RESEARCH ARTICLE

Baseline Proteomic and Biochemical Characterization of Lens and Aqueous Humour Proteins in Native Sheep: Establishing Molecular References for Early Ocular Disease Detection

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Abstract

Early diagnosis of ocular diseases in livestock is limited due to the lack of baseline molecular reference data for intraocular proteins. The present study aimed to characterize biochemical and proteomic profiles of lens and aqueous humour proteins in healthy sheep and to isolate major crystallin fractions from ovine lens tissues. Eyes from clinically healthy sheep (n = 10) were collected from a local abattoir, and aqueous humour and lens tissues were processed for biochemical and electrophoretic analysis. Total protein concentration was estimated using a colorimetric method, and protein profiling was performed using SDS-polyacrylamide gel electrophoresis (SDS-PAGE). Lens proteins were further purified using DEAE anion-exchange chromatography with sodium chloride gradient elution. The mean total protein concentration was significantly higher in lens homogenates (32.45 ± 1.28 mg/mL) compared with aqueous humour (1.86 ± 0.14 mg/mL), indicating the selective permeability of the blood-aqueous barrier. SDS-PAGE analysis revealed protein bands ranging from 10-55 kDa in aqueous humour and dominant crystallin bands between 18-30 kDa in lens extracts. Chromatographic purification of ovine lens proteins resulted in several fractions, with a prominent band observed at approximately 18.4 kDa in fractions eluted with 0.4 M NaCl. This band corresponded to gamma crystallin based on molecular weight estimation. The findings provide baseline biochemical references for ocular proteins in sheep and demonstrate an effective approach for purification of crystallin proteins from ovine lenses. These molecular insights may facilitate future biomarker discovery for early diagnosis of ocular diseases in livestock.

Key words: Aqueous humour, Lens crystallins, Native sheep, Ocular proteins, SDS-PAGE.

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Introduction

Ocular health is a vital determinant of livestock welfare and productivity, as vision governs essential grazing, social, and predator-avoidance behaviours. Impairments such as keratitis, uveitis, and cataracts are common but often diagnosed only in advanced stages via field examination. Consequently, understanding the biochemical composition of ocular fluids is critical for early biomarker identification (Gelatt, 2014). The aqueous humour maintains ocular homeostasis, regulates pressure, and nourishes avascular tissues. While the blood-aqueous barrier strictly limits protein entry, disturbances like inflammation or oxidative stress alter this composition, making the fluid a prime candidate for molecular disease investigation (Goel *et al.,* 2010). Recent proteomic advances using mass spectrometry have enabled the characterization of aqueous humour proteins involved in immune regulation and metabolic pathways. Differentially expressed proteins identified through these technologies serve as potential diagnostic biomarkers (Du *et al.,* 2024). Specifically, proteomic shifts in diabetic cataracts (Xu *et al.,* 2023) and the application of machine-learning to these datasets (Wang *et al.,* 2024) highlight the diagnostic potential of this fluid.

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The crystalline lens maintains transparency through a highly organized structure of crystallins, which account for 90% of its soluble protein (Bloemendal *et al.,* 2004). These are classified into alpha (α), beta (β), and gamma (γ) families; alpha crystallins act as molecular chaperones, while beta and gamma types ensure structural and optical integrity (Wistow, 2012). Gamma crystallins, specifically, are crucial for refractive power, but their modification or oxidative damage often leads to protein aggregation and cataractogenesis (Truscott, 2005). Despite extensive research in humans, proteomic data for livestock ocular tissues remains limited. Establishing baseline biochemical and electrophoretic profiles in these species is essential for detecting early molecular shifts. Hence, this study was designed to characterize the baseline profiles of aqueous humour and lens proteins in sheep and purify gamma crystallins from bovine lenses using ion-exchange chromatography. These findings aim to establish molecular reference data to support future biomarker discovery for livestock ocular diseases.

Materials and Methods

Sample Collection

The study was made on slaughtered animals; hence formal animal ethical committee approval was not obtained. Eyes from clinically healthy adult sheep (n=10) were obtained immediately after slaughter at a licensed municipal abattoir in Korutla and Hyderabad, Telangana (India). Animals were visually examined before sample collection to ensure the absence of ocular abnormalities. Immediately after enucleation, the eyeballs were rinsed with sterile physiological saline to remove adhering contaminants and transported to the laboratory in ice-cooled containers. All samples were processed within 1 h of collection to prevent protein degradation (Gelatt, 2014).

Collection of Aqueous Humour

Aqueous humour samples were collected aseptically by inserting sterile insulin syringes at the corneoscleral junction under sterile conditions. Approximately 0.2-0.3 mL of aqueous humour was aspirated from each eye. The collected samples were transferred into sterile microcentrifuge tubes and centrifuged at 1,000 x g for 10 min at 4 °C to remove cellular debris. The clarified supernatant was stored at –20 °C until further biochemical analysis (Goel *et al.,* 2010).

