# Susceptibility of Ovine Lens Crystallins to Proteolytic Cleavage by Calpain during Cataract Formation: A 2-DE Approach

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## Background

In humans, cataracts are a major cause of blindness. As such, extensive research is being pursued to elucidate the exact mechanism by which cataracts form. Currently many people endure risky operations to remove the cataract-affected lens, which impedes vision. An understanding of the process of cataractogenesis may uncover an alternative, less invasive treatment. Crystallin proteins in the lens are known substrates of a family of enzymes known as the calpains. It is proposed that calpain proteolysis of crystallins disrupts their structure and functional organization resulting in the opacification of the lens. A line of sheep at Lincoln University, inherit cataracts and offer a unique opportunity to study the mechanism of cataract initiation and progression in lens (Figure 1). Lens specific calpain, Lp82 and ubiquitous calpain II activity have both been measured in these sheep lenses. Examination of crystallin modification and proteolysis during ovine cataractogenesis may help reveal if calpain is responsible for cataract formation in the sheep. Consequently, the ovine lens may provide an alternative model for cataract research, as the cataract is reliably reproduced and develops over a short time period.

## Purpose

The purpose of this experiment was to identify and map crystallins in the normal ovine lens and to describe crystallin truncations. Truncations subsequently form during cataractogenesis and may be attributed to ubiquitous calpain II and lens specific calpain Lp82.

Figure 1.

## Methods

**Animal Model:**

Coopworth lambs genetically predisposed to cataract formation were bred at Lincoln University. Lambs that have the "cataract gene" develop opacities within the first 8 weeks of life.

**Ovine Crystallin 2-DE Map:**

Lens crystallins were isolated from normal-eyed sheep and homogenised in dissection buffer. The soluble and insoluble proteins were separated by 2-DE using an immobilised pH gradient in the first dimension. Molecular weight separation of proteins from IPG strips was completed on 12% SDS-PAGE gels[1]. Gels containing coomassie stained proteins[2] were imaged on a flatbed scanner and then protein spots were cut from 2-DE gels. Proteins were digested with trypsin and analysed by electrospray ionisation mass spectrometry using an LCQ ion trap mass spectrometer. Peptides from gel digests were separated by reverse phase chromatography and the mass spectra collected during liquid chromatography using a full mass scan to determine the relative abundance of each ion and tandem mass spectrometry (ms/ms) scan on the most abundant ions to collect collision-induced dissociation (CID) spectra. Analysis of CID spectra to determine the amino acid sequence of peptides was performed using Sequest software as described by Yates *et al.* (1999)[3]. The search used a database of all known proteins including all crystallins from human, rat, mouse and cow.

**Cataract Related Crystallin Modifications:**

Normal and cataract lenses were treated as above and the mass of each peptide measured. Modified crystallin masses were predicted and the crystallin modifications during cataract formation identified.

## Results

A 2-DE map of ovine crystallins was created from the soluble cortex proteins of a normal 0.8 g lens approximately 10 months old (Figure 2). The ovine lens possessed the normal complement of crystallin found in other mammals. The identities of each are shown in Figure 2, and are based on the identification of at least 4 peptides matching bovine crystallin peptides during Sequest searches using a database of all known bovine protein sequences.

The insoluble cortex proteins from the same normal lens were also separated using 2-DE. As in the water-soluble fraction, crystallins were the major components of the water-insoluble fraction.

The appearance of multiple spots for several of the crystallins especially βB3 (Figure 2), indicated that post-translational modifications were abundant in the young ovine lens. The highly modified αA-crystallin produced a spot that was more acidic and at slightly higher molecular weight compared to the major αA spot corresponding to phosphorylation. A peptide obtained during the in-gel digestion of this spot was identified by Sequest analysis as peptide 120-145 of bovine αA, containing a phosphorylated serine at residue 122. The phosphorylated form of αA was more abundant in the insoluble fraction when compared with the soluble fraction and was nearly equal in abundance to the unmodified form of αA (Figure 3).

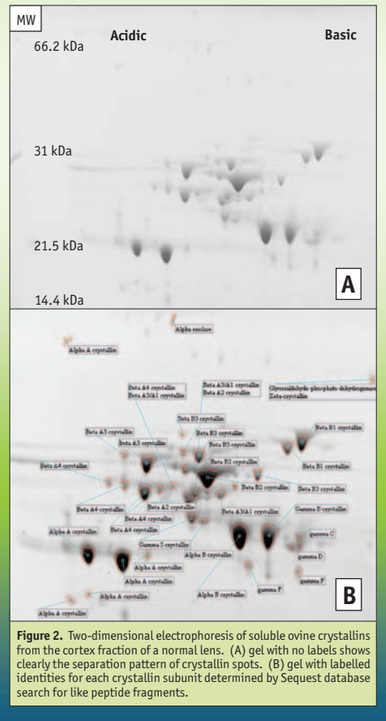


**Figure 2.** Two-dimensional electrophoresis of soluble ovine crystallins from the cortex fraction of a normal lens. (A) gel with no labels shows clearly the separation pattern of crystallin spots. (B) gel with labelled identities for each crystallin subunit determined by Sequest database search for like peptide fragments.

