A novel type of crystallin in the frog eye lens

35-kDa polypeptide is not homologous to any of the major classes of lens crystallins

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The nucleotide sequence of a cloned DNA coding for the 35-kDa polypeptide of the eye lens of the frog (*Rana temporaria*) has been determined. The sequence without connectors and poly(A) tract is 889 nucleotides in length and shows no homology with sequences coding for other classes of crystallins: α-, β-, γ- or δ-crystallins. The sequence contains one reading frame 675 nucleotides in length, an apparently intact 3'-non-translated region with the polyadenylation signal sequence and a poly(A) tract; the 5'-non-translated region is lost along with part of the coding region; this accounts for about 1/4 of the total mRNA length. The secondary structure prediction according to the Ptitsin–Finkelstein method shows the presence of predominantly β-strands with only a few α-helical regions. We conclude that the 35-kDa polypeptide from the frog eye lens belongs to a new class of eye lens crystallins for which we propose the name ϵ-crystallin.

Recombinant DNA *DNA sequence* *Protein structure* *Ophthalmology* *Eye lens*

1. INTRODUCTION

The diversity of crystallins – the structural proteins of the vertebrate eye lens – is believed to be limited to the 4 main structurally different families of polypeptides: α-, β-, γ- and δ-crystallins [1]. α- and δ-crystallins show no homology with each other or with β- and γ-crystallins, while β- and γ-crystallins, having a significant sequence homology, are included in one superfamily of proteins. δ-Crystallins have only been found in birds and reptiles, where they are present in place of γ-crystallins.

The use of recombinant DNA technology resulted in the isolation and structural characterization of genes coding for all the main classes of eye lens crystallins [2–10].

We have determined here the nucleotide sequence of the cloned DNA coding for a major 35-kDa polypeptide from the eye lens of the frog (*R. temporaria*). During the chromatographic fractionation of the frog eye lens extracts this polypeptide was found in the fraction of oligomeric proteins with an apparent molecular mass of 200 kDa.

The results obtained demonstrate that the cDNA coding for the 35-kDa polypeptide shows no detectable homology with cDNAs coding for other major classes of crystallins: α-, β-, γ- and δ-crystallins. On the basis of this result we conclude that the 35-kDa polypeptide belongs to a new class of crystallins for which we propose the name ϵ-crystallin.

2. MATERIALS AND METHODS

2.1. Recombinant plasmid pRt(1)95

The recombinant plasmid pRt(1)95 coding for

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the 35-kDa polypeptide was described and partially characterized in [8].

2.2. Plasmid DNA preparation

Plasmid DNA was isolated according to the method in [11] and additionally purified by the CsCl–EtBr density gradient centrifugation.

2.3. DNA sequencing

The nucleotide sequence of DNA was determined using the method in [12] after labelling of the DNA termini with DNA-polymerase I (Klenow's fragment) and [α-^{32}P]dNTP or with polynucleotide kinase and [γ-^{32}P]ATP.

2.4. Fractionation of eye lens extracts [13]

Fresh frog eye lenses were stirred in a glass beaker on a magnetic stirrer in a solution containing 50 mM Tris–HCl (pH 7.0), 0.1 mM EDTA, 1 mM DTT for 3 h. The turbid solution was aspirated and centrifuged for 30 min at 30 000 × *g*. The supernatant (3 ml) was then applied to an Ultrogel AcA 34 column (25 × 100 cm). The column was eluted at 15 ml/h, with the above buffer containing 0.2 M NaCl. Fractions of about 5 ml were collected. The polypeptide composition of the fractions was determined by SDS–polyacrylamide gel electrophoresis (SDS–PAGE).

3. RESULTS

3.1. 35-kDa polypeptide of the R. temporaria *eye lens*

Eye lens extracts from wintering frogs were fractionated on an Ultrogel AcA 34 column. The profile obtained is presented in fig.1. Studies of the polypeptide composition of different peaks which will be described in detail in another paper have demonstrated that peak II with the apparent molecular mass of about 200 kDa contains predominantly a polypeptide with a molecular mass of about 35 kDa as determined by SDS–PAGE (see fig.1b).