Lens Isolation and Preparation of Lens Homogenate

Following aqueous humour collection, the lenses were carefully dissected from the eyeballs under sterile conditions. The isolated lenses were washed with chilled phosphate buffered saline (PBS; 0.025 M, pH 7.0) to remove adhering ocular tissues. The lenses were weighed and homogenized in chilled PBS buffer using a glass homogenizer to obtain a uniform suspension. The homogenates were centrifuged at 12,000 x g for 15 min at 4 °C and the supernatant containing soluble lens proteins was collected for further analysis (Bloemendal *et al.,* 2004).

Estimation of Total Protein and SDS-PAGE Analysis of Ocular Proteins

Total protein concentration in aqueous humour and lens homogenate samples was determined using a standard colorimetric protein estimation method. Bovine serum albumin (BSA) was used as the standard protein for preparation of the calibration curve. Absorbance values were recorded using a UV-visible spectrophotometer and protein concentrations were calculated from the standard curve (Lowry *et al.,* 1951).

Protein profiles of aqueous humour and lens homogenates were analyzed using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) following the method described by Laemmli (1970). Protein samples were mixed with loading buffer containing SDS and β-mercaptoethanol and heated at 95 °C for 5 min to ensure complete denaturation. The samples were then loaded onto polyacrylamide gels and electrophoresed under constant voltage conditions. After electrophoresis, gels were stained with Coomassie Brilliant Blue R-250 to visualize protein bands. Molecular weight markers were used to estimate the molecular weights of the separated proteins.

Purification of Lens Crystallin Proteins using Ion-Exchange Chromatography

Lens proteins were purified using diethylaminoethyl (DEAE) anion-exchange chromatography. The crude lens protein extract was loaded onto a DEAE-cellulose column previously equilibrated with phosphate buffer (pH 7.5). Unbound proteins were washed with equilibration buffer, and bound proteins were eluted using a stepwise sodium chloride gradient ranging from 0.2 M to 2.0 M NaCl. Eluted fractions were collected sequentially and monitored at an absorbance of 280 nm using a spectrophotometer to detect protein peaks (Scopes, 1993).

Electrophoretic Analysis of Chromatographic Fractions

Protein fractions obtained from ion-exchange chromatography were subjected to SDS-PAGE to evaluate purity and determine molecular weight distribution. The presence of distinct protein bands corresponding to crystallin proteins was identified by comparison with molecular weight markers.

Statistical Analysis

Protein concentration values were expressed as Mean ± Standard error (SE). Electrophoretic band patterns were analyzed qualitatively, and molecular weight estimation was performed using standard protein markers.

Results and Discussion

Comparative Analysis of Total Protein Concentration

The biochemical quantification revealed a pronounced contrast in the protein concentration of the two ocular

compartments. The mean protein concentration in lens homogenate was 32.45 ± 1.28 mg/mL, while the aqueous humour showed a significantly lower concentration of 1.86 ± 0.14 mg/mL. The significantly higher protein density in the lens was consistent with the findings of Bloemendal *et al.* (2004), who noted that crystallins must constitute nearly 90% of the soluble protein fraction to maintain the refractive index and transparency necessary for vision. In contrast, the dilute nature of the aqueous humour observed in our sheep samples is justified by the selective permeability of the blood-aqueous barrier. As stated by Goel *et al.* (2010), this barrier strictly regulates protein entry to prevent light scattering while simultaneously supporting nutrient transport to avascular tissues.

Electrophoretic Profiling (SDS-PAGE)

SDS-PAGE analysis (Fig. 1) provided a visual mapping of these protein fractions, revealing distinct banding patterns between the crude and purified samples (Table 1). Lens Proteome (Lanes A & C) of crude extracts displayed multiple bands from 10 to 55 kDa. The dominant, high-intensity band at ~18 kDa was a critical finding. This ~18 kDa fraction likely represents α-crystallins. Our results supported the earlier observation of Horwitz (2003), who identified these as small heat-shock proteins acting as molecular chaperones to prevent protein aggregation. Furthermore, the presence of fractions in the 20-30 kDa range likely encompasses β- and γ-crystallin variants, which is in complete agreement with the established molecular organization of mammalian lenses described by Bloemendal *et al.* (2004). Whereas, aqueous humour proteome (Lanes E & D) profile was characterized by fewer and less intense bands, specifically at 50-55 kDa and 20-21 kDa. The 50-55 kDa band identified in our study likely represents albumin-like proteins. This corresponds with the previous findings of Chowdhury *et al.* (2010) and Saccà *et al.* (2007), which established that many aqueous humour proteins originate from plasma filtration across the ciliary body epithelium. Our observation of lower intensity bands confirms the controlled diffusion mechanisms required to maintain the optical clarity of the intraocular environment.

