LENS PROTEINS

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I. INTRODUCTION

The lens is an entirely epithelial structure, which arises in the embryological stage of vertebrates from the surface ectoderm. The lenticular epithelial cells lie beneath a semipermeable membrane, the lens capsule, and form a monolayer covering the anterior surface of the organ. Near the so-called equator the cells elongate and differentiate into fiber-like cells. Concomitantly, cell division ceases and the fibers ultimately become anucleate. Thus the lens represents a pure well-defined cell population, suitable for a variety of fundamental studies.

The lens is unique in many respects. The whole system is separated from the blood-stream. It maintains its size and shape throughout life by constant addition of new lens fibers differentiated from the subcapsular monolayer.

Lens cells do not die. The epithelial cells originating from the early fetal life are found back as differentiated fibers in the core (or nucleus) of the lens (Figure 1). One can imagine what this means for the proteins in the nucleus. Since there is virtually no protein turnover, proteins synthesized at the embryonic stage, for instance in human lens, are still present in the lens nucleus 70 or more years later. Therefore, most changes observed in protein structure or conformation are due to aging.

A lens contains approximately 35% by weight of structural proteins. These proteins were classified in 1894 into so-called water-soluble crystallins and a water-insoluble part designated albuminoid.[1] The subclassification of the water-soluble part of mammalian lenses into α-, β-, and γ-crystallin survived nine decades of intensive research. However, systematic biochemical investigations of the water-insoluble lens components, which started only a few years ago,[2-7] showed that albuminoid represents more than just an insoluble α-crystallin fraction, as initially has been thought.[8-15] For this reason it has become meaningless to maintain the old name albuminoid. In this paper a more extended discussion will be devoted to the water-insoluble lens proteins than in previous reviews in which emphasis was centered primarily on the water-soluble crystallins. The reader is referred to these latter surveys.[16-21]

The structural lens proteins are considered to be organ-specific. The term organ-specificity describes a serologic property. Organ-specificity of the first order designates an antigen that is characteristic of a particular organ with a single species. Organ-specificity of the second order describes an antigen characteristic of a special organ occurring in many different species.[22] Lens crystallins have been supposed to fulfill both criteria. Strictly the lens is rather a single tissue than an organ situated in another organ, the eye. Therefore, the term organ-specificity is for lens antigens inappropriate. Moreover,

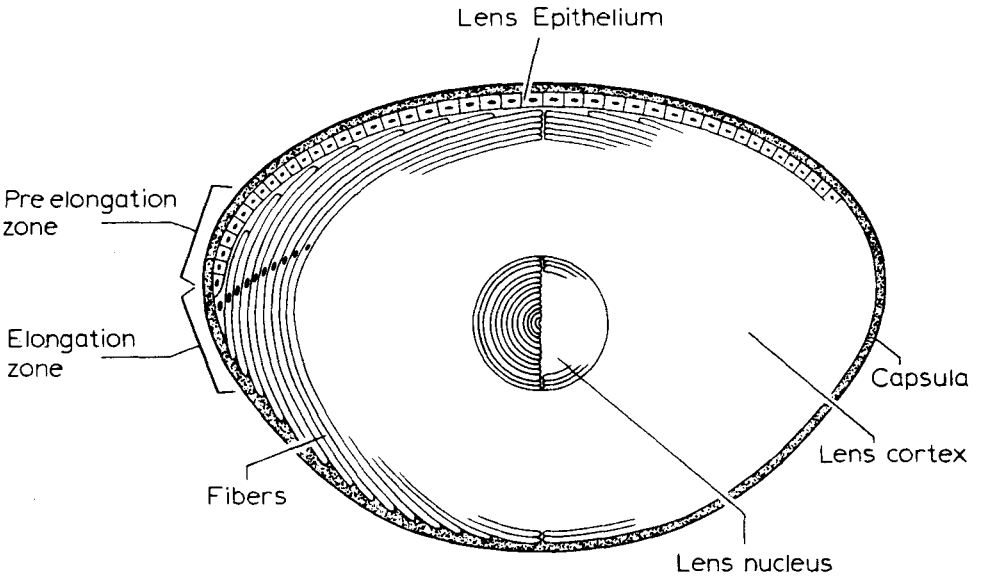


FIGURE 1. Schematic drawing of a mammalian eye lens.

several authors demonstrated that lens antigens are not restricted to lens tissue, but can be detected in other ocular tissues such as iris, cornea, retina, and aqueous humor, and even in skin and brain.[23-25] Since lens antigens are detected immunologically only in ocular tissues derived from primitive ectoderm, in skin and in brain, but not in tissues originating from mesoderm or endoderm, lens crystallins — in particular from mammalian sources — should be defined as specific for ectodermal tissues but not for species. For this reason and as the structural lens proteins are conserved in evolution,[26] I shall confine this report to a discussion of the hitherto best studied species, namely the crystallins derived from calf lens. Many of the separation methods and properties are similar for a variety of crystallins from other species. Incidentally rodent or chick lens crystallin will also be dealt with in more detail if typical results have been obtained with these kinds of protein.

II. WATER-SOLUBLE LENS PROTEINS

A. Crystallins

After homogenization of lens tissue and centrifugation at 10,000 × g the supernatant fraction can be separated into four major fractions: α, β_H, β_L, and γ (Figure 2). Each fraction, with exception of γ-crystallin, consists of a mixture of multimeric proteins. Molecular weight estimates are given in Table 1. By gel filtration on Agarose Biogel® A-5m, A-15m or A-50m, two groups of α-crystallin aggregates are separated, one of which amounts to more than 5×10^7 dalton.[27-31] The high molecular weight α-crystallin designated HM-α can also be separated from the other crystallin fractions by gel filtration on Sepharose®-CL-6B.[32]

1. α-Crystallin

α-Crystallin is best defined of the multimeric lens proteins. The native protein is an aggregate with an average molecular weight of 800,000. The composing subunits have been designated αA_1 and αA_2 (A = acid) and αB_1 and αB_2 (B = basic) on the basis of their

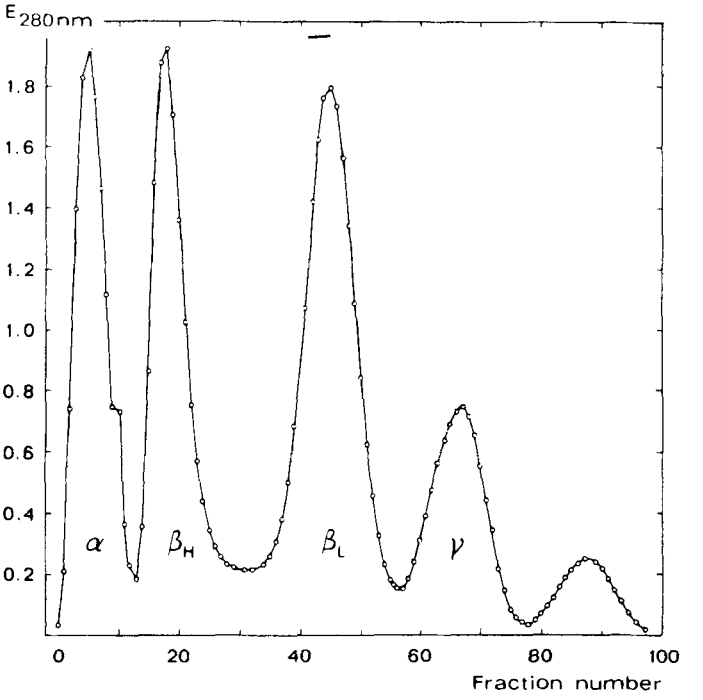


FIGURE 2. Gel filtration pattern of the water-soluble crystallins from calf lens on a Sephadex® G200 column.

Table 1
MOLECULAR WEIGHTS OF THE SOLUBLE CRYSTALLINS ESTIMATED BY GEL FILTRATION (PEAK FRACTIONS)

Crystallins	Molecular weight
α	800,000
β_H	160,000
β_L	50,000
γ	20,000

migration behavior in alkaline urea gels (Figure 3). A number of preparative isolation techniques for the α-crystallin subunits have been reviewed elsewhere.[20] Recently, we succeeded in separating these subunits by a new procedure called chromatofocusing.[32a] This method is based on a separation technique developed by Sluyterman and Elgersma.[32b] By equilibrating our columns in 6 *M* urea, αB_2, αB_1, αA_2, and αA_1 could be obtained well-resolved in a single step at about 75% yield[32c] (Figure 4). The molecular weight of each subunit amounts to 20,000.[33] Sequence studies revealed about 60% homology between the two kinds of chains.[33-34] The synthesis of αA_2 crystallin is directed by a 14S mRNA, which contains virtually twice the information required for the synthesis of a 20,000 dalton polypeptide.[35] On the other hand, the 10S mRNA that encodes αB_2 corresponds rather well to an encoded polypeptide of 20,000,[37] taking into account the nucleotides of the 5′ cap structure,[38,39] and the 3′ poly(A) track.[40,41]

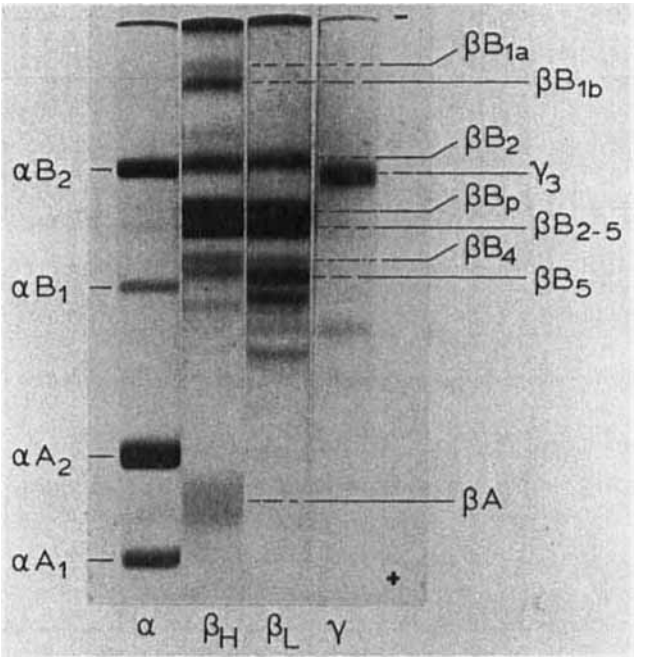


FIGURE 3. Migration of crystallin subunits in 6 M urea-containing polyacrylamide gels at alkaline pH.

Since hitherto no bicistronic (or polycistronic) messengers* have been detected in any eukaryotic system, the very long αA_2 mRNA would be a notable exception. After our first studies on this messenger[42] we mentioned this possibility,[36] albeit also other interpretations, such as noncoding regions or secondary structure, had to be envisaged. Actually we provided some evidence for regions of secondary structure in the αA_2 messenger.[43] Chen and Spector concluded from product analyses, 2-D oligonucleotide maps, and molecular hybridization that the 14S crystallin mRNA is indeed a bicistronic message.[44,45] However, in our experiments, performed routinely with 14S lens message, reproducibly only the newly synthesized αA_2 chain was found, irrespective a cell-free translation system[46,47] or microinjection into living oocytes[48–50] was used. Although sequencing of the corresponding gene will provide us with the definite answer, there is already the following argument against the bicistronic nature of the 14S lens mRNA. Lens messengers are capped at their 5′ end.[38,39] If cap analogues are added to an incubation mixture containing a reticulocyte lysate supplemented with isolated 14S mRNA, no translation takes place. If now an internal initiation site would be available, the cap analogue would not inhibit translation of the nucleotide sequence that encodes the "second" gene product.

The ratio of the two component polypeptide chains αA:αB is not constant during development. Biosynthetic studies revealed that αB is more abundant in the central epithelium than αA, whereas the reverse situation was observed in lens fibers.[51,52] The final ratio found in the fiber is about αB:αA=1:3.[53]

* A polycistronic mRNA is a messenger that contains more than one initiation site and differs fundamentally from a pseudopolycistronic one that encodes a polyprotein (precursor). The gene product of the latter is split into several proteins after translation.

