparative SDS–PAGE and were removed from the gel material by electroelution. Rabbits were injected with 2 mg of protein in the presence of complete Freund's adjuvant, and blood samples were taken after 3 weeks. Electroblot transfer and immunoautoradiography with [^{125}I]protein A were performed as in [11].

3. RESULTS AND DISCUSSION

The sea lamprey water-soluble lens extract contains a major polypeptide component of M_r 48000, which is in the molecular mass range of the sauropsidan δ-crystallin subunits (fig.1A). However, its elution volume on a gel filtration column, calibrated with calf lens crystallins of known M_r, shows that it behaves like a monomer, in contrast to the tetrameric δ-crystallin (fig.2). Two-dimensional gel electrophoresis of chicken and lamprey lens extracts shows that the 48-kDa protein focuses

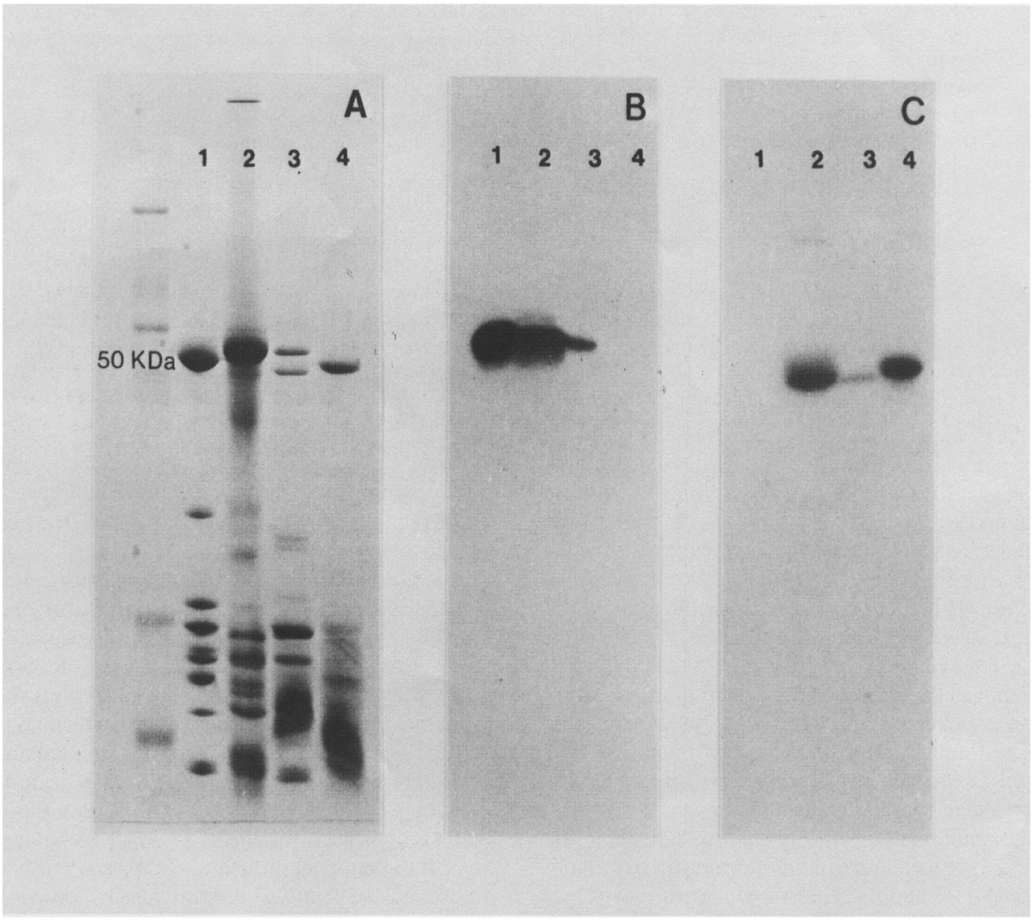

Fig.1(A). SDS–PAGE of water-soluble lens proteins of (1) chicken (*Gallus domesticus*); (2) alligator (*Alligator mississippiensis*); (3) turtle (*Pseudemys scripta-elegans*); (4) sea lamprey (*Petromyzon marinus*); (B) Immunoblotting of the same gel with antiserum against chicken δ-crystallin; (C) Immunoblotting with antiserum against lamprey 48-kDa protein.

