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Bovine lens 23, 21 and 19 kDa intrinsic membrane proteins have an identical amino-terminal amino acid sequence

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We have isolated bovine lens intrinsic membrane proteins (MP) having molecular masses of 19, 21 and 23 kDa. Limited amino acid sequence analysis of the amino-terminal portion of each of these polypeptides revealed a 100% homology in sequence for the number of residues determined (20 amino acids). Northern blot analysis of bovine lens mRNA using a labeled antisense oligonucleotide probe common to the amino acid sequence of these three peptides revealed a single band having an apparent molecular size of 0.8 kb. Taken together, these findings suggest a genetic commonality between these polypeptides.

Amino acid sequencing; Lens membrane protein; mRNA; Synthetic antisense oligonucleotide

1. INTRODUCTION

The vertebrate lens is a transparent tissues whose main function is to focus light onto the retina. The physicochemical properties of lens constituents, particularly lens proteins, appear geared towards maintaining its transparency. Perturbances in these physicochemical balances often alter lenticular transparency, resulting in lens opacity (cataract). Extensive research has been carried out to isolate and characterize the soluble lens proteins, known as crystallins, which constitute about 90% of the total lens protein content [1–4]. Less attention has been given to the lens membrane proteins, which perform several vital functions such as cell to cell communication, maintenance of homeostasis, and preserving the integrity of the tissue. In cataractous lenses, an association of crystallins with plasma membrane proteins has been demonstrated [5–7]. To date, most of the studies concerned with lens membrane proteins have involved the isolation and characterization of the most abundant lens plasma membrane protein, MIP26 (major intrinsic protein) [8–13]. Recently, two other lens intrinsic plasma membrane proteins having apparent molecular masses of 70 kDa (sheep [14–16] and bovine lenses [17]) and 17 kDa (bovine lenses [18]) have also been isolated and characterized. In the present communication we report the isolation and limited amino acid sequence analysis of three low molecular mass lens intrinsic membrane proteins from calf. Of interest was our finding that all three polypeptides had the same amino-terminal amino acid sequence, at least for the first 20 amino acids, indicating that these three polypeptides are genetically related.

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2. MATERIALS AND METHODS

2.1. *Lenses*

Calf eyes were procured from a slaughterhouse within 30 min of decapitation. Lenses were dissected carefully and frozen at −70°C until use.

2.2. *Isolation of lens intrinsic membrane proteins*

Lenses were thawed and the capsules were removed. The decapsulated lenses were homogenized in 50 mM Tris-HCl (pH 7.4), 5 mM EDTA, 10 mM 2-mercaptoethanol, 0.2% (w/v) sodium azide and total lens plasma membranes were isolated

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essentially as per the protocol described by Russell et al. [19]. Alkali-insoluble membranes (containing exclusively intrinsic membrane proteins) were prepared [19] and washed with 5 mM sodium phosphate buffer (pH 8.0) to lower the pH, washed further with deionized H_2O, and lyophilized. Known amounts of membranes (usually 50 to 100 μg) were dissolved in sample buffer and individual polypeptides separated on 0.1% (w/v) SDS/15% (w/v) PAGE mini-gels according to the method of Laemmli [20]. Cortical and nuclear regions of the lens were separated as per previous methods [8], and individual polypeptides were separated on SDS-PAGE as described above.

2.3. *Amino acid sequencing*

Usually 200 to 400 μg of lens membranes were dissolved in 200 μl of sample buffer and separated on preparative 0.1% (w/v) SDS/15% (w/v) PAGE gels. Separated polypeptides were electroblotted onto PVDF membranes (Immobilon, Millipore Corporation, Bedford, MA) and stained with Coomassie blue according to the protocol described by Matsudaira [21]. Desired polypeptide bands were cut out and loaded onto a gas-phase amino acid sequencer (Applied Biosystems) for sequence determination.