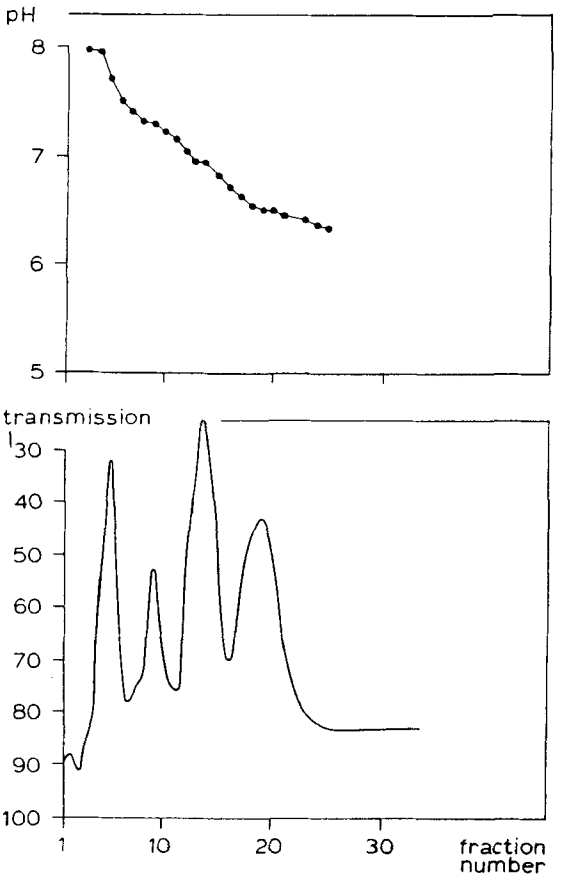


FIGURE 4. Fractionation of the four subunits of calf α-crystallin by chromatofocusing. Column: 0.9 × 30 cm. Bed height 25 cm; Sample: 140 mg of purified α-crystallin; Elution conditions: start buffer 0.025 *M* imidazole-HCl, pH 7.4; Elution buffer 0.0075 mmol/pH unit/mℓ polybuffer 74, pH 6; Flow rate: 13 mℓ h^{-1}; All buffers contain 6 *M* urea.

About 30% of the primary translation products αA_2 and αB_2 is only post-translationally modified by deamidation into αA_1 and αB_1. Hence, in prenatal calf lenses as well as upon *in vitro* synthesis, both αA_1 and αB_1 are lacking.[54-57]

When a lens ages, the molecular weight of the α-crystallin aggregate increases while part of the subunits undergo C-terminal degradation.[58,59] The question as to whether this degradation of chains is due to enzymic action or, alternatively, to "spontaneous" breakdown has still to be answered.

Quite surprisingly, lenses of rodents contain an additional α-crystallin chain. The α-nature of an extra chain (αA^{Ins}) was proven in rat lens by precipitation with an antiserum directed against purified calf α-crystallin which lacks the αA^{Ins}.[60] This polypeptide contains at its NH_2-terminal side and on the COOH-terminal side sequences which, when added together, form the "normal" αA_2 primary structure. However, between amino acid residues 63 and 64 (as compared to the calf sequence) there is an insert of 22 amino acids.[61] Also, this exceptional phenomenon awaits the elucidation of the structure of the corresponding gene. In an attempt to putatively explain the existence of the insert, we put

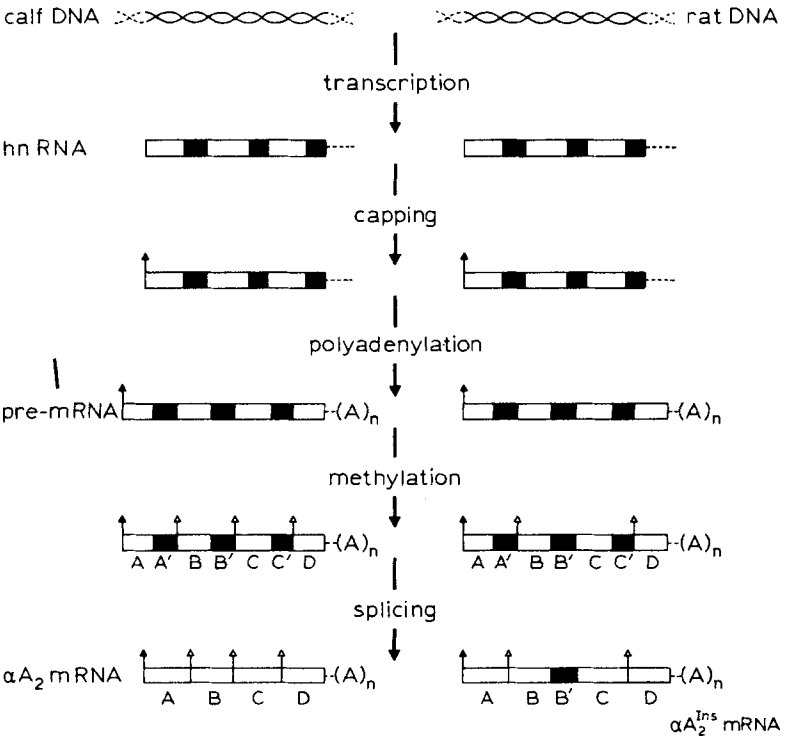


FIGURE 5. Tentative splicing scheme of calf and rat lens αA-mRNA.

forward the idea that the splicing mechanism of rodent αA mRNA may deviate in some respects from that operative in other species (see Figure 5).[62,62a]

Studies upon the biosynthesis in cell-free systems and in living oocytes[49] were suggestive for a model of the α-crystallin aggregate in which a core of αA chains is required before αB chains copolymerize. This in concert with limited proteolysis experiments which suggest that most αB chains are surface exposed.[63] A model for the architecture of the α-crystallin polymer has been proposed recently.[64]

As stated before the crystallins can completely be dissociated into their component chains by 6 M urea treatment. Correct reassembly of the α-crystallin chains can be achieved even in the presence of β and γ chains, provided proper concentration conditions are chosen.[65,66] Siezen et al.[66] provided evidence that also the degraded chains, shortened at the C-terminus, are involved in polymerization of α-crystallin aggregates.[67]

2. β-Crystallin

Originally β-crystallin was believed to be a single protein. However, after the introduction of modern electrophoretic and chromatographic procedures it became evident that β-crystallin is much more complex. Upon gel filtration on Sephadex® G200 the β-fraction is separated into two major fractions which have been called β_H (H = high molecular weight) and β_L (L = low molecular weight), respectively.[68-70] By application of well-chosen buffers[71] or on Sephacryl® S200[72] further fractionation of β_L-crystallin can be achieved. Unfortunately an answer to the question: "how many different β-crystallin aggregates are present in the water-soluble lens fraction" cannot be given yet. The component polypeptides of both β_H and β_L have been analytically resolved by gel electrophoresis[70,73,74] and on a preparative scale by chromatography on DEAE cellulose (compare also Figure 3).[75] The results showed that the two aggregates have a number of

polypeptide chains in common. Among the shared chains is the major β-crystallin constituent βB_P (B = basic; P = principal). The original investigations by Herbrink and Bloemendal were done on calf lens.[70] Zigler and Sidbury were able to show that the occurrence of βB_P in the β-crystallin fraction of both β_H and β_L was a general feature of the vertebrate species.[76] Ramaekers et al.[77] extended these studies and found also the βB_P chain as main polypeptide in β_H and β_L. This implies a highly conservative character of βB_P during evolution and may reflect a crucial role of this chain for the functional integrity of the β-aggregates.

Basic urea gel electrophoresis shows the minimum number of β_H to be at least nine and that of β_L at least eight. Upon SDS gel electrophoresis five bands of different molecular size are seen in β_H and four in β_L. Ten and more components can be distinguished upon isoelectric focusing.[77a] The isofocusing patterns change considerably upon aging.[77b] However, all of these bands by no means represent primary gene products. Peptide mapping revealed that a number of them are very closely related.[75] However, the exact relationship (deamidated chains, shortened chains?) has not been established yet.

In Figure 6A the elution profile of soluble lens protein from nine different species is shown. The gel filtration pattern resembles to a great extent the original pattern of calf lens. The βB_P chains can be seen in all species by subsequent gel electrophoresis (Figure 6B).

Recently we resolved the complete primary structure of the βB_P chain.[78] To our surprise we had to arrive at the unexpected conclusion that βB_P contains not only an internal duplication but reveals also homology with γ-crystallin (Figure 7). The mRNA's that encode the β-crystallin subunits are found in the 10S region on sucrose density gradients.[79] However, there is one exception. The β_H fraction is characterized at the subunit level by two polypeptides with an approximate molecular weight of 32,000 and 31,000, respectively (compare Figure 3). These chains have been designed βB_{1a} and βB_{1b}.[80] Thorough studies revealed that only for the 32,000 species a messenger could be detected. This mRNA with a sedimentation value of about 12S can be purified by a rather simple centrifugation procedure.[80] In accord with the lack of an mRNA for the 31,000 βB_{1b} chain is the finding that this polypeptide arises gradually from the 32,000 species. Thus, in very young calf lenses there is almost no βB_{1b}, whereas in old cow lenses the βB_{1a} chain is lacking (Figure 8). Peptide mapping carried out in my laboratory revealed no detectable differences between the two fingerprints. At this moment all explanations concerning the transition from βB_{1a} to βB_{1b} are mere speculation, albeit a number of possibilities have to be envisaged. For instance, glycosylation, deamidation, or a very limited proteolysis might be responsible for this transition. In this connection one has to keep in mind that minute changes in amino acid composition can give rise to striking differences in apparent mobilities on SDS-polyacrylamide gels.[81] Therefore, the estimated difference of 1000 dalton between βB_{1a} and βB_{1b} may turn out to be considerably less as soon as the true relationship between these polypeptides has been established. Vermorken et al.[80] provided already some evidence that the βB_{1a} chain is required in order to achieve β_H assembly. Translation of lens polysomes or the isolated total population of lens mRNAs gives rise to the formation of the crystallins in cell-free systems and in living oocytes. *De novo* synthesis is also observed in the homologous lens cell-free system.[82] However, in all cases the β_H aggregate is undetectable. This presumably reflects the necessity of maturation or aging of some component of the multimeric protein β_H. According to Vermorken et al.[80] βB_{1a} might be a good candidate for this requirement.

3. γ-Crystallin

Gamma crystallins are found in the low molecular weight fraction of the soluble lens

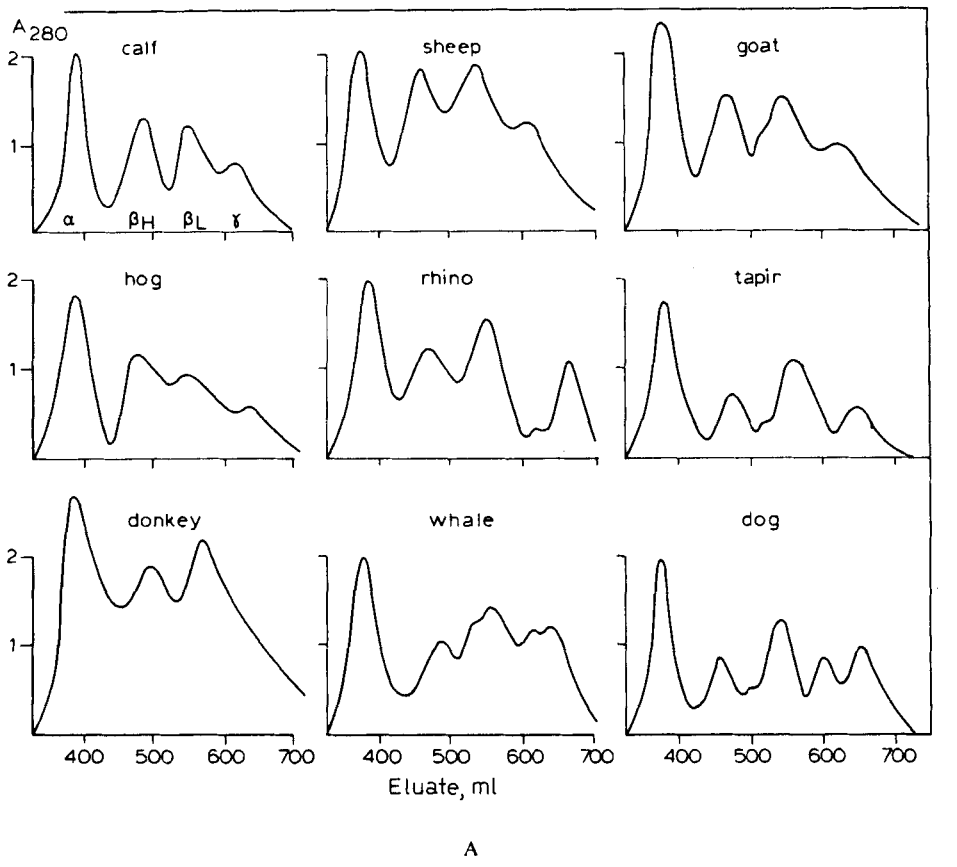


FIGURE 6. (A) Gel filtration patterns of the water-soluble crystallins from nine different species; (B) Gel electrophoretic analysis of the β_L- and β_H-fractions from 6(A). Note that the β-crystallins from all species share the βB_P-chain. 1 = dog, 2 = hog, 3 = sheep, 4 = goat, 5 = calf, 6 = tapir, 7 = rhino, 8 = donkey, 9 = whale, 10 = marker αcrystallins from calf.

proteins.[83,84] In contrast to α- and β-crystallin, γ-crystallins are not N-terminally acetylated.[85] By SE Sephadex® chromatography γ-crystallin fractions, obtained after Sephadex® G200 gel filtration, can further be subfractionated into four fractions, one of which belongs actually to the β-crystallins.[84,85] This is a 28,000 molecular weight protein blocked in N-terminal position.[86,87] This protein is called β_s (s = slow). As reported by Croft its N-terminus is tryptophan.[87] So far an N-terminally acetylated tryptophan is quite unusual, and therefore this finding certainly requires confirmation by other laboratories.