The polypeptide with the apparent molecular mass of 35 kDa accounts for a significant proportion of the polypeptides synthesized in the rabbit

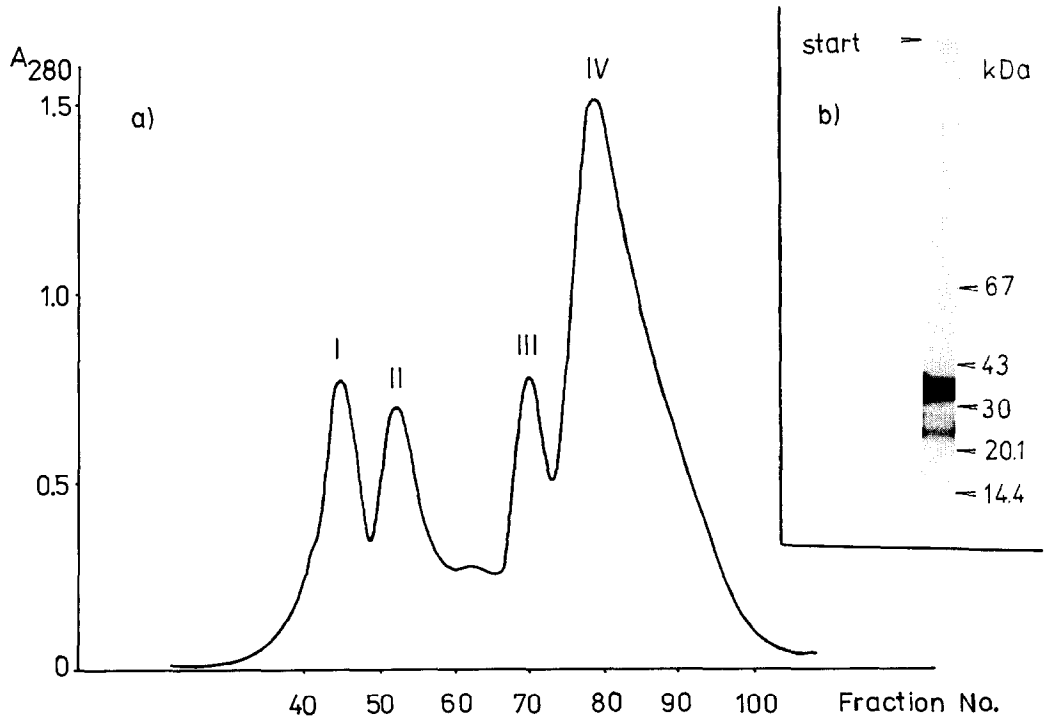

Fig.1. (a) Elution profile of the frog (*Rana temporaria*) eye lens extract from an Ultrogel AcA 34 column. (b) Gradient SDS–PAGE of the material from peak II. The lane was overloaded to detect possible contaminating proteins. Positions of molecular mass markers are shown on the right.

reticulocyte cell-free system programmed by the total frog lens poly(A)$^+$ RNA [8]. This polypeptide was not precipitable by the antisera precipitating frog lens α-, β- and γ-crystallins. On the other hand the labeled polypeptide synthesized in the cell-free system could be immunoprecipitated with an antiserum against peak II. This was taken as evidence that the 35-kDa polypeptide present in the translation products of the frog lens mRNA corresponds to the material of peak II. Since the recombinant cDNA clone pRt(1)95 described in [8] coded for the 35-kDa polypeptide in the hybrid-selected translation test we have performed sequencing of its DNA to establish the identity of the clone and to characterize the structure of the corresponding polypeptide.

3.2. *The nucleotide sequence of plasmid pRt(1)95*

The cleavage map of the pRt(1)95 cDNA insert for several restriction endonucleases is shown in fig.2. In addition this figure illustrates the sequencing strategy. More than 90% of the sequence was determined independently for the two complementary chains.

The total length of the sequence without connectors and poly(A) tract is equal to 889 nucleotides (fig.3). Only the coding strand is shown. The identification of the coding strand is based on the presence of the poly(A) tract and a universal polyadenylation signal sequence (positions 868–873). The coding region is followed by a 3′-non-translated region having a length of 114 nucleotides.

A computer translation of the obtained sequence has demonstrated that there is only one long open reading frame with the length of 675 nucleotides. Numerous stop codons are present in the remaining 5 reading frames.

The length of the mRNA determined by hybridization of a Northern blot with the labeled pRt(1)95 DNA is equal to 1200 nucleotides (see section 4). The length of the cloned pRt(1)95 cDNA is 951 nucleotides (889 nucleotides plus 62 nucleotides of the poly(A) tract). Therefore at least 3/4 of the mRNA sequence is present in the cloned cDNA.

