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Identification of hydrophobic sites on UV irradiated beta crystallins from bovine lenses

The lens of the eye is an avascular tissue of a complex network of proteins surrounded by a capsule. The three major protein families: alpha, beta, and gamma crystallins account for 95% of the lens proteins. These crystallins over time may change from their posttranslational structure due to environmental factors and aging. During aging and cataractogenesis, lens proteins show increased aggregation. To determine the role of hydrophobic sites in lens protein aggregation we performed a beta crystallin aggregation assay and investigated whether there is increased exposure of hydrophobic sites prior to aggregation. Lens proteins were fractionated in a Sephadex G-200 column and the beta crystallin peak was collected. Beta crystallins in 50mM phosphate buffer (pH 7.4) were irradiated for 3 hours at 295 nm. Prior to irradiation and at every half hour interval the 360nm scattering was recorded and an aliquot of the sample was mixed with bis-ANS for the fluorescence to be measured (Ex390/Em490). Both, fluorescence and light scattering increased in parallel during the experiment. However, bis-ANS interaction studies have shown that there is an increased exposure of hydrophobic, bis-ANS binding sites in beta crystallins exposed to UV light, prior to the formation of light scattering protein aggregates. This suggests to us that initial exposure of hydrophobic sites in beta crystallins is necessary for the protein aggregation. Similar results were obtained when the beta crystallins were denatured by chemical method involving Cu^{2+} ions and H_2O_2 . Studies are underway to identify the newly exposed hydrophobic sites in UV-treated beta crystallins.