On contrast to the other crystallins, γ-crystallin could be crystallized[83,88,89] and subjected to X-ray diffraction. At 5.5 Å resolution it could be shown that the molecule is arranged in two globular domains with a radius of approximately 25 Å. The whole molecule appeared to be an ellipsoid with main axes of about 55 × 30 × 25 Å.[90]

Unlike α-crystallin, the similarity of γ-crystallin between vertebrates is not great. Despite the fact that γ-crystallin is a monomeric protein, the electrophoretic patterns of for instance calf and dogfish γ-crystallin are affected by high urea concentrations.[91,92] Li suggested that this is due to removal of ions which are involved in the secondary structure of the molecule.[92]

γ-Crystallin is responsible for a phenomenon which has been called "cold cataract".

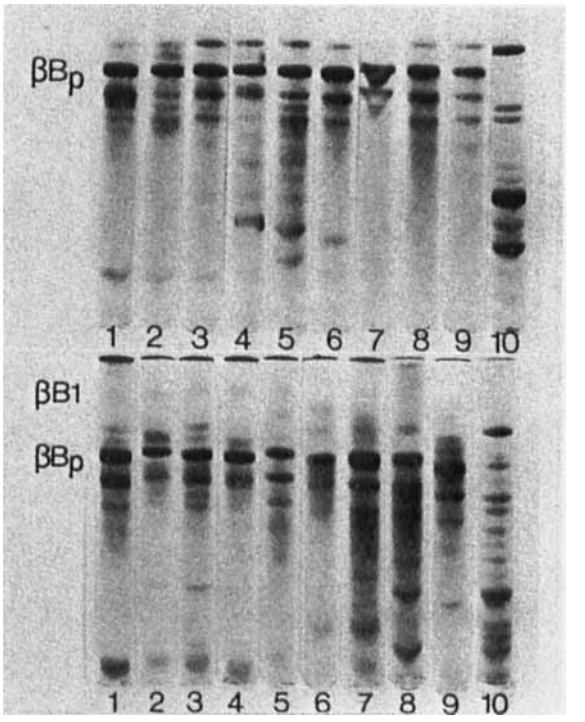


FIGURE 6B

Lenses become opaque upon cooling below 4° C. The protein starts to precipitate but dissolves again upon raising the temperature.

The occurrence of γ-crystallin in birds and reptilians has been questioned.[93] McDevitt and Croft[94] pointed out that pigeon γ-crystallin differs remarkably from several other vertebrate γ-crystallins. The number of primary gene products in the γ-crystallin fraction is still uncertain. Our experiments with calf and rat lens 10S mRNA strongly suggest that only very few (three or four at most!) primary chains exist at least in calves. Slingsby and Croft[95] suggested that at least four nonalleleic genes are coding for the calf γ-crystallin. On the other hand, by isotachophoresis about 16 γ-components could be resolved.[96] Undoubtedly the major part arises either by posttranslational modification or artifactual changes due to the separation medium.

Glycine is found to be the N-terminal amino acid residue both in γ-crystallin from calf[83] and rat[97] lens. Earlier work by Papaconstantinou provided evidence for a role of γ-crystallin as marker protein in calf lens fibrogenesis.[98] Russell et al.[99] demonstrated that the γ-crystallin concentration in the soluble lens fraction decreases during development of cataract in mice. They also showed that this loss of γ-crystallin during opacification of the lens is in part due to leakage.[100]

4. δ-Crystallin

Lens proteins from birds and reptiles contain a crystallin species designated originally FISC ("first important soluble crystallin") because of its early appearance in chick lens development.[101] This class of lens proteins is now generally indicated by the greek letter δ.

Most investigations were carried out on chick δ-crystallin. The protein can easily be isolated by gel filtration.[102] Analysis of δ-crystallin by thin layer isoelectric focusing reveals seven components in a narrow pH range between 5.18 and 5.34.[103] Since these

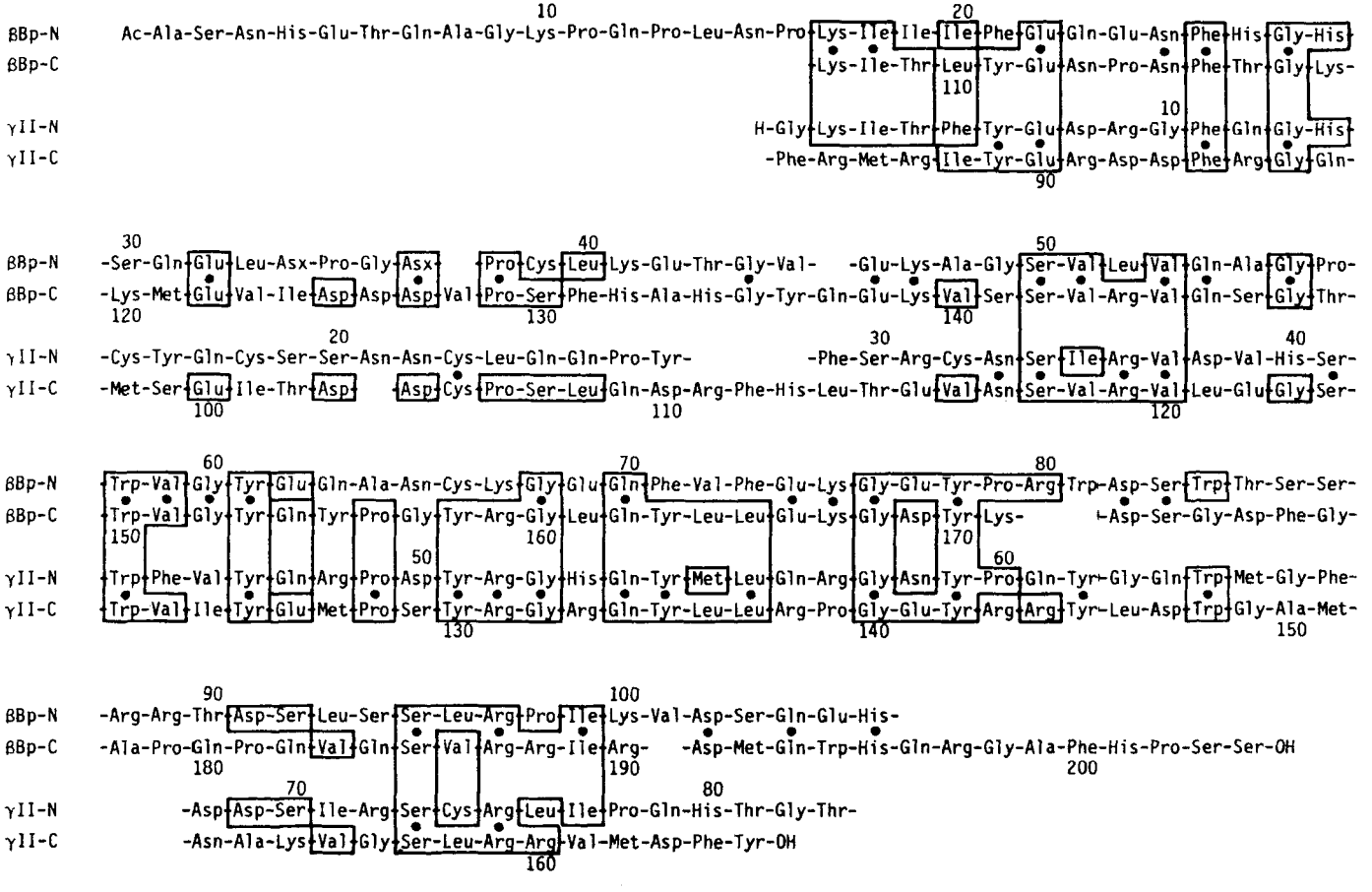


FIGURE 7. Optimal alignment of N- and C-terminal halves of bovine βB_P and γ-crystallin fraction II[215] sequences. Interchain identical residues in homologous positions of βB_P and γ II half-chains are enclosed in boxes. Intrachain sequence homology between the N- and C-terminal parts of βB_P or γ II is emphasized by dots.

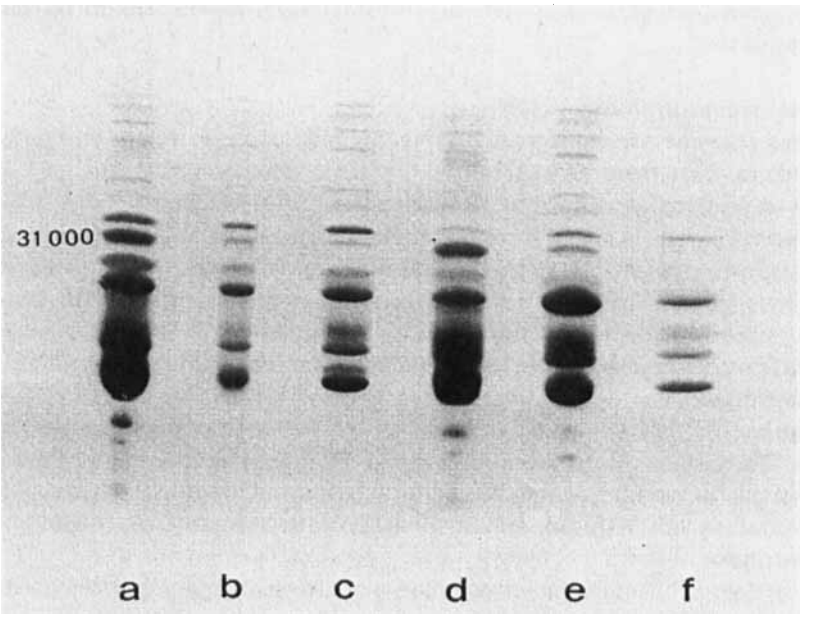


FIGURE 8. Sodium dodecyl sulfate gel electrophoretic pattern of the total lens proteins present in different lens fractions. (A) The calf lens nucleus; (B) the calf lens inner periphery; (C) the calf lens outer periphery; (D) the cow lens nucleus; (E) the cow lens inner periphery; (F) the cow lens outer periphery. The polypeptide with molecular weight 31,000 (βB_{1b}) is indicated. Note that young lens fibers have almost exclusively βB_{1a} (C, F), while old ones comprise virtually only βB_{1b} (D).

components are immunologically identical, their primary structure is presumably closely related. The native molecule has a molecular weight of about 200,000[104,105] and consists of four subunits. In SDS gels containing 7 M urea this protein can be resolved into two polypeptides that differ only slightly in molecular weight. Peptide mapping suggests a very high degree of similarity between the two kinds of subunits.[106] Bhat and Piatigorsky[107] described the molecular cloning and partial characterization of δ-crystallin cDNA. The inserted cDNA was estimated to represent at least 69% of the δ-crystallin coding sequence.[107] The authors provided evidence that minimally two nonalleleic δ-crystallin genes exist in chick and turkey chromosomes. The number of intervening sequences has been estimated to at least 14. The sequence complexity of the δ-crystallin mRNA has been determined by hybridization with cDNA. Only a single species could be detected.[108] A notable structural difference between δ-crystallin and all other crystallin types is the α-helical conformation of the former. This was concluded both from Raman spectra and circular dichroism.[109,110] This conformation is supposed to give the soft consistency to bird lenses, and ultimately allows their strong deformation upon accommodation.[111]

B. Noncrystallins

Besides the bulk of water-soluble crystallins the eye lens contains a great variety of other water-soluble lens proteins among which numerous enzymes, for instance, those involved in DNA-, RNA-, and protein biosynthesis, in proteolysis, and the enzymes of the glycolytic pathway (cf. Ref. 111a). It is beyond the scope of this report to deal with these proteins, in particular since none of them have been purified to homogeneity. Neither do they differ fundamentally in any functional aspect from the corresponding

enzyme protein in other tissues. There is, however, one notable exception: bovine leucine aminopeptidase.