2.4. *RNA blot analysis*

Total cellular RNA from calf lenses was isolated using the single-step protocol of Chomczynski and Sacchi [22]. Poly(A)$^+$ RNA was purified using oligo(dT)-cellulose chromatography as outlined by Aviv and Leder [23]. The poly(A)$^+$ RNA was size fractionated on 1% (w/v) agarose gels after denaturation with glyoxal and dimethyl sulfoxide, as described by McMaster and Carmichael [24]. RNA was transferred from the gel to nytran membrane filters (Schleicher & Schuell Inc., Keen, NH) according to the method of Thomas [25]. The membranes were baked for 2 h at 80°C under vacuum and prehybridized by incubation in prehybridization buffer (6 × SSC, 5 × Denhardt's, 0.05% (w/v) sodium pyrophosphate, 0.05% (w/v) SDS, 100 μg/ml of boiled salmon sperm DNA) overnight at 37°C. The membrane was then hybridized in hybridization buffer (6 × SSC, 1 × Denhardt's, 0.05% (w/v) sodium pyrophosphate, 100 μg/ml of yeast tRNA) containing 6–8 × 10^6 cpm of oligonucleotide probe (sequence used was determined from a portion of the MP19 amino acid sequence, table 2) for 48 h at 60°C. End labeling of synthetic oligonucleotide mix with [γ-^{32}P]ATP (spec. act. 3000 Ci/mmol, NEN) was done as per the protocol described by Duby et al. [26]. The membrane was washed in 6 × SSC, 0.05% (w/v) sodium pyrophosphate for 10 min (4 times) at room temperature and for 30 min at 65°C. The membrane was air dried and exposed to Kodak X-Omat AR X-ray film for 2 days with an intensifying screen at −70°C.

3. RESULTS AND DISCUSSION

SDS-PAGE analysis of bovine lens intrinsic membranes revealed four stained bands migrating below the prominent MIP26 band, having molecular masses of 24, 23, 21 and 19 kDa (fig.1, lane d). Limited sequence analysis of the MP24 kDa band, just under MIP26, revealed that this polypeptide was related to MIP26, probably as a degradation product. When lens nuclear and cortical intrinsic membranes were analyzed separately, quantitative differences were noted in the overall pattern of polypeptides between these two regions of the lens (fig.1, lanes b and c, respectively). Bands 23, 21 and 19 kDa were found to be quantitatively 3-fold higher in the nucleus than in the cortex of the lens.

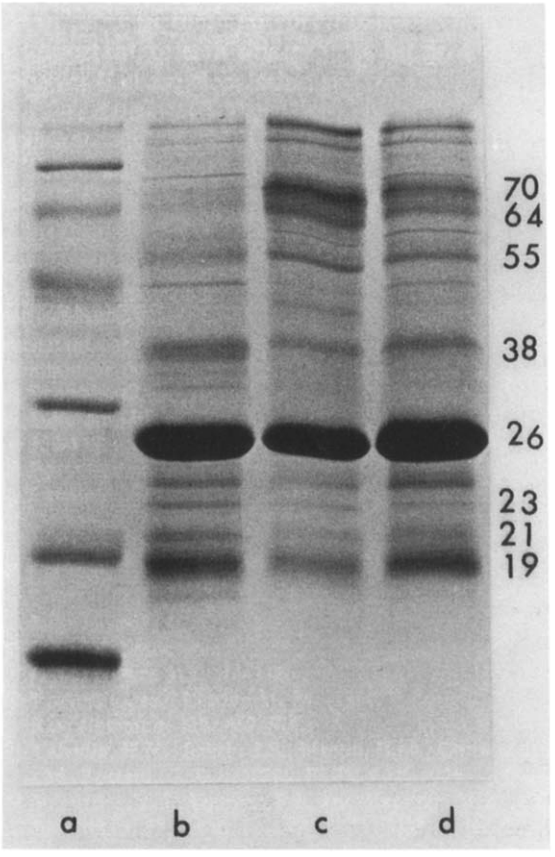

Fig.1. SDS-PAGE analysis of bovine lens intrinsic membrane proteins. Lane a, molecular mass markers (from top: phosphorylase *b*, 97.4 kDa; albumin, 66.2 kDa; ovalbumin, 42.7 kDa; carbonic anhydrase, 31 kDa; trypsin inhibitor, 21.5 kDa; and lysozyme, 14.4 kDa). Alkali-insoluble proteins: lane b, lens nucleus; c, lens cortex; and d, whole lens.

Among the low molecular mass lens intrinsic membrane proteins, MIP26 and MP19 have been well characterized by other investigators [18,27–30]. MP23 and MP21 have been routinely observed by earlier investigators in SDS-PAGE gels of either urea-insoluble or alkali-insoluble lens membrane proteins, however, these polypeptides were identified as αB and αA crystallins [18]. As is evident