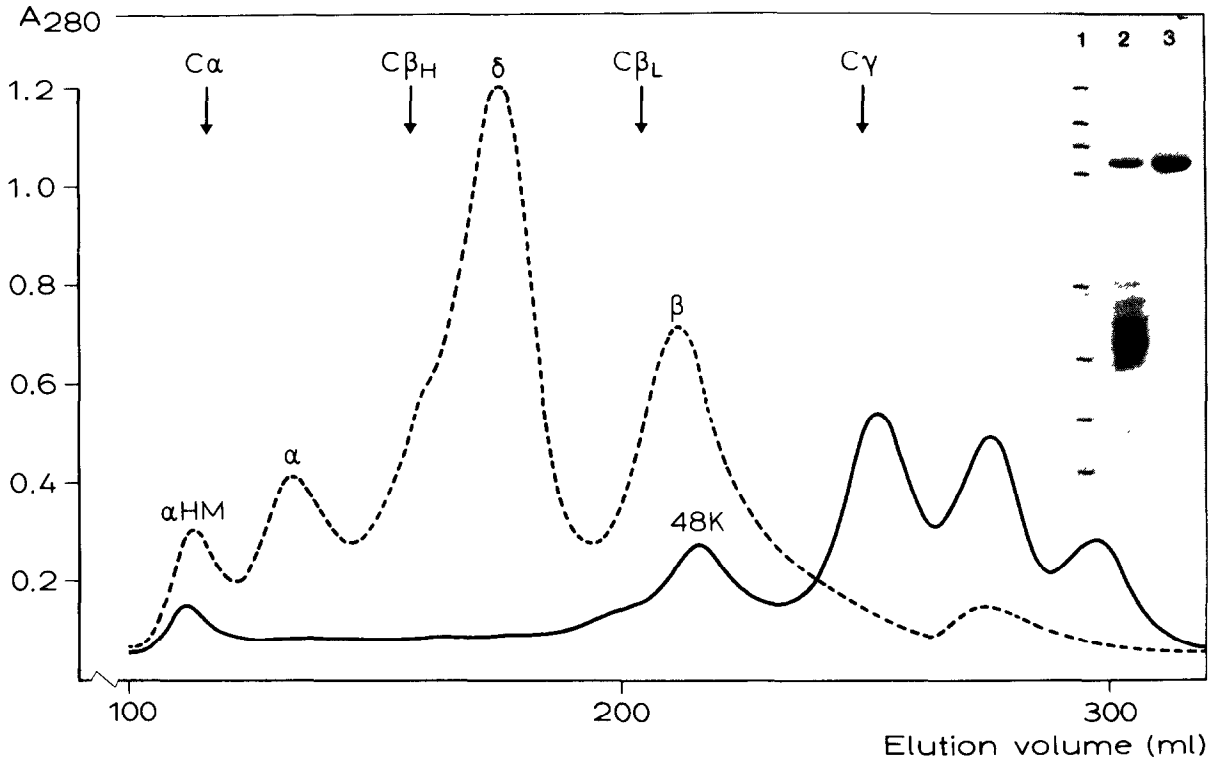

Fig.2. Comparative gel filtration on Ultrogel AcA-34 of lens extract of chicken (– – –) and sea lamprey (——): Arrows indicate the elution volumes of calf α-crystallin (M_r 800000), β_{High}-crystallin (M_r 180000), β_{Low}-crystallin (M_r 50000) and γ-crystallin (M_r 20000). Insert: SDS-PAGE of (1) marker proteins: cytochrome *c* (12400), myoglobin (17000), α-crystallin A (20000), chymotrypsinogen A (26000), ovalbumin (45000), leucine aminopeptidase (54000), bovine serum albumin (68000) and phosporylase A (93000); (2) lamprey lens extract; (3) 48-kDa protein.

at an isoelectric point of about 8, while the δ-crystallin subunits are found in the pH 5–6 region (fig.3A,B). Amino acid analysis of the 48-kDa protein (Table 1) does not, amongst others, reveal the low value for tyrosine, and the high values for threonine and leucine which are characteristic for δ-crystallins [4,7]. Peptide mapping followed by amino acid analyses of 22 tryptic peptides, accounting for about 30% of the total polypeptide chain, did not reveal any significant resemblances to δ-crystallin peptides either.