1. Leucine Aminopeptidase (LAP)

As far as enzymes are concerned LAP is the only example which until now may be considered, at least from a quantitative point of view, as typical for lens. LAP can routinely be isolated according to Hanson et al.[112] This enzyme belongs to a class of aminopeptidases which occur in different tissues of animals and plants and also in microorganisms. It is one of the few proteolytic enzymes of which no sequence data were hitherto available. During the last two years, however, the primary structure of the enzyme has been solved in my laboratory.[113,114] It might well be that this knowledge will be helpful for a better insight into the exact mode of action of this enzyme which is not yet fully understood.

Lenticular LAP has a molecular weight of 326,000 and is composed of six identical subunits. The enzyme catalyzes the cleavage of unblocked amino acid residues from the NH_2-terminus of peptides and proteins. Its activity is highest toward hydrophobic and aliphatic amino acids. Peptide bonds involving proline, β-aspartyl and γ-glutamyl bonds resist hydrolysis.

In the eye lens LAP may be involved in the breakdown of organelle proteins since upon terminal differentiation from epithelium to fiber cells, nuclei, and mitochondria and their component molecules disappear completely. Structural proteins like α- and β-crystallin will not be attacked due to their blocked NH_2-termini. The reason that the unprotected αN-terminal glycine of γ-crystallin is not cleaved may be due to a favorable folding of the native protein that presumably renders the N-terminus inaccessible for LAP action. However, this assumption has to be sustained by experimental evidence.

Studies on the pH dependence of the catalytic reaction and on chemical modification of the enzyme revealed the involvement of the side chains of His, Cys and Tyr as essential groups in the catalytic process.[115–119] From the results of these studies Müller-Frohne derived the model depicted in Figure 9.[120] In this model the substrate is bound to the hydrophobic groups of the enzyme through the side chains of its amino acid side and via its amino group and carboxy oxygen to the Zn ion. In particular His and Cys are supposed to be the binding residues for the zinc ion. Nucleophilic attack on the peptide bond can be exerted by an OH^--group or another nucleophilic group of the enzyme. A proton donating group close to the peptide NH-group may have a rate-enhancing effect on the catalytic step.

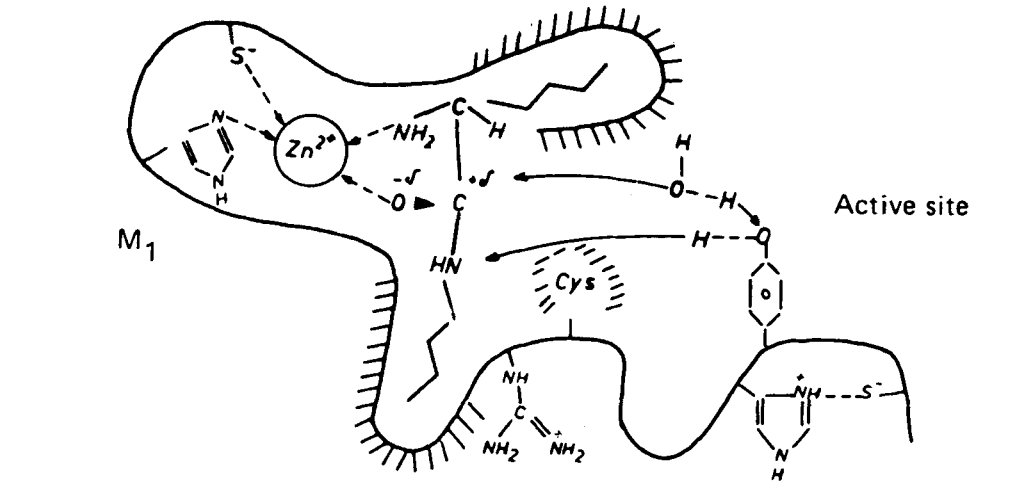
Electron microscopy revealed that the quaternary structure of LAP is rather unusual[121] and to some extent comparable to the quaternary structure of aspartate transamylase.

A number of properties of the bovine enzyme have been reviewed by Hanson and Frohne.[122]

2. FM (fast moving) Crystallin

FM crystallin is a monomeric acidic water-soluble lens protein occurring in rather low quantities. This protein, formerly named pre-α-crystallin due to its high electrophoretic mobility at alkaline pH,[123] has been described in cattle and in a few other species. The old designation is misleading since hitherto no relationship with α-crystallin, neither immunologically nor structurally, could be detected. In fact the function of this protein is completely obscure.[124]

The so-called pre-α-crystallin found in some anuran and urodelan amphibians is antigenically unrelated to FM crystallin.[125] Like γ-crystallin this protein seems to be restricted to lens fibers.[126] The protein has recently been purified to homogeneity.[127]



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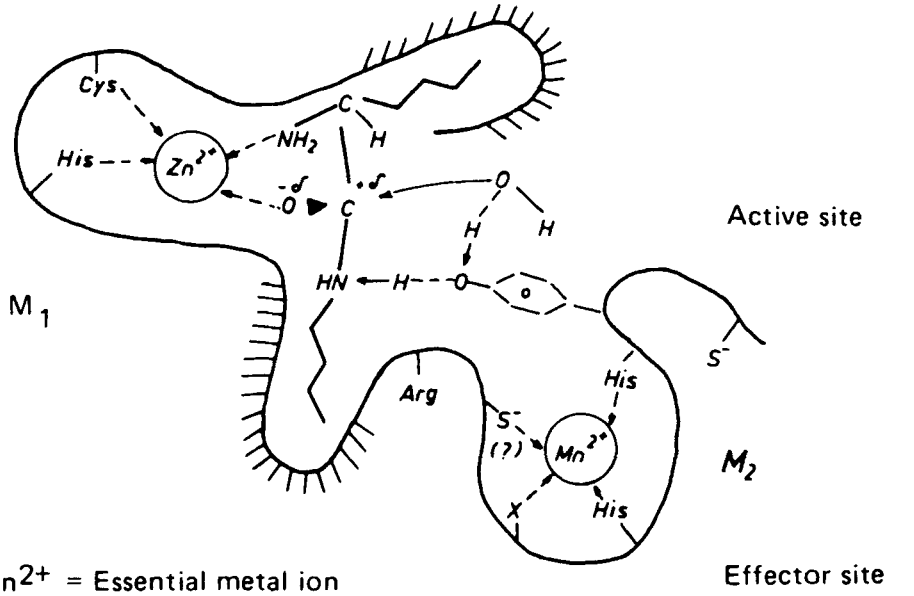

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FIGURE 9. Model of the active site of leucine aminopeptidase. (A) Nonactivated enzyme; (B) Mn^{2+}-activated enzyme. (From Müller-Frohne, M., *Opthalmic Res.*, 11, 377, 1979. With permission.)

3. RNase Inhibitor

A natural inhibitor of alkaline RNase has been described in a great variety of tissues.[128-132] This inhibitor has also been found in ocular tissues.[130,133,134] Despite the fact that only a small amount of water-soluble lens protein represents the inhibitor, it can be

purified rather easily in a two steps procedure. Therefore, lens tissue belongs together with placenta[131,132] to the most suitable sources for the isolation of the inhibitor which is a useful substance in protecting polyribosomes and messenger RNA from degradation.[135-141] The structure of the inhibitor is hitherto unknown.

4. G-Actin

Albeit gel filtration of the water-soluble lens fraction (for instance on Sephadex® G200) yields purified crystallins highly satisfactory for various studies, it should be kept in mind that very often even an additional purification step based on another parameter than molecular size does not yield proteins that are completely pure. Sometimes minor impurities can be detected by two-dimensional electrophoresis according to O'Farrell,[142] but in many cases the contaminants are virtually below the limits of detection. Only after the application of specific methodologies does the presence of these contaminants become apparent. A notable example of such an impurity in the water-soluble crystallins is G-actin, one of the two forms of the protein subunit of microfilaments. Since the molecular weight of this protein is about 43,000, it will be eluted together with the β_L fraction upon separation by gel filtration.

However, due to the fact that DNase I binds actin,[143] one can construct an affinity chromatography column of DNase I linked to agarose that specifically binds lens G-actin.[144] The protein absorbed to these affinity columns is eluted by discontinuous guanidine-HCl solutions. Actin comes off from the column together with an appreciable amount of α-crystallin, which under the conditions of the experiment for some unknown reason also sticks to the column matrix. For a final purification, preparative polyacrylamide gel electrophoresis is applied.[144] Amino acid analysis, peptide mapping, and electrophoretic behavior (see Figure 10) show that calf lens G-actin is indistinguishable from calf thymus and rabbit skeletal muscle actin.

The purified protein has been used to provoke a specific antiserum in order to demonstrate, by indirect immunofluorescence, the location of actin *in situ*. The immunofluorograph is strongly suggestive for a concentration of lens actin in close proximity to the plasma membranes. Possible implications for the process of visual accommodation have recently been discussed by comparing the distribution of actin in accommodating and nonaccommodating species.[145]

III. WATER-INSOLUBLE LENS PROTEINS

As mentioned earlier, in the past the water-insoluble part of the mammalian eye lens has only been studied incidentally.[11,146-149] In 1972 this rather neglected part of the lens became an object of more intensive studies.[2,150] The reason for this new interest was the fact that the lens fiber plasma membranes were recognized as the major constituents of the water-insoluble fraction. Later on our group[33,144,151-153] and that of Maisel[154,155] became aware that in addition to plasma membranes also the lenticular matrix remains in fiber ghosts after removal of the lens crystallins. This matrix or cytoskeleton can be isolated as a complex with the plasma membranes provided proper ionic conditions are maintained during the preparation.[156] In Figure 11 it is depicted how a bovine lens can completely be dissolved by subsequently applying: water extraction, urea treatment, and sodium dodecylsulphate dissolving. Roughly the water-soluble fraction contains the crystallins; the urea-soluble fraction comprises, in addition to water-insoluble crystallins, the cytoskeletal proteins and some extrinsic membrane components; whereas the SDS-soluble fraction is characterized by the occurrence of the intrinsic membrane protein subunits to be dealt with later.

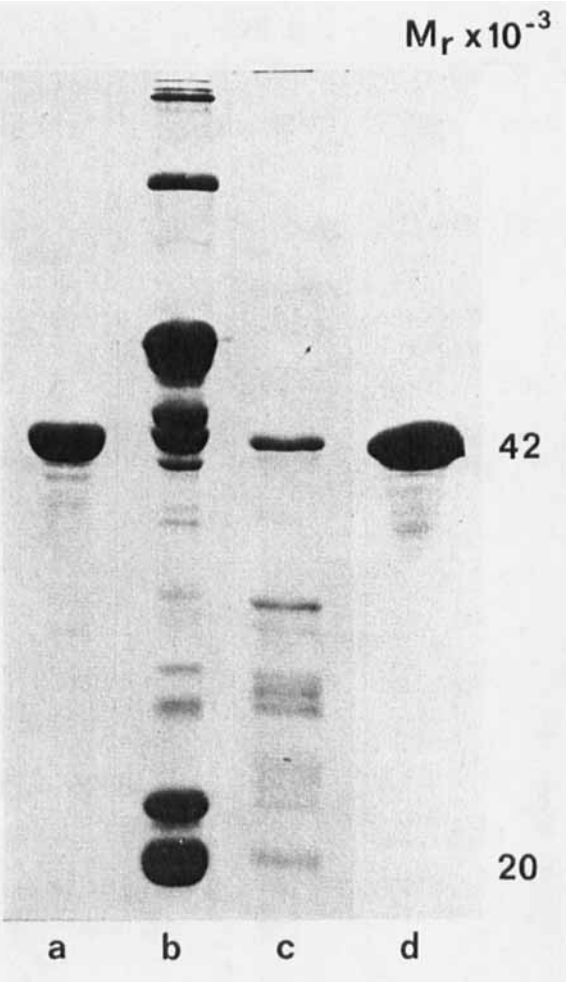


FIGURE 10. SDS-polyacrylamide gel electrophoresis of purified G-actin from calf lens (d). For comparison rabbit muscle actin (a), the water-insoluble calf lens fraction (b), and a crude preparation from calf lens extracted with 2-{[tris(hydroxymethyl)methyl]amino}-ethanesulfonic acid are also shown.