Densitometric Quantification

Quantitative densitometry confirmed the structural dominance of the ~18 kDa lens band with a normalized intensity of 1.00 ± 0.06 (Table 2). A significant observation in our results was the shared 20-21 kDa band detected in both tissues (0.74 ± 0.03). This indicates a degree of molecular resemblance within the eye. Previous proteomic investigations by Chowdhury *et al.* (2010) and Saccà *et al.* (2007) have demonstrated that certain crystallin fragments or stress-response proteins may diffuse into the aqueous humour from adjacent tissues during normal physiological turnover. Additionally, our detection of low-molecular-weight

Table 1: Lane-wise distribution of protein bands detected by SDS-PAGE in lens and aqueous humour samples of native sheep

Approx. Molecular weight (kDa)	Lane A (Crude lens)	Lane B (Marker)	Lane C (Purified lens)	Lane D (Purified AH)	Lane E (Crude AH)	Interpretation
50-55	+	Protein ladder			+	Albumin-like protein fraction
~50	+	Protein ladder			±	Minor structural protein
25-30	+	Protein ladder			–	Lens crystallin variants
2021	+	Protein ladder			+	β-crystallin / globulin-like fraction
~18	++	Protein ladder	+++	++	++	Conserved intraocular protein
10-15	±	Protein ladder			+	Low molecular weight proteins

Symbols: +++ strong, ++ moderate, + detectable, ± faint, – absent

Table 2: Quantitative densitometric analysis of SDS-PAGE protein bands in lens and aqueous humour proteins of native sheep (Mean ± SE)

Band No.	Molecular weight (kDa)	Sample type	Normalized band intensity*	Probable protein identity
1	50–55	Aqueous humour (AH)	0.82 ± 0.04	Albumin-like protein
2	~50	Lens	0.31 ± 0.03	Structural-associated protein
3	20–25	Aqueous humour	0.56 ± 0.05	Globulin-like proteins
4	20–21	Lens & AH	0.74 ± 0.03	β-crystallin fraction
5	~18	Lens	1.00 ± 0.06	α-crystallin dominant band
6	~18	Aqueous humour	0.48 ± 0.04	Conserved low-MW protein
7	10–15	Aqueous humour	0.29 ± 0.02	Regulatory proteins
8	25–30	Lens	0.67 ± 0.05	Crystallin variants

*Band intensity normalized to total lane density (relative units).

proteins (10-15 kDa) in the aqueous humour (0.29 ± 0.02) justified the earlier work of Richardson *et al.* (2009), who identified similar regulatory peptides and signaling molecules involved in ocular immune modulation and homeostatic signaling.

The electrophoretic patterns observed in native sheep are largely consistent with those reported in other mammalian species, suggesting that the basic molecular architecture of ocular proteins is highly conserved across vertebrates. By establishing these baseline reference data, our study necessitates the potential for bioinformatics and machine-learning to utilize these protein profiles for disease prediction. Ultimately, this characterization provides the molecular reference data necessary for detecting early biochemical shifts in livestock ocular diseases.

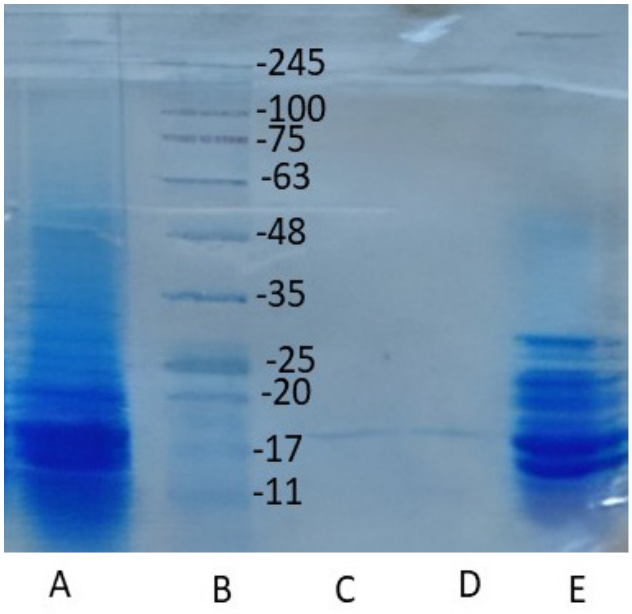

Fig. 1: SDS -PAGE profile of lens and aqueous humour proteins in native sheep. Lane A: Crude lens proteins; Lane B: Protein ladder (Molecular weight marker); Lane C: Purified lens proteins; Lane D: Purified aqueous humour proteins; Lane E: Crude aqueous humour proteins.

Conclusion

The main goal of the current study was to identify the electrophoretic profiles of lens and aqueous humour proteins in native sheep breeds, which clearly showed differences between the two components in terms of protein composition and concentration. The lens was characterized by a high concentration of proteins, including a major protein of ~18 kDa, which is an indicator of crystallins. The aqueous humour, on the other hand, showed a low concentration of proteins, including a few weak bands of proteins involved in transport and regulation. The presence of homologous electrophoretic bands indicates that there is a proteinaceous profile that is common between the components, which could be an indicator of a biochemical interface for inter-tissue nutrient exchange. The results of this study are significant in establishing a reference base for understanding protein composition in the eye and can be a guide for future research on lens physiology and disease states like cataracts in livestock.

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