Immunoblotting of the lens extracs shown in fig.1A, using antiserum against chicken δ-crystallin, gives the expected reaction with the δ-crystallin bands of chicken, alligator and turtle, but not with any of the lamprey polypeptides (fig.1B). On the other hand, the antiserum against the 48-kDa protein not only reacts with the lamprey component, but also evokes a similar reaction with a band just below δ-crystallin in the turtle and alligator lens extracts (fig.1C).

In the course of further comparative immunoblotting a number of vertebrate water-soluble lens extracts were studied, including mammals (calf, horse, rhesus monkey, and the Australian spiny anteater), amphibians (*Rana esculenta, Xenopus laevis*), reptiles (caiman, rattlesnake, monitor lizard), birds (emu, penguin, gannet, peking duck, buzzard, coot, gull, pigeon, budgerigar, cuckoo and eagle owl), fishes (river lamprey, dogfish, alligator gar, cichlid and carp). A clear immunological reaction appeared with a ±48-kDa band in lens extracts of all reptiles, a number of avian species (emu, penguin, gannet, duck, gull, cuckoo) and with the lens extracts of alligator gar and river lamprey. The anti-48-kDa-serum did not react with any other protein band of any lens extract subjected to the immunoblotting

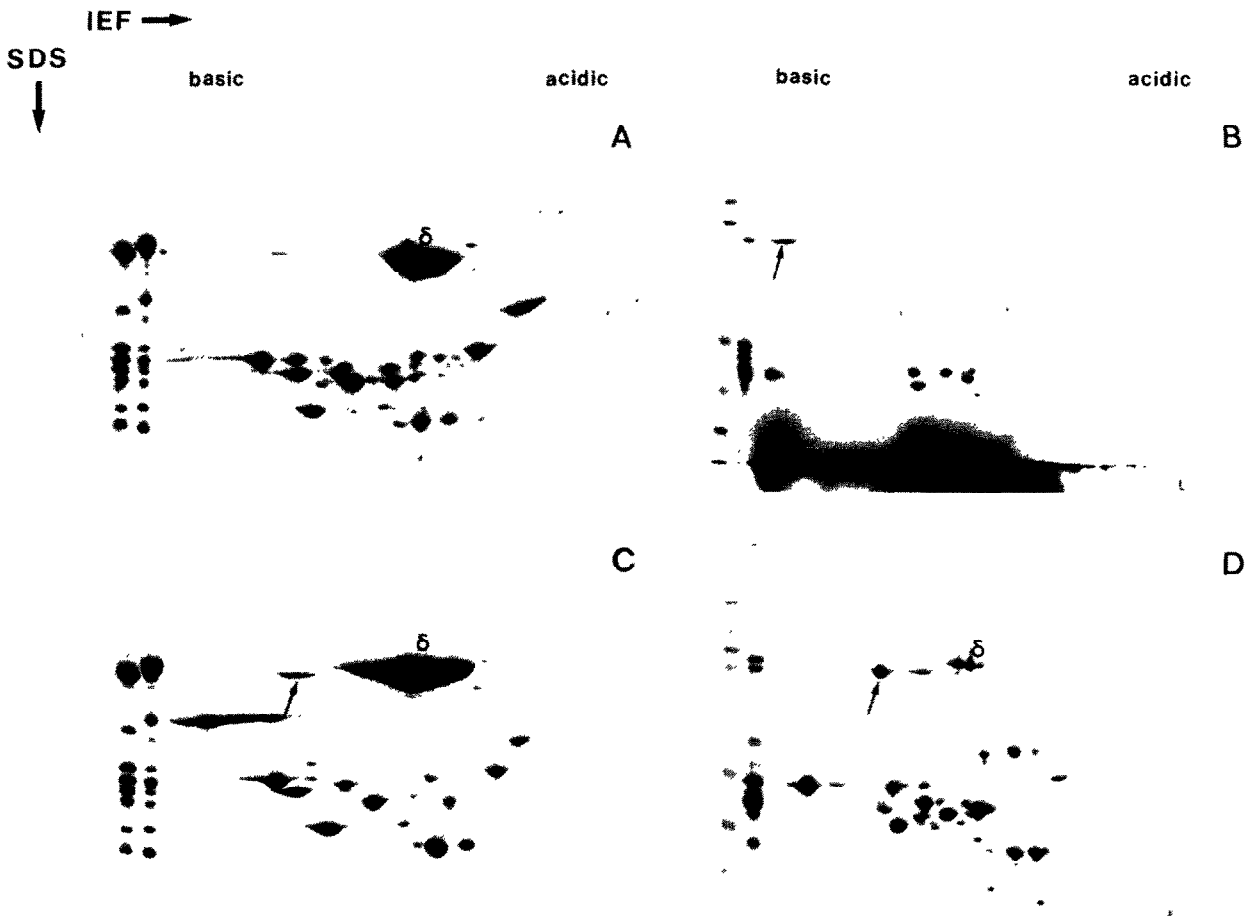

Fig.3. Two-dimensional gel electrophoresis of lens extracts of chicken (A), sea lamprey (B), peking duck (C) and turtle (D), using 1% ampholine (pH 3.5–10); 1.3% ampholine (pH 7–9) and 2.6% ampholine (pH 6–8) in the focusing gels. Arrows indicate the 48-kDa protein in lamprey and the corresponding components in Peking duck and turtle. The turtle component apparently occurs in different charge forms, probably due to deamidation. Reference lanes of fig. 3A and 3C contain lens extracts of chicken and Peking duck, respectively.

procedure (not shown). Comparative gel filtration of lens extracts from alligator, lizard, turtle and peking duck clearly showed the presence of the 48-kDa-related component in the β_{Low}-fractions, which is an indication of its monomeric behaviour in these species. Two-dimensional gel electrophoresis of the lens extracts of Peking duck and turtle (fig.3C,D) pointed out that the protein in birds and reptiles, as compared to the lamprey component, has a lower isoelectric point, of about 6. In fact, the two-dimensional gel of chicken lens extract (fig.3A) also reveals a minor spot in a position corresponding to the duck 48-kDa component. It thus can not be excluded that a small amount of the 48-kDa protein, although not detected by one-dimensional gel immunoblotting, is present in chicken lens as well.