3.3. *Lack of homology between the nucleotide sequence of pRt(1)95 and gene sequences of other classes of crystallins*

Using the dot-matrix procedure, the nucleotide sequence of the pRt(1)95 clone has been compared

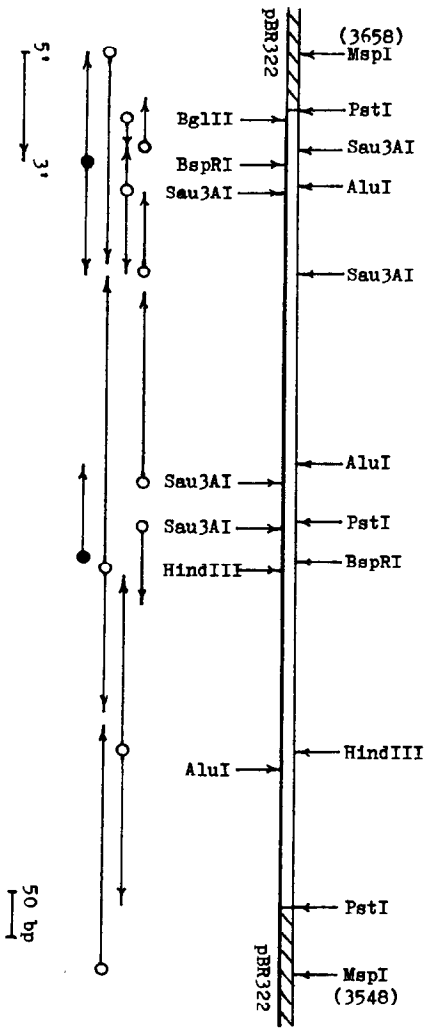

Fig.2. Cleavage map of the ϵ-crystallin cDNA cloned in the recombinant plasmid pRt(1)95 and the strategy of sequencing. The sequence was read from the corresponding sites labeled with [γ-^{32}P]ATP (—●—) or [α-^{32}P]dNTP (—○—). 5′⟶3′: direction of mRNA translation.

with the nucleotide sequences of cloned cDNAs coding for other classes of *R. temporia* crystallins: αA_2-crystallin [10], 23-kDa β-crystallin (in press) and two γ-crystallins [9,14]. No significant homologies have been found.

Furthermore, the structure of pRt(1)95 cDNA did not contain any internal duplications of the type found in the β-γ-crystallin superfamily [3,7,9].