A. The Urea-Soluble Lens Proteins (USL)

1. Starting Material

a. Preparation

As a routine procedure the methods described by Kibbelaar and Bloemendal can be applied.[144,157] Lens cortices are homogenized in 0.05 M Tris-HCl buffer at pH 7.5, centrifuged at 15,000 × g, and the pellet is washed very thoroughly so that the last wash does not contain more material absorbing at 280 nm than 0.05 mg/ℓ. The final pellet is suspended in 0.05 M Tris-buffer containing 6 M urea, 0.05 M NaCl, and 0.001 M EDTA, and is then adjusted to pH 8.6. The suspension is stirred for 2 to 3 hr. All manipulations are carried out at 4°C. Thereafter the solution is centrifuged in rotor 30 of a Spinco® preparative ultracentrifuge at 100,000 × g for 90 min. The supernatant contains the USL fraction.

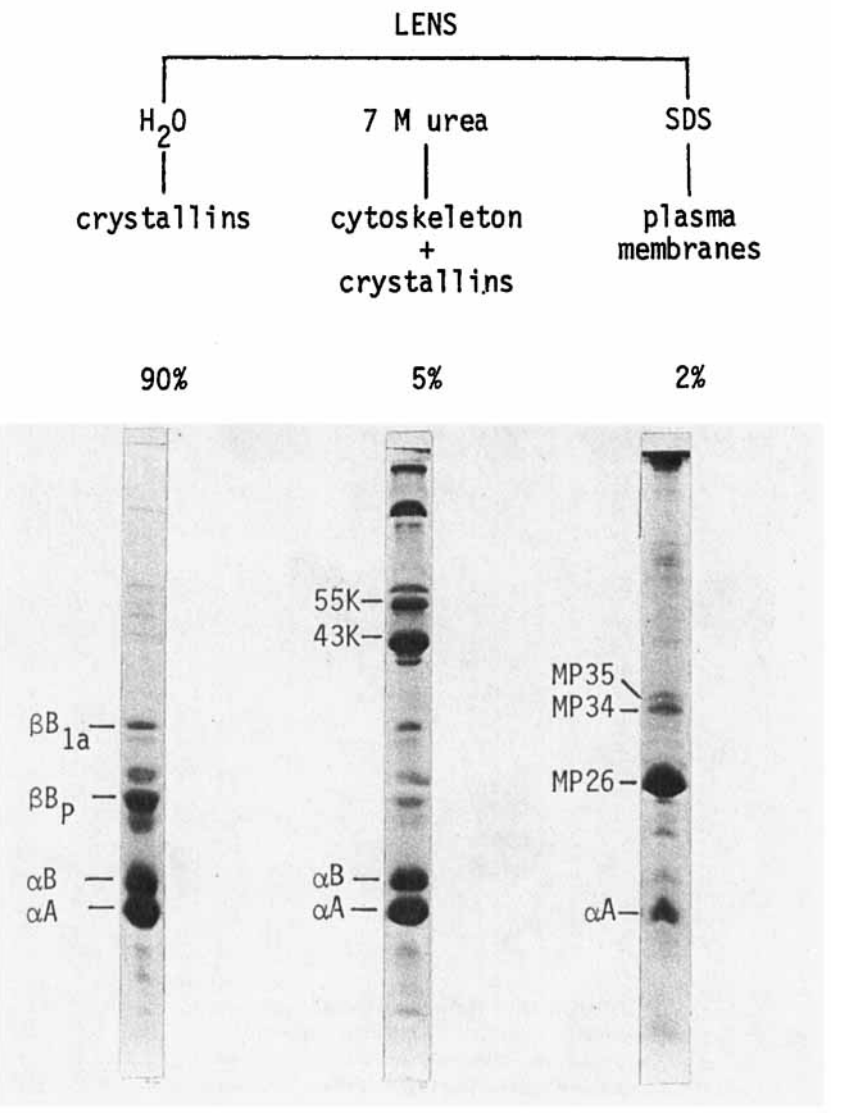


FIGURE 11. Fractionation of calf lens fiber cells into a water-soluble, urea-soluble and SDS-soluble part, followed by SDS-polyacrylamide gel electrophoresis.

b. Gel Filtration of USL

About 50 mg of USL protein is applied to a column (150 × 1.5 cm) packed with Sephadex® G200 superfine and equilibrated in the 6 M urea-Tris buffer. Four fractions merge from the column (Figure 12). Each fraction was analyzed on a SDS-polyacrylamide slab gel. Fraction I contains a major 100,000 dalton band in addition to an 200,000 dalton component and a component with an apparent molecular weight of about 43,000 which represents actin, as has been shown by coelectrophoresis of pure rabbit skeletal actin. Peak II comprises as main components polypeptides in the 43,000 and 56,000 molecular weight range, respectively. In this region the microfilament protein subunit (actin: 43,000) and the intermediate-sized filament protein subunit (vimentin: 56,000) occur. However, in particular on overloaded gels also other components of almost similar molecular weight coincide with the two cytoskeletal proteins. Fraction III

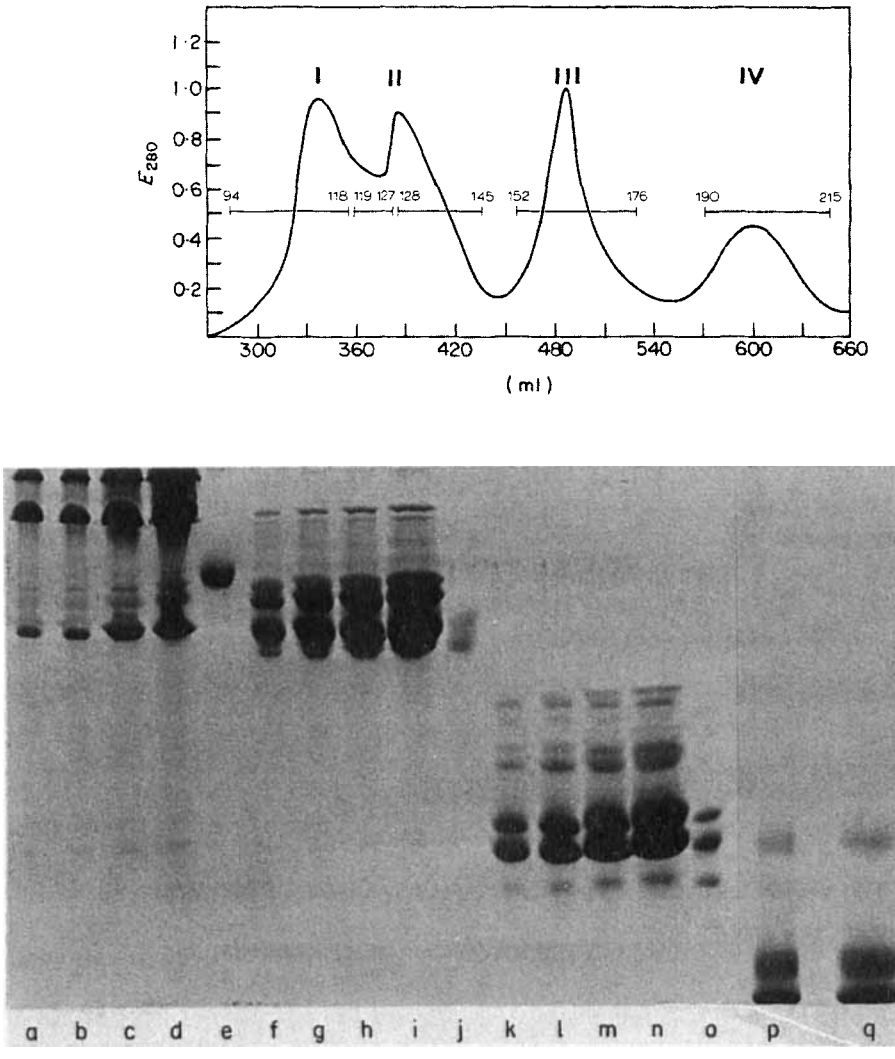


FIGURE 12. Gel filtration pattern of the urea-soluble fraction (USL) of lens fiber cells on a Sephadex® G200 column equilibrated in a 6 M urea-containing Tris buffer, followed by SDS-polyacrylamide gel electrophoresis.

shows the typical pattern of the soluble crystallins, although the ratio between the individual polypeptides differ from the pattern of soluble crystallins obtained by water extraction. Under peak IV, two hitherto unidentified polypeptides of 13,000 and 10,000 molecular weight are located. The latter component may well correspond to the 9,600 molecular weight polypeptide found by Roy and Spector in human lens.[158,159]

2. F-Actin

Actually, calf lens actin is the only cytoskeletal protein subunit from lens tissue which has been characterized by biochemical and immunochemical techniques. Kibbelaar et al.[144,160] provided the most extensive evidence for the actin nature of the 43,000 dalton lens protein. Maisel and Perry[161] concluded that actin is present in chick lens fiber cells from their observation that a 43,000 dalton protein derived from these cells comigrated with actin. More definitive proof, both in chick and in mouse lens fiber cells, was given by the biochemical studies of Mousa and Trevithick.[162] The latter authors also studied the

changes in actin content during differentiation in rat lens. This protein appeared as a major component of the proteins in new-born lens epithelium but as a minor component in new-born lens fibers. The percentage of actin both in new-born and mature fiber cells was about 1% of the total protein and the ratio β/γ-actin was 1.2. In contrast, in the epithelial cells the percentage of actin appeared to be 10.7% and the ratio β/γ-actin 2.2. These figures should be taken with great care since the authors applied a scanning method of the stained proteins in their polyacrylamide gels.[163] Confirmation by a more direct estimate, for instance determination with the aid of DNase I[164], would be worthwhile. Mousa and Trevithick were unable to demonstrate actin in the lens nucleus, which is in contradiction with the findings of Kibbelaar et al.[145] who detected this protein with the aid of immunofluorescence also in rat lens nucleus.

a. Actin and Cataract

The most common form of lens opacities is the so-called senile cortical cataract.[165] Creighton et al.[166] hypothesized that the degeneration of globular-like structures and disruption of normal morphology are responsible for the loss of visual acuity. This globular degeneration could artificially be provoked by treatment of the lens with cytochalasin D.[167] Since it was known that the latter drug strongly affects actin microfilament organization,[168] the conclusion was drawn that disorganization of actin filaments leads to the observed degeneration of the globules.

3. Nonactin 43K Protein

Garner et al.[169] isolated a 43,000 dalton polypeptide from the water-soluble and the water-insoluble fraction of human cataractous lenses which, as far as amino acid compositions and immunological properties are concerned, are closely related. Originally the authors suggested that this polypeptide was formed by posttranslational cross-linking of two water-soluble 20,000 dalton polypeptides.[170] Later on it was put forward that the 43,000 dalton component represents a direct gene product which is not identical to actin.[171] Nevertheless it is very striking that this polypeptide shares a number of properties with actin, namely its occurrence in a water-soluble and a urea-soluble form, an identical molecular weight, a high content of aspartic and glutamic acid, and the localization of the water-insoluble species in the membrane region.[172] Spector et al. suggest that the 43,000 dalton polypeptide may act as nucleation site on the membrane for the formation of large protein aggregates by interaction with soluble crystallins which may cause cataract.

4. Vimentin

Recently great interest has been focused on the intermediate-sized (IF) or 10-nm filaments.[173] These filaments have been subdivided according to the embryonic origin of the cell and the type of protein subunits.[174] For instance, prekeratin is the major polypeptide of IF from epithelial origin, whereas mesenchymal cells contain 10-nm filaments of the vimentin class. Strikingly, in lens — which is thought to be of epithelial origin — vimentin is the major protein subunit found in the IF. We demonstrated the presence of vimentin with the aid of immunofluorescence (Figure 13) applied to epithelial cells in tissue culture[175,176] after detection of IF by electron microscopy in intact lenses and in isolated lens fiber cells.[177-179] The only preliminary biochemical characterization of lenticular vimentin has hitherto been reported by Kibbelaar et al.[144] The protein was obtained in a purified form by preparative electrophoresis on polyacrylamide gel slabs. The results were highly suggestive for the occurrence of vimentin in a soluble and an insoluble form as in the case of actin.