These data allow us to conclude that the lamprey 48-kDa lens protein is not detectably related to δ-crystallin, nor to the α-, β- or γ-crystallins. It represents a new class of crystallins with a scattered distribution among vertebrates. It is immunologically related to the turtle 46-kDa (pI 6.2) polypeptide observed but not further characterized [12].

The presence of the 48-kDa protein in distantly related taxa with structurally very different lenses, such as lampreys, the fish superorder Holostei,

Table 1

Amino acid composition of lamprey 48-kDa lens protein compared with literature values for chicken δ-crystallin [7] and bovine α- β_{Low}- and γ-crystallins [1] (residues/1000 residues)

	48-kDa[a]	δ	α	β_{Low}	γ
Asp	111	71	86	85	116
Thr	39	76	34	30	22
Ser	54	96	103	81	42
Glu	105	130	105	146	131
Pro	38	23	81	59	58
Gly	91	56	60	91	93
Ala	99	80	44	49	32
Cys	16	3	5	9	42
Val	69	80	58	63	48
Met	20	8	12	10	37
Ile	65	74	47	35	42
Leu	76	151	87	62	81
Tyr	31	8	31	41	76
Phe	37	22	76	43	48
His	24	12	39	45	37
Lys	93	72	48	54	24
Arg	45	39	73	61	111

[a] Values are the average of duplicate analyses after 24, 48 and 72 h of hydrolysis. Values for threonine and serine are obtained by extrapolation to zero time hydrolysis; valine and isoleucine have values for 72 h hydrolysis

reptiles and several avian species, while the protein is not detected in many other, sometimes closely related taxa, makes it difficult to attribute a specific structural-functional significance to this protein, nor can it be seen as a characteristic phylogenetic trait. This protein clearly is the product of an evolutionarily old gene, which has largely or completely been silenced in the eye lenses of the investigated mammals, amphibians, teleost fishes, shark, and in many birds, while it is still expressed in other groups. Especially intriguing is the situation among birds, where the 48-kDa component occurs scattered over different orders. It would be of interest to establish whether the gene for this protein still occurs in the chromosomal DNA of species which seem to have lost the component in their eye lenses, and if so, to study the structural changes that led to its inactivation.

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