ABSTRACT

Cornea, a highly refractile and avascular tissue, is a part of the outermost coat of the eye. It owes its transparency to the ultrastructural organization of collagen and proteoglycans, the two main structural proteins of the cornea. The uniform diameter of the collagen fibrils and the equal spacing between them results in destructive interference of the scattered light, thus leading to transparency. Proteoglycans are thought to play an important role in maintaining the two parameters of the collagen molecules - diameter and fibrillar distance. Much of the experiments carried out address this issue using methods such as electron microscopy and low angle X-ray diffraction.

The present thesis concentrates on studying the ultrastructural features of the cornea and its components using spectroscopy. Fluorescence is a rather appealing method as it makes it possible to selectively study the proteoglycans which have the tryptophan (Trp) residue as an endogenous fluorescence probe (collagen lacks Trp residues and has very few tyrosine residues). Further, structural studies on proteoglycans using optical spectroscopic methods such as circular dichroism or Raman spectroscopy, in general, poses problems as the amide chromophores of the glycosaminoglycan chains overlap with the amide spectral region of the protein leading to complication in analysing the data. Here, the Trp residues can be used as intrinsic probes to report on the microenvironment, solvation and dynamics of the region around this residue.

Cornea, being the outermost layer, is susceptible to exogenous oxidizing agents apart from endogenous sources. Unlike the eye lens, which turns opaque with age leading to cataract, no such opacity develops in the cornea with age. Besides the various protective mechanisms in the tissue against oxidative agents, the cornea has a regenerative epithelium, though the interior layers, stroma and endothelium have essentially no turnover. The principal line of defense is therefore the epithelium. This work attempts to study the changes in the tissue and its components following oxidative damage, and the role of aldehyde dehydrogenase, a major soluble protein of the corneal epithelium, in combating such an assault.

Chapter I gives an overview of the morphology of the cornea and its ultrastructural organization, along with theories accounting for the transparency of the tissue. The proteins
of the cornea mainly constitute collagen and proteoglycans, the principal extracellular matrix proteins of the corneal stroma. Some of the important features of these molecules are discussed. The effect of ultraviolet radiation on the cornea and the mechanisms to prevent or counteract oxidative damage to the tissue are summarized.

In situ studies of the intact cornea would provide insights on its ultrastructural organization and on the changes accompanying oxidative damage. Chapter II summarizes the results of such a study. The cornea exhibits the phenomenon of red edge excitation shift (REES), indicating a restricted mobility around the Trp residues, with the proteoglycans being the main contributor to such a property. REES has been used as a tool to study the effect of UVB radiation on both intact bovine cornea and isolated proteoglycans. Such in situ studies were extended to human corneas, so as to look for age-dependent changes, if any, in the fluorescence properties of the tissue. Over one hundred human corneas were analyzed using synchronous excitation spectroscopy. In most cases there were no significant changes with age but in some tissues, a highly fluorescent band was seen, which was identified to be a glycosylated product of collagen, based on similar fluorophores which could be generated in vivo in diabetic rats. Preliminary studies on the sclera, which is the opaque continuation of the cornea, were carried out. The tissue could be rendered partially transparent by treating it with buffers of low pH and opacity could be restored by transferring the tissue back to a buffer of neutral or slightly alkaline pH.

Keratan sulphate proteoglycan (KSPG) is one of the major proteoglycans of the cornea. Though its importance in the ultrastructural organization of the tissue is well known, the modes of interaction and the physical properties of the molecule as a whole are not clear. Chapter III explores the conformation, stability and the interactions of KSPG. The conformation of the molecule was probed using the methods of steady state and time resolved fluorescence spectroscopy. KSPG exhibits REES very similar to that of the intact cornea itself, indicating that the Trp residues in KSPG are in a motionally restricted environment. Here, it can be speculated that such a restriction of mobility could be imposed by the glycosaminoglycan chains of the proteoglycans, as one of the Trp residues of KSPG is present in a leucine-rich region very close to a potential glycosylation site. The molecule is hydrophilic in nature but at the same time has significant surface hydrophobicity. The stability of the molecule was assessed by subjecting it to various denaturing conditions. Disulphide
bonds in the molecule were found to be important for stability. Its dual interaction, with collagen on one hand and lipids on the other, indicate it to act more as a filler molecule in the tissue than as a binding glue.

Dermatan sulphate proteoglycan, is the other major proteoglycan of the stroma. Conformational studies of this molecule are presented in Chapter IV. The lone Trp residue of this molecule exhibits a structured emission band but yet is in a polar environment, as indicated by the quenching features of its fluorescence, and the isotope effect on the emission lifetime of the Trp residues when studied by time resolved spectroscopy. Denaturation studies point towards the fact that the emission occurs from two different environments. We suggest that this situation could result from the differential glycosylation of the molecule.

Aldehyde dehydrogenase (ALDH) is the major soluble protein of the cornea. It is also referred to as "transparentin" as its occurrence in the tissue during development parallels the onset of transparency. The precise role of this enzyme in the tissue is not clear, but recent reports speculate that it may have a structural role to play. Chapter V aims at looking for a possible role of this enzyme in the cornea. ALDH was found to prevent hydroxyl radical-induced (oxidative) crosslinking of the test protein RNase A; ALDH itself was found to be rather stable to hydroxyl radical treatment. This protective ability of ALDH towards other proteins seems to arise from the direct quenching of hydroxyl radicals by the free sulphhydryl groups of the Cysteine residues. Blocking the -SH groups leads to loss of this protective ability. ALDH is also thought to prevent UV damage to the tissue by absorbing it. Experiments were hence carried out to look for the effects of UVB radiation on its structure and activity. There was a fall in the activity of the enzyme with increased periods of irradiation, but no significant change in secondary structure was visible. The tertiary, and the oligomeric (quaternary) state of the enzyme, were found to be altered as indicated by changes in the surface hydrophobicity and hydrodynamic properties. Irradiation also results in the formation of high molecular weight covalent crosslinks. Under these conditions, ALDH partially inhibited the photoinduced aggregation of the eye lens f-crystallin. The mechanism by which this occurs may be due to the quenching of hydroxyl radicals generated during UVB radiation, as well as by the availability of exposed hydrophobic surfaces for binding. Thus, ALDH can play an important role in the cornea by preventing oxidative damage to proteins in the cytoplasm.