Maisel et al.[180] reported on regional differences in the polypeptide composition of the

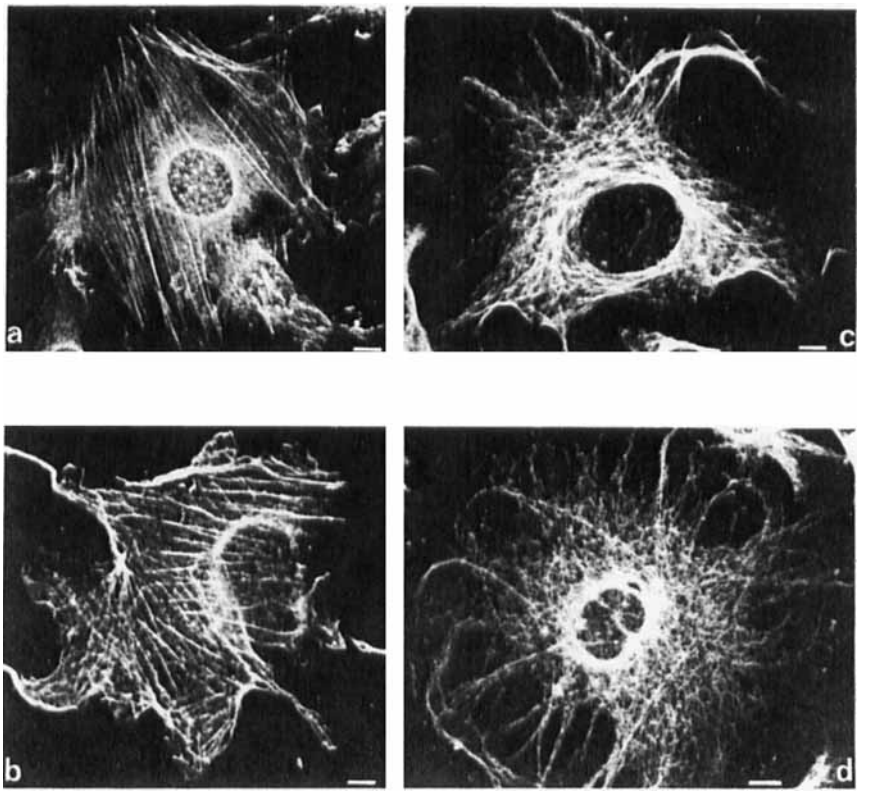


FIGURE 13. Visualization by immunofluorescence of cytoskeletal proteins in cultured hamster lens cells grown in normal and in SV40-transformed monolayer culture. Actin-filaments in (a) normal monolayer or (b) transformed monolayer culture; intermediate-sized filaments in (c) normal monolayer or (d) transformed monolayer culture; and (c) and (d) are visualized with the aid of antibodies directed against vimentin. As a control also antiprekeratin serum was used. The latter reaction was negative.

urea-soluble fraction in chick lens. High molecular weight components present in the cortical fibers had markedly decreased in the urea-soluble fraction from the lens nucleus. This observation could be correlated on the morphological level with the absence of intermediate-sized filaments from the nuclear fibers. A similar situation was demonstrated in bovine lens.[181] Actin appeared to occur only in trace amounts in the nuclear urea-soluble fractions from both chick and cattle.

B. The SDS-Soluble (Urea-Insoluble) Lens Proteins (UIL)

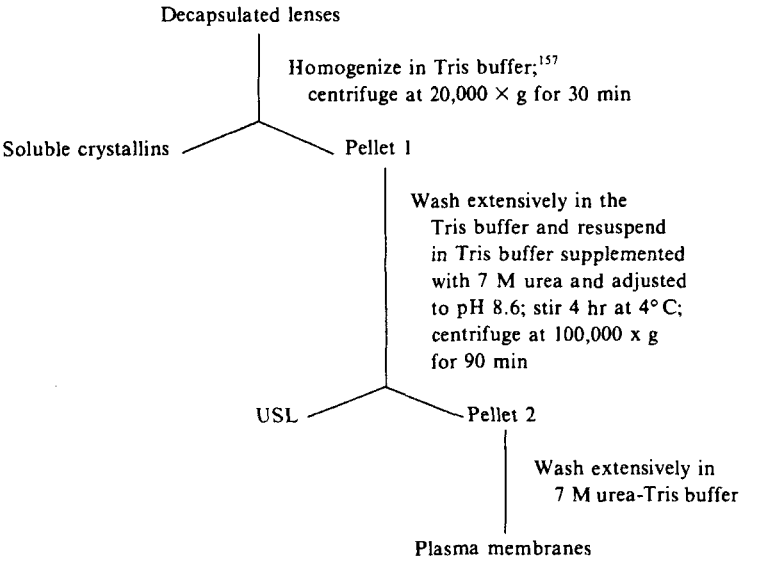
1. Starting Material

a. Preparation of Lens Membranes

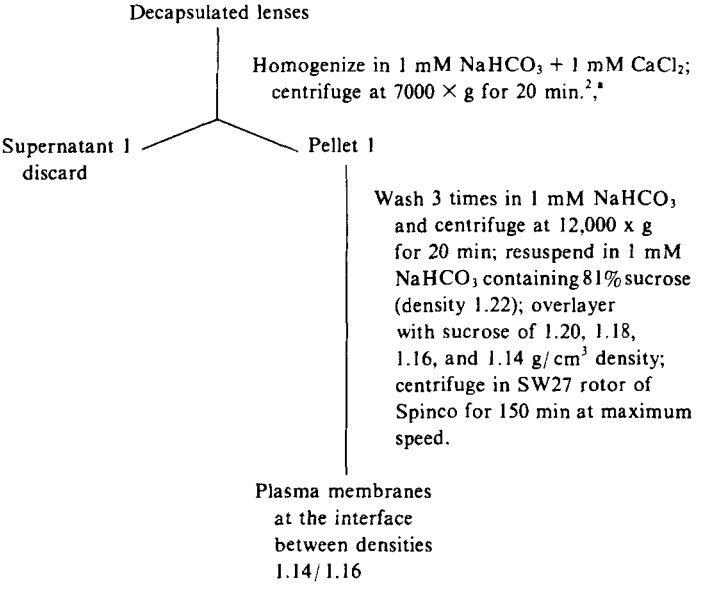
Previous studies in our laboratory[2,3,151] indicated that the urea-insoluble fraction of calf lens contains a considerable amount of plasma membranes, containing typical intrinsic proteins. Recent investigations showed that also human UIL is biochemically and morphologically identical to the human lenticular plasma membrane.[182]

For the preparation of the lens plasma membrane fraction two procedures can be followed, which are summarized in schemes 1 and 2. The first method, virtually confined to water and urea extraction, is based merely upon the (in)solubility properties of the membranes. In the second procedure both density and solubility properties of the membranous material are explored.

Scheme 1
ISOLATION OF LENS FIBER PLASMA MEMBRANES BASED UPON UREA EXTRACTION



Scheme 2
ISOLATION OF LENS FIBER PLASMA MEMBRANES BY DENSITY GRADIENT CENTRIFUGATION



[a] The procedure can be scaled down to one or two lenses with the modification as described by Horwitz et al.[185]

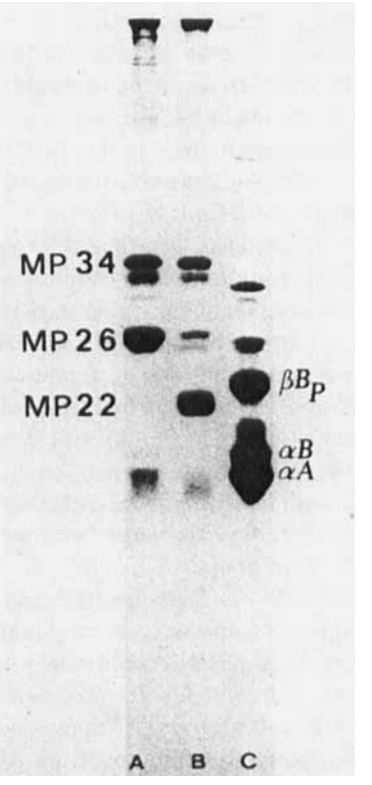


FIGURE 14. SDS-gel electrophoresis pattern of lens fiber plasma membrane proteins. (A) urea-insoluble fraction; (B) aged urea-insoluble fraction (note the decrease of MP26 and the appearance of MP22. In both lanes MP34 occurs as a doublet); (C) total water-soluble lens fiber proteins (for comparison).

2. The Major Plasma Membrane Proteins

a. MP26

In order to facilitate discussion between students of lens plasma membranes and to avoid the introduction of fancy names, we introduced a rational nomenclature for lens membrane proteins.[7] In this nomenclature membrane protein is indicated by the capitals MP followed by the apparent molecular weight in kilo daltons. Hence the two major protein components of calf lens fiber plasma membranes are named MP26 and MP34, respectively (compare Figure 14). We observed in some experiments, in particular upon storage or extensive urea treatment of plasma membranes isolated by flotation, that a 22,000 polypeptide appeared on the gel pattern, while MP26 decreased or even disappeared. Later on the same phenomenon has been reported by other workers for MP26 from human lens.[182-185] Since both MP26 and MP22 are urea-resistent but are released from membranes after extraction with chloroform methanol,[186] they obviously represent intrinsic membrane proteins.

Electron microscopy of cortical lens fiber plasma membranes obtained after extensive urea washing reveals the presence of a large number of junctional elements. Actually, when UIL is thoroughly extracted with deoxycholate, the MP26 component represents about 95% of the remaining protein material. This observation strongly suggests that the

junctional core consists almost exclusively of MP26 as intrinsic protein component. Freeze fracturing micrographs of detergent-isolated lens junctions show the occurrence of 9 nm particles appearing in linear rows or loosely packed in polygonal arrays. The architecture of the lens fiber junctions deviates from that of a typical gap junction.[187]

Alcalá et al.[5] raised the question whether or not MP26 is identical to βB_P whose molecular weight is also almost 26,000. However, amino acid analysis and peptide mapping revealed that the two proteins are definitely different.[157] In this connection it has to be kept in mind that in general both similarity as well as differences in molecular weights of different proteins estimated by sodium dodecylsulfate gel electrophoresis may be misleading as long as no additional chemical characteristics are available.

Roy et al.[184] reported that in human lenses MP26, as well as its degradation product MP22, reacts positively with periodic-Schiff's reagent indicating the glycoprotein nature of these components. Horwitz and Wong[188] showed that both bovine and human MP26 lost a 4000 dalton peptide upon cleavage by *Staphilococcus aureus* protease. In contrast "native" MP22 resisted further proteolysis. It was concluded that all these proteins possess similar composition and conformation. Additional chemical evidence was obtained by comparison of α-chymotryptic maps obtained from the purified proteins isolated by preparative gel electrophoresis.

Wong et al.[189] observed that MP26 is heat-sensitive and aggregates even in sodium dodecylsulfate solution to a high molecular weight component. We observed that heating for 2 min at 100° C of a calf lens fiber plasma membrane preparation diminishes already considerably the amount of protein which can penetrate on SDS-containing polyacrylamide gel. Broekhuyse and Kuhlmann[190] reported that one can greatly avoid this undesirable association by reducing the temperature to about 37° C and increasing the duration of solubilization up to 3 hr. The temperature could be raised to 80° C if the time of heating was no longer than 10 min. Addition of 1% dithiothreitol had a marked effect. It is thought that this reducing agent promotes deaggregation.[191]

There is now general agreement between students of lens plasma membrane proteins that MP26 is the principal intrinsic protein of lenticular fiber membranes from various species. However, species specificity was observed by Alcalá et al.[182] in that the reaction of an antiserum directed against chick lens MP26 and human lens plasma membranes was negative.

b. MP34

MP34 is a rather intriguing lens membrane constituent. Originally[2] we found this component as a fuzzy band on polyacrylamide gels run according to the procedure of Weber and Osborn.[192] The molecular weight estimated following the method of Shapiro et al.[193] appeared to be about 37,000 to 38,000.