No homology has been found between the sequence of pRt(1)95 and the known part of the chicken δ-crystallin sequence [15]. We conclude

```
1
CTGGAGAGATCTCTGAGGGATGTTGGAATGGATTATCTGGATCTGTTCCTTATGCACTGGCCTGTCTCTCTTAAGCCTAGTGGAGCTTCT
LeuGluArgSerLeuArgAspValGlyMetAspTyrLeuAspLeuPheLeuMetHisTrpProValSerLeuLysProSerGlyAlaSer

                100
GATCCCTCCGATAAGGACAAGCCTTTCATCTATGATAATGTGGACCTTTGTGCTACATGGGAGGCTCTAGAGGCACGCAAAGATGCAGGT
AspProSerAspLysAspLysProPheIleTyrAspAsnValAspLeuCysAlaThrTrpGluAlaLeuGluAlaArgLysAspAlaGly

                        200
TTAGTGAGATCCCTCGGAGTATCAAACTTTAACCGCAGGCAGCTGGAACGTATCCTGAACAAACCAGGACTGAAGTACAAGCCAGTTTGC
LeuValArgSerLeuGlyValSerAsnPheAsnArgArgGlnLeuGluArgIleLeuAsnLysProGlyLeuLysTyrLysProValCys

                                300
AACCAGGTGGAGTGTCATGTATATTTAAATCAAAACAAACTTCACTCCTACTGCAAATCCAAGGACATCGTTTTGGTGACTTACAGCGTC
AsnGlnValGluCysHisValTyrLeuAsnGlnAsnLysLeuHisSerTyrCysLysSerLysAspIleValLeuValThrTyrSerVal

                                        400
TTGGGCTCACACAGAGACAGGAACTGGGTGGACCTCAGCTTGCCAGTGCTACTTGATGATCCAATTTTGAATAAAGTTGCTGCTAAGTAC
LeuGlySerHisArgAspArgAsnTrpValAspLeuSerLeuProValLeuLeuAspAspProIleLeuAsnLysValAlaAlaLysTyr

                                                500
AATCGCACCTCTGCAGAGATCGCCATGCGCTTCATTCTCCAGAAGGGAATTGTGGTCTTGGCCAAAAGCTTCACCCCTGCTCGTATCAAG
AsnArgThrSerAlaAspIleAlaMetArgPheIleLeuGlnLysGlyIleValValLeuAlaLysSerPheThrProAlaArgIleLys

                                                        600
CAAAACCTTGGGGTCTTTGAATTTGAACTGAAACCTGAAGATATGAAATCACTTGAGAGCCTAGACAGAAACCTACATTATGGACCTTTT
GlnAsnLeuGlyValPheGluPheGluLeuLysProGluAspMetLysSerLeuGluSerLeuAspArgAsnLeuHisTyrGlyProPhe

                                                                700
AGAGAGGTGAAACAGCACCCAGAATACCCCTTCCACGATGAGTACTGAAGACCAACTGAGTGCCAACGCAGTCTCCAAGAAGATGCTTTC
ArgGluValLysGlnHisProGluTyrProPheHisAspGluTyrEND

                                                                        800
TGTATTATATATGTAAAGCTTTAGTAGAGCTGCACTCTGTTACATACAGAAAAAATAACATTTAGTCATTTGCCCAGTATTTATAAAGCA

                                                                                951
TTAATGATTGGATGGGAGCGTTATGTCTTATGCCATGTTGCCTTATGCATAACAACCAATAAATTTAATGCTGAACATCAAA....AAAA
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Fig.3. Nucleotide sequence of the frog (*Rana temporaria*) ϵ-crystallin cDNA and the corresponding amino acid sequence. The polyadenylation signal is underlined.

that the sequence coding for the 35-kDa polypeptide is unrelated to that of any other classes of crystallins and that the 35-kDa polypeptide may be considered as a novel class of frog eye lens crystallins: the ϵ-crystallin.

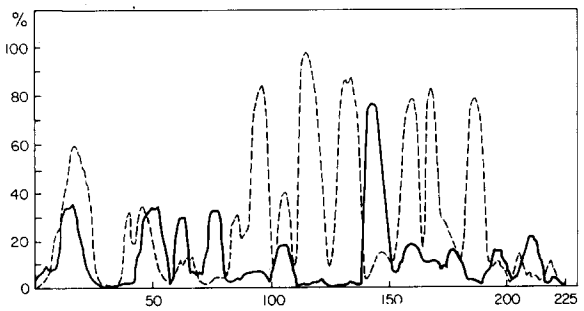

Fig.4. Predicted secondary structure of the sequenced portion of the *R. temporaria* ϵ-crystallin. Abscissa: number of amino acid residues along the protein chain. Ordinate: probability of a given residue being in the α-helical (——) or β-strand (– – –) structure.

3.4. Predicted secondary structure of the known part of the 35-kDa polypeptide

Availability of the partial amino acid sequence of the 35-kDa protein has enabled authors in [16] to perform a secondary structure prediction using their algorithm.

The result of this prediction shown in fig.4 as a probability map demonstrates that the 35-kDa polypeptide is rich in β-strands; there are only a few α-helical regions. The results of the prediction will be compared with the measured CD spectra of the 35-kDa polypeptide when these become available.

4. DISCUSSION

The present results demonstrate that the known part (about 3/4) of the 35-kDa polypeptide from the frog eye lens lacks any homology with other known classes of crystallins.

To exclude the possibility that the 35-kDa polypeptide coded by pRt(1)95 is a minor non-represen-

tative fraction of the total 35-kDa lens polypeptide (assuming that it was heterogeneous) we performed Northern blot hybridization with the total lens poly(A) RNA using labeled pRt(1)95 as a specific probe. This result was shown in fig.3 of [10]. At that time, when structural information about the cDNA of pRt(1)95 was not available, we thought that the 35-kDa polypeptide having a molecular mass in the range of β-crystallins was one of these proteins. This point of view should now be revised in the light of the structural data obtained and we refer to the 35-kDa polypeptide as ϵ-crystallin (see below).

The signal intensity from RNA hybridizing with pRt(1)95 in the Northern blot is similar to that for αA_2-crystallin mRNA. We conclude that RNA corresponding to pRt(1)95 belongs to an abundant class of eye lens mRNA and corresponding pRt(1)95 is a clone representative of this major RNA fraction.

On the basis of all the data obtained we conclude that the 35-kDa-polypeptide should be considered as a novel class of crystallins. In [17] we have proposed that it be named ϵ-crystallin. Recently we learned that Dutch authors gave the name ϵ-crystallin to the new crystallin which they discovered in the eye lens of the Peking duck and the mallard [18,19].

By the criteria of molecular mass (35 kDa for the frog eye lens and 39 kDa for the Peking duck) and the oligomeric nature of the native proteins they may well be similar. This question will be finally settled after the structural data on the duck eye lens crystallin become available.

The discovery of a new class of crystallin in the frog eye lens raises a number of interesting questions. Which other taxons contain this class of crystallin? What is its evolutionary origin and its role in the eye lens?

The presence of the new crystallin class in the frog eye lens plus other observations on the detection of new types of crystallin polypeptides in the lamprey [20] and duck [18] changes our view on the diversity and evolution of eye proteins. It appears that the number of different proteins belonging to crystallins is actually greater than previously thought and that α- and β-crystallins – the most conservative members of the ensemble – may be complemented by apparently different proteins in different taxonomic groups to form the structure providing a transparent and stable eye lens.

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