from table 1, the amino-terminal end of these peptides was not blocked, whereas the αB and αA crystallins are blocked at the NH_2-terminus [31]. This finding itself indicated that these two peptides are not αB and αA crystallins, as has been reported by other investigators [18]. The most interesting observation among our findings was the 100% identity of amino acid sequences between the 23, 21, and 19 kDa polypeptides (table 1). Mulders et al. [18] have recently characterized a 17 kDa membrane protein from calf lens and found that it was a lens fiber-specific intrinsic membrane protein. The peptide which we have identified as MP19 (19 kDa) in the present study is probably the same as that reported by Mulders et al. [18]. Since these three peptides have the same amino acid sequence, at least for the number of residues determined, we surmised a genetic commonality between the peptides. Are these proteins products of different genes and/or the same gene with possible alternative splicing? In order to address this question, we have made an antisense oligonucleotide common to these three peptides (table 2 shows oligonucleotide used) end-labeled with [^{32}P]ATP and used as a probe for the analysis of lens total poly(A)$^+$ RNA. If the peptides were products of three different genes or a single gene with alternative splicing, then one would expect to identify at least three separate messages on the northern blot. As is evident from fig.2, only one band with an apparent molecular size of 0.8 kb was observed upon lens poly(A)$^+$ RNA blot analysis with the oligonucleotide probe. This result suggests that these three polypeptides are products of a single gene. However, based on our present data, we cannot totally rule out the possibility of either splicing and/or very similar genes because we cannot resolve differences of 50 to 75 nucleotides, which were at most needed for the observed differences in the sizes of polypeptides, in messages on Northern blots. Since these three peptides have identical N-terminal amino acid sequences and apparently a single message, it is possible that the smaller peptides are derived from the larger one by proteolytic degradation from its C-terminal end. Proteolytic degradation is not an unusual event in lens tissue. For example, recently, Kistler et al. [32,33] and ourselves [17] demonstrated a similar proteolytic degradation relationship for MP70, MP64 and MP38. Horwitz and Wong [28] and Alcala et al. [30] have also reported that MP24 is a cleavage product of MIP26. Indeed, our amino acid sequence data for MP24 also revealed that this peptide has resulted due to a C-terminal cleavage from MIP26. A computer search for homologies to known amino acid sequences indicated that MP23, MP21 and MP19 are not related to any proteins so far known, including crystallins and MIP26.

Table 1

Amino-terminal amino acid sequence of bovine lens MP23, MP21, and MP19

	Residue number																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
MP23	Met	Tyr	Ser	Phe	Met	Gly	Gly	Gly	Leu	Phe	Cys	Ala	Trp	Val	Gly	Thr	Ile	Leu	Leu	Val
MP21	Met	Tyr	Ser	Phe	Met	Gly	Gly	Gly	Leu	Phe	Cys	Ala	Trp	Val	Gly	Thr	Ile	Leu	Leu	Val
MP19	Met	Tyr	Ser	Phe	Met	Gly	Gly	Gly	Leu	Phe	Cys	Ala	Trp	Val	Gly	Thr	Ile	Leu	Leu	Val

Table 2

Sequence of antisense oligonucleotide constructed to probe bovine lens mRNA

Peptide		Met	Tyr	Ser	Phe	Met	Gly	Gly	Gly	Leu	Phe	Cys	Ala	Trp	Val	
Codons	5′	ATG	TA$^{T}_{C}$	$^{TC}_{AG}$X	TT$^{T}_{C}$	ATG	GGX	GGX	GGX	$^{C}_{T}$TX	TT$^{T}_{C}$	TG$^{T}_{C}$	GCX	TGG	GTX	3′
Probe	3′	TAC	AT$^{A}_{G}$	III	AA$^{A}_{G}$	TAC	CCI	CCI	CCI	IAI	AA$^{A}_{G}$	AC$^{A}_{G}$	CGI	ACC	CA	5′

All four nucleotides are represented by an X to indicate variations in the third codon position. Inosine (I) was used in several instances to 'fill-in' for variations in codon use

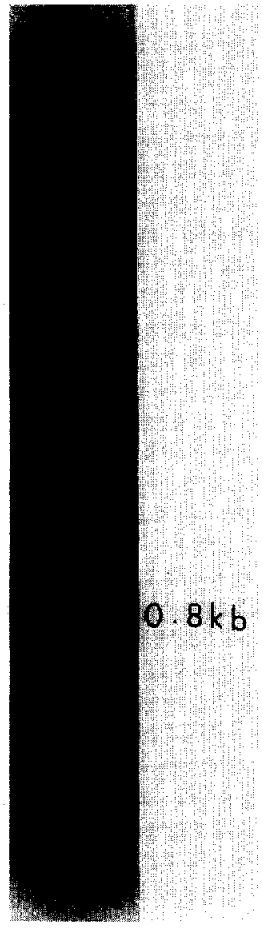

Fig.2. Northern blot analysis of bovine lens poly(A)$^+$ RNA (1 μg).

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