In later studies[3] on Laemmli gels[194] an apparent molecular weight of about 34,000 was calculated. However, careful examination even of the older patterns revealed that MP34 frequently exists as a doublet with a molecular weight of 34,000 and 35,000, respectively. Interestingly, MP34 is found both in plasma membranes isolated from epithelial cells and in lens fibers[195] (Figure 15). On the other hand, MP26 is a typical fiber cell membrane constituent, presumably the major protein component of the large junctional structures observed in the electron microscope. Anti-MP26 serum does not react either with isolated MP34 or with lens epithelial membranes showing that MP26 synthesis occurs exclusively in lens fiber cells. This notion renders MP26 a very useful marker in differentiation studies.[196]

MP34 is very likely identical to a bovine lens plasma membrane fraction designated EEP by Bouman et al.[197] This designation was chosen since the protein can readily be extracted by EDTA-solution from the membranes. In fact this fraction contains two

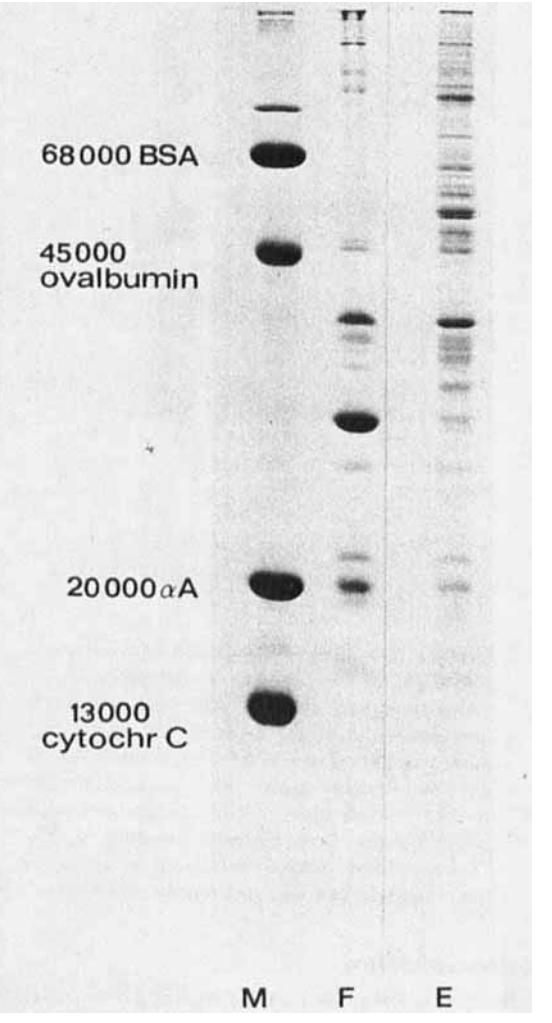


FIGURE 15. SDS-gel electrophoresis pattern of lens plasma membrane proteins derived from lens fibers (F) and epithelial cells. (E) Marker proteins (M) for comparison.

proteins, MP35 and MP32, which may well be similar to the doublet observed in our experiments. On a total membrane protein basis the recovery of MP32 and MP35 is only 5%. Bouman et al.[197] consider the EEP fraction as extrinsic lens membrane protein, presumably linked to the membrane by calcium ions. The authors conclude that the amino acid composition of EEP is different from the composition of α-, β-, and γ-crystallin, respectively. However, since EEP comprises two different antigenic components, such a comparison is not conclusive. In order to completely rule out the possibility that, for instance, the 32,000 molecular weight membrane component is related to βB_{1a} or βB_{1b}, peptide maps of the isolated components would be required.

In contrast to MP26, MP34 is not subject to gradual proteolytic degradation upon aging. This may mean that the latter protein is protected against enzyme attack thanks to a hitherto unknown feature. Detailed structural data upon MP26 and MP34-35 are highly desirable in order to elucidate this problem.

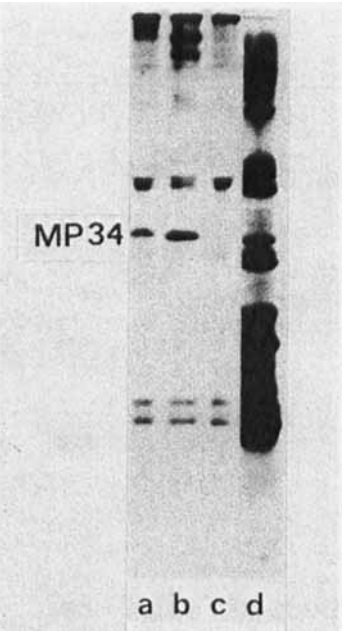


FIGURE 16. Biosynthesis of MP34 in vitro. Autoradiograph of the SDS-gel electropherogram of protein immunoprecipitated with total antiplasma membrane serum. (a) antiserum directed against total plasma membrane protein from lens epithelium; (b) antiserum directed against total plasma membrane protein from lens fiber cells; (c) antiserum directed against bovine serum albumin (control); (d) the [^{34}S]-methionine labeled translation products of "free" lens polyribosomes in a reticulocyte lysate.

3. Biosynthesis of MP34 and MP26

Extensive studies on the biosynthesis of water-soluble lens proteins have been reported from our laboratory.[19,36,37,42,46-48,50-52,60,79,80,82,198,199] The analysis of newly synthesized products was greatly facilitated since all crystallin subunits migrate in a molecular weight range between 18,000 and 32,000. In contrast, membrane and cytoskeletal proteins are located above this range except MP26. We observed that the lens cell-free system[82] manufactured, in addition to the soluble crystallins, polypeptides that coelectrophorese with plasma membrane components.[200] Lens polyribosomes or the poly(A)$^+$-RNA derived therefrom when translated in a heterologous cell-free system direct also the synthesis of noncrystallin polypeptides.[201]

As mentioned previously the biogenesis of the lens fiber cells is accompanied with the appearance of MP26 as major fiber plasma membrane constituent.[195] In an attempt to verify whether or not MP26 and MP34 are synthesized in the lens cortex, a reticulocyte lysate was supplemented with polyribosomes isolated from lens cortices. After incubation immunoprecipitation was performed with an antiserum directed against lens fiber membranes. From Figure 16 it can be concluded that only the synthesis of MP34 is directed by the lens fiber polyribosomes. This striking observation was originally poorly understood. It was thought that the result was due to low antigenicity of MP26. Alternatively it could not be excluded that MP26 might have interacted with an unidentified component from the heterologous lysate, since with some antisera not only MP34 but also an additional polypeptide in the 100,000 to 200,000 region was

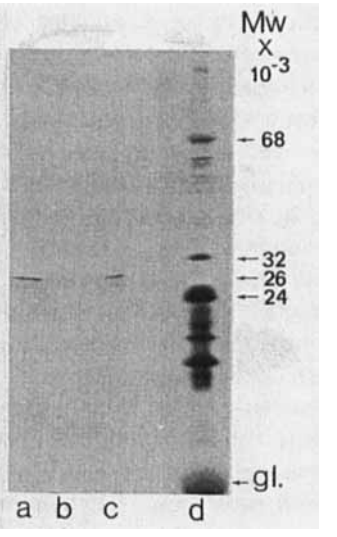


FIGURE 17. SDS-polyacrylamide gel electrophoretic patterns of MP26 immunoprecipitate in a reticulocyte lysate incubated with DNase I-isolated polysomes (a), with "free" polysomes (b), or with deoxycholate isolated polysomes (c), and polypeptides synthesized in a reticulocyte lysate under the direction of "free" lens fiber polyribosomes without immunoprecipitation (d).

precipitated. The latter assumption seemed to be rather plausible because only antisera directed against lens fiber membranes caused the precipitation of the high molecular weight component, whereas antiserum to lens epithelial membranes precipitated MP34 exclusively.[194]

Nevertheless the solution of this problem was found after we became aware that a certain class (or classes) of lens polyribosomes is (are) associated with the fiber plasma membrane-cytoskeleton complex.[152,153,156,196] These polysomes can be released from the latter complex by DNase I treatment. Since it is well-established that DNase I depolymerizes actin filaments,[143] it may well be that part of the lens polyribosomal population is attached to the plasma membrane—cytoskeleton complex via actin. Interaction between polyribosomes and cytoskeletal elements has also been suggested to occur in HeLa cells,[202,203] ascites cells,[204] acrosomes,[205] kidney cells, and fibroblasts.[206]

The DNase-released lens polyribosomes, when added to a reticulocyte lysate, direct virtually the synthesis of one major product. The nature of the newly synthesized product was deduced from three observations:

1. Its molecular weight as estimated after SDS-polyacrylamide gel electrophoresis appeared to be 26 kilo dalton.
2. It precipitated specifically with MP26 antibodies.
3. A component with molecular weight of 22,000 arose upon "aging" of the radioactive translation product (Figure 17).

4. Complex Formation Between Lens Proteins

a. Interaction Between Crystallins

One of the unanswered questions about lens crystallins is: "how are these proteins

arranged *in situ*? Is the lens fiber cell merely a sac filled with these soluble proteins in the form of a gel or do they occur as more or less ordered structures?" Albeit this question has not been solved yet, several attempts have been made in order to approach the problem.

First, one should like to know whether or not, under physiological conditions, the crystallins can form protein — protein complexes. In recombination experiments we were able to show that dissociated subunits of the crystallins reassociate properly to "native" aggregates provided the concentration of each polypeptide component is kept low, a condition presumably comparable to the situation at the moment of biosynthesis *in vivo*.[65,66] On the other hand, at higher concentrations, "artificial" hybrids between α- and β-crystallin can be obtained. In a recent series of investigations Manski and colleagues[207-209] searched for interactions between crystallin molecules. These authors, who explored selective filtration and immunochemical methods as analytical tools, observed that part of the cortical lens protein did not filter through a membrane having a cut-off point of 300,000 daltons. The aggregate contained also β-crystallin (molecular weight 150,000-200,000), whereas in the corresponding nuclear fraction also γ-crystallin (molecular weight about 20,000) was found. After immunoprecipitation with anti-α-crystallin serum the nuclear crystallin complex yielded filterable β- and γ-crystallin. From this observation it was concluded that interaction of α-crystallin with β- and γ-crystallin rather than self-aggregation of the latter proteins led to the complex formation. Manski et al.[207] suggest that this complex formation is a continuous process which firstly takes place only between α- and β-crystallin. Upon maturation also γ-crystallin becomes complexed and with progressing age the initial α-β complex binds more free γ-crystallin.[208] The formation of the complex, which is dependent upon ionic conditions, is characterized by extremely weak forces, since all complexes dissociated in a constant electric field, yielding the free crystallins.[209] The immunoadsorbens method worked exclusively with the soluble crystallins but not with the urea-soluble ones.