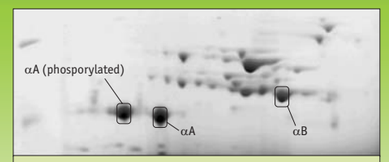


**Figure 3.** Two-dimensional electrophoresis of ovine crystallins from the insoluble cortex fraction of the lens with the identities of αB, αA and αA phosphorylated crystallin spots.

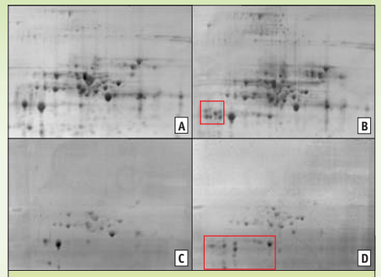


**Figure 4.** Two-dimensional electrophoresis gels of the soluble and insoluble fractions of lenses from lambs with mature cataracts and their age-matched controls (no cataracts). A) total soluble protein pattern from a normal lens. B) total soluble protein pattern for a lens with mature cataracts. C) total insoluble protein pattern for a normal lens. D) total insoluble protein pattern for a lens with mature cataracts. The red boxes indicate α-crystallins in the soluble and insoluble fractions of the mature cataract lens which have been extensively degraded (panels B and D). Truncations to insoluble crystallins in panel D are calpain specific.

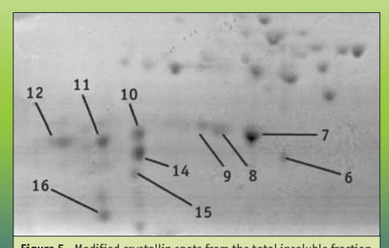


**Figure 5.** Modified crystallin spots from the total insoluble fraction of a mature cataract lens. Each numbered spot corresponds to a different crystallin and these crystallins and their modifications are identified in Table 1. Spots 14 and 15 missing 5 and 11 residues respectively are calpain specific truncations

**Table 1.** The number of amino acids missing from C-terminally truncated crystallins produced in the insoluble fraction of a mature cataract lens. Each spot is numbered in Figure 5. Alpha truncation sites are based on eluted whole mass measurements.

| Spot | Crystallin | Mass | Predicted Mass with modification | Modification |
|---|---|---|---|---|
| 6 | αB | 18,858.9 | 18,859.6 | -12aa |
| 7 | αB | 18,615.3 | 19,614 | -5aa |
| 8 | αB | 18,613.4 | 19,614 | -5aa |
| 9 | αB + $PO_4^{-3}$ | 19,693.1 | 19,694 | -5aa |
| 10 | αA | 19,875.5 | 19,875.1 | intact |
| 11 | αA | 19,446.9 | 19,445.7 | -5aa |
| 12 | αA | 19,446.7 | 19,445.7 | -5aa |
| | αA + $PO_4^{-3}$ | 19,525.7 | 19,525.7 | -5aa |
| 14 | αA | 19,445.9 | 19,445.7 | -5aa |
| 15 | αA | 18,719.9 | 18,718.9 | -11aa |
| 16 | αA | 17,613.5 | 17,613.7 | -22aa |
| | | 17,880.0 | 17,879.0 | -19aa |

2-DE gels of soluble and insoluble lens proteins from sheep with mature hereditary cataracts and normal age matched controls show pronounced differences in crystallin migration for both αA- and αB-crystallin. In normal lens soluble and insoluble fractions αA- and αB-crystallin were relatively intact (Figure 4 panels A and C) compared to the cataract lens soluble and insoluble fractions, where the intact forms of normal and phosphorylated αA-crystallin were decreased in concentration. Concurrently, numerous partially degraded forms of αA-crystallins as well as partially degraded forms of αB-crystallin appeared as a series of smaller protein spots of varying size and pH (Figure 4 panel B and D). Truncation of α-crystallins at their C-terminus was more extensive in the insoluble fraction of cataractous lenses than in the soluble fraction (compare Figure 4 panels B and D). While intact α-crystallin was observed in the soluble fraction of cataract lenses, it was nearly absent from the insoluble fraction.

C-terminally truncated ovine αA-crystallins with masses of 19 446 and 18 720 was observed. These decreases in mass were identical to the decreases observed in bovine and rat αA-crystallins after they were proteolysed by calpain II and Lp82. These truncated α-crystallin species corresponded to masses expected when 5 or 11 residues were removed from the C–terminus by Lp82 and calpain II respectively. This suggests that both Lp82 and calpain II were activated in ovine lenses developing mature cataracts. However, the 2-DE protein spot for the –5 Lp82 specific species was more abundant than the calpain II specific –11 species (Figure 5 and Table 1, spot 14 and 15 respectively). α-Crystallin can function as a molecular chaperone to prevent aggregation and insolubilisation of other lens proteins[4]. Calpain proteolysis may render the protein ineffective as a chaperone and cause increased insolubilisation of other crystallins leading to opacification.

## Conclusion

Crystallins in the normal ovine lens appear similar to those of other mammals. Both calpain II and Lp82 cleavage sites on α-crystallin were found in the insoluble fraction of cataract lenses. However, the greater abundance of truncated αA-crystallin missing 5 residues from its C-terminus, suggested that Lp82 is preferentially activated in ovine lens. This data provides further support for calpain activation in the sheep cataract. It also provides the first evidence for increased Lp82 activation in a non-rodent species during cataract formation.

## References

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