These highly interesting studies of Manski's group still need some additional controls. It cannot completely be ruled out that traces of proteins other than crystallins are involved in the observed complex formation. The soluble crystallins, in general considered to be pure proteins after separation by gel filtration, are by no means 100% homogeneous entities. In particular on overloaded gels one can easily detect various "impurities". A notable example is G-actin.[144] One should exclude the possibility that such filament-forming molecules play a role in crystallin complex formation. In connection herewith, studies by Maisel and Perry,[161] Bloemendal et al.,[65] and Benedetti et al.[151] should be kept in mind. In these investigations, by electron microscopy, giant stranded structures were visualized in the water-soluble lens fraction from chick lens[210] and calf lens extracts[65] (Figure 18). The major part of the "subunits" of these structures has the exact dimension of α-crystallin globules. If part of the strands would be formed by actin one may assume that the decoration with α-crystallin renders the total structure water-soluble. The occurrence of both α- and β-crystallin antigens in the observed water-soluble filaments was unequivocally proven by Maisel and Perry[161] with the aid of immunoprecipitation.

b. Interaction Between Newly Synthesized Crystallins and Noncrystallin Protein with Membranes

Bracchi et al.[211] reported that α-crystallin interacts with plasma membranes provided the protein is aged. This conclusion was based on dialysis experiments with isolated crystallin fractions. During our studies on lens membrane assembly *in vitro* we verified whether or not newly synthesized lens proteins were inserted into membranous structures.[212,213] It appeared that of the crystallins, αA_2 polypeptides almost exclusively became associated with heterologous membranes (Figure 19).[212]

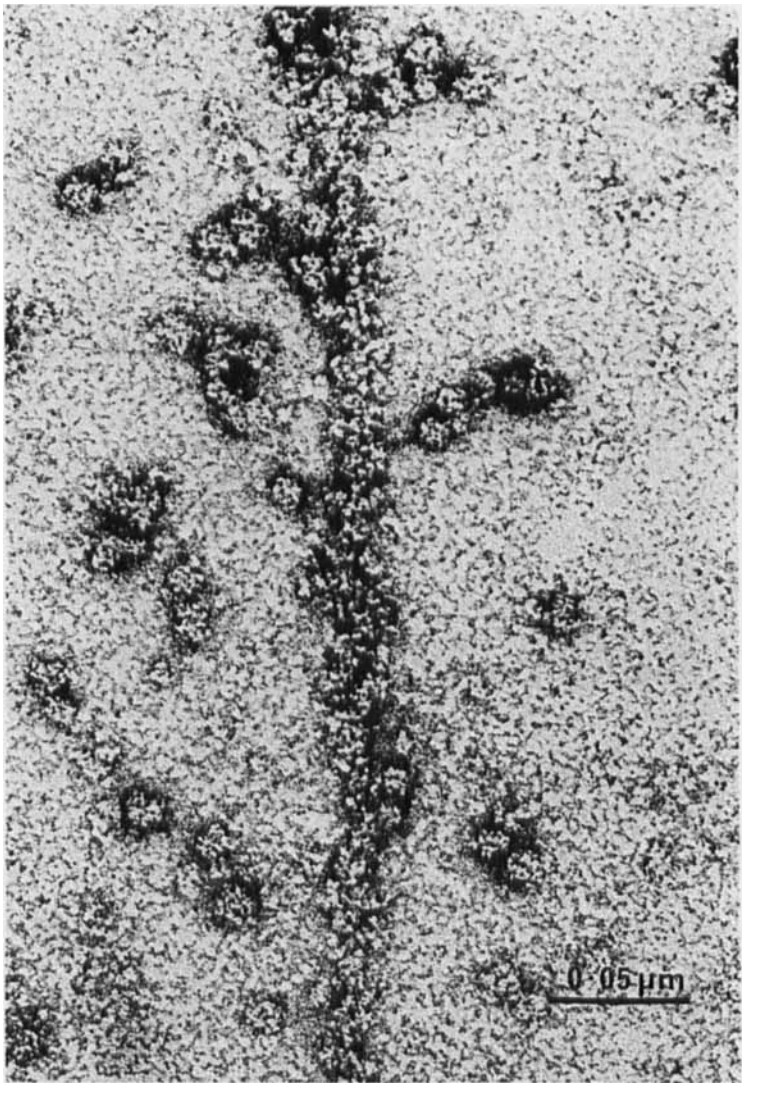


FIGURE 18. Electron micrograph of giant crystallin molecules found in the water-soluble fraction of calf lens fibers.

The experiments were carried out as follows. A reticulocyte lysate was supplemented with lens polyribosomes. After incubation the reticulocyte membranes were isolated as described for lens plasma membranes,[2] and the proteins analyzed by SDS-gel electrophoresis. Aging of α-crystallin results among other phenomena in deamidation of the composing polypeptides. Thus, αA_2 gives gradually rise to αA_1.[55] Our studies clearly demonstrated that the transition from αA_2 to αA_1 is no prerequisite for insertion of the former polypeptide into plasma membranes.

A variation of the experiments described was also carried out.[213] Again lens polyribosomes were translated in the reticulocyte lysate. After isolation of the newly synthesized products purified lens plasma membranes were added and the incubation continued. Only αA_2 chains interacted with the plasma membrane and became urea-insoluble.[213] Strikingly, αB chains do not interact with the membranes.

When the plasma membranes are isolated under conditions maintaining the complex

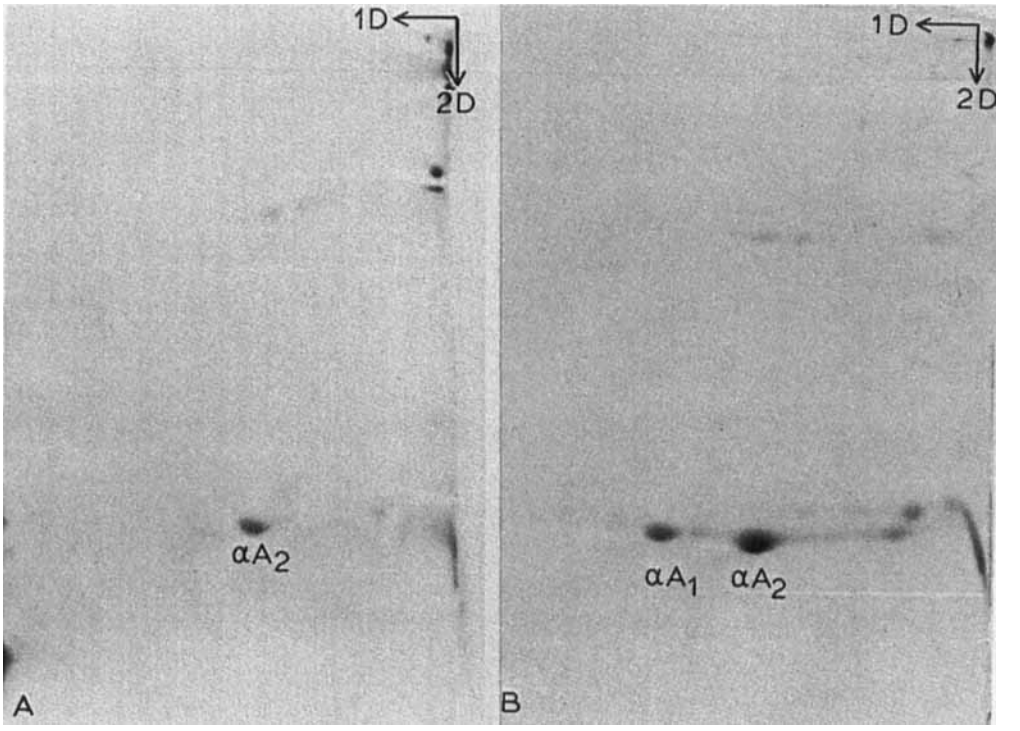


FIGURE 19. 2-D electrophoresis of newly synthesized polypeptides in the water-insoluble lens fraction. (A) Autoradiograph (note that only αA_2 is synthesized, but not αB_2); (B) If unlabeled αA chains are added as carrier, the position of αA_1 is also seen upon staining.

with the cytoskeleton, newly synthesized actin becomes also attached to the membranes in an almost stoichiometric ratio (1:1) with αA_2 polypeptides. It remains to be established whether this finding has implications for the *in situ* organization of the "water-soluble" lens proteins, in particular in the formation of the giant "soluble" structures occasionally observed in the electron microscope (compare Figure 18).

Recent studies revealed that also newly synthesized vimentin, the protein subunit of lenticular intermediate-sized filaments, is found to be associated with membranes which have been added to an incubation mixture containing reticulocyte lysate and lens fiber polyribosomes.[214] This observation leads to another problem which remains to be solved: "do there exist, on lens plasma membranes, different binding sites for αA_2 crystallin polypeptides and vimentin, respectively, or does αA_2 serve as protein cofactor or nucleation site in the formation of intermediate-sized filaments?"

At any rate, our reconstitution experiments are suggestive for the existence of such binding or recognition sites on the plasma membranes. Evidence is accumulating that the major subunit of α-crystallin A_2 can attach to heterologous and homologous plasma membranes. As far as the "water-insoluble" α-crystallin is concerned the following mechanism seems to be operative. Newly synthesized αA_2 polypeptides interact with the plasma membrane. Only thereafter αB_2 chains join the complex to form the high molecular weight α-crystallin aggregate. This model is in accord with a proposal for the architecture of native α-crystallin[64] and studies upon its biosynthesis in cell-free systems or oocytes,[48] where it was found that a core of αA chains is required before αB polypeptides can copolymerize to form the α-crystallin aggregate.

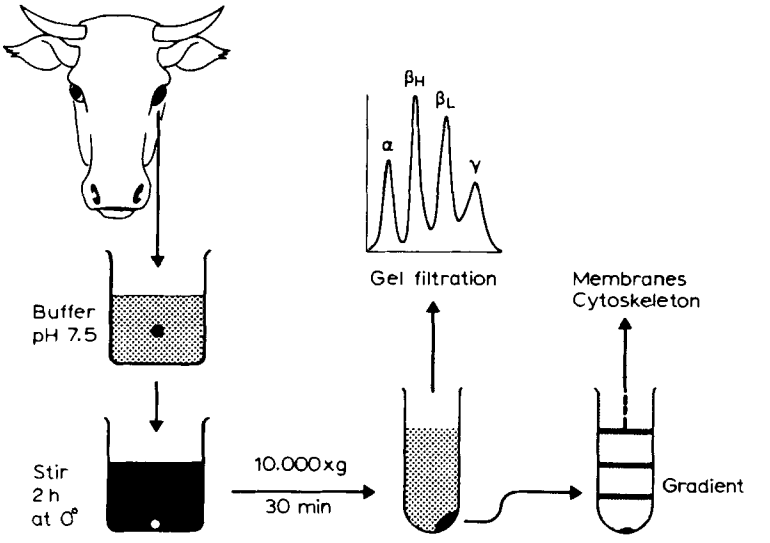


FIGURE 20. Summary of the isolation techniques of the crystallins and the water-insoluble fractions from bovine lens.

IV. CONCLUDING REMARKS

In the preceding sections various features of lens proteins have been described. Many characteristics of the major structural lenticular proteins, including the complete primary structure of the crystallin subunits αA_2, αB_2, βB_P, and γII, are available at present. Attempts to thoroughly characterize the insoluble lenticular fraction, in particular the proteins of the plasma membranes and the cytoskeleton, were only started a few years ago. Fortunately, problems, scarcely imagined previously, can now be investigated with success and with techniques only recently developed. The results undoubtedly will bring us closer to full understanding of the organization and function of the eye lens in normal and disordered states.

In order to follow a certain line in the story of lens proteins, some contributions to the field have been omitted from this review. I regret that it was, at least in my opinion, not feasible to include a discussion of all of these efforts. I would like to console colleagues neglected in this way by assuring them that all the data from my own laboratory have not been quoted in this paper. Actually, progress in lens research is being made so rapidly, that from the very moment this review will be set in print revision and extension will be required. Perhaps others who have courage and energy would like to undertake this task.

Finally, I want to emphasize once more[33] the usefulness of the lens system for model studies in molecular and cellular biology. In order to encourage newcomers to the field of lens research, I outlined the simplicity of the system, as far as the isolation techniques of various fractions are concerned, in a single schematic representation (Figure 20). The fact that the lens cytoskeleton and the lens plasma membranes appear (presumably for the first time) in such a scheme is not only due to the author's present research interest, but also to his conviction that these items are likely to become of paramount interest in the very near future.

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ADDENDUM

After finishing the foregoing review, a few new developments in the field of lens research have been reported. To the large arsenal of fractionation procedures for crystallins, the new technique of chromatofocusing has been added. This method enables us to separate in high yield the four composing subunits of α-crystallin in a single step.[216] Also, various γ-crystallin species, differing only slightly in pI values, are well resolved after chromatofocusing.[217] The molecular cloning of mRNA sequences encoding rat lens crystallins has been described by Dodemont et al.[218] Furthermore, it could be shown by sequence studies that the unusual length of the 14S rat lens mRNA encoding αA_2-crystallin is due to a long noncoding region at the 3'-end.[219] Computer analysis revealed that this message has only a single reading frame for a polypeptide of 20,000. This finding solves the problem raised above concerning the monocistronic nature of the α-crystallin A_2-mRNA.

Very recent results obtained in our laboratory with cloned lenticular vimentin cDNA probes are strongly suggestive that, contrary to actin genes, the vimentin gene does not occur in a multigene family.[220] Blundell et al[221] reported the three-dimensional structure of bovine γ-crystallin II at 2.6 Å resolution. They showed that the protein has a two-dimensional β-structure folded into four similar Greek key motifs. Using predictive and computer graphics techniques, it could be demonstrated that βB_P, like γII, also should have a three-dimensional structure involving four structurally homologous motifs.[222] However, extensions exist of the polypeptide chain both at the C- and N-terminus. These extensions may be involved in self-association of βB_P chains.

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