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ONSET OF OXIDATIVE DAMAGE IN α -CRYSTALLIN BY MASS SPECTROMETRY AND ITS IMPLICATIONS FOR CATARACTOGENESIS

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α -Crystallin is the most abundant protein within the ocular lens of the eye. The oxidative damage to this protein by reactive oxygen species (ROS) is the primary cause of cataract formation. Analysis of lens tissue has revealed that high molecular weight cross-linked proteins as well degradation products accumulate in the eye as the cataract progresses. Yet the underlining mechanisms and nature of these products during cataract development remains incompletely understood.

In collaboration, we have recently developed a new approach with which to probe the structures and interactions of proteins through their treatment with a high flux of radicals on millisecond timescales [1-5]. At these timescales (<50 msec), proteins have shown to be resilient to oxidative damage and their structures remain intact and can be probed. It has been noted, however, that as the reaction time becomes greater the yields of degradation and cross-linked products correspondingly increase (>50-100msec). Thus this biophysical approach, coupled to mass spectrometric analysis, provides a convenient means with which to probe the onset of protein structural damage associated with highly reactive radical species. This in turn enables us to investigate processes of physiological relevance to disease and aging.

This presentation will describe the results of our studies to explore the onset of oxidative damage in the form of protein degradation and cross-linking throughout the subunits of α -crystallin. Our results show that certain hydrophilic domains within each subunit show a high propensity to damage and thus are of importance to the early onset of cataractogenesis.

(1) S.D. Maleknia, M.R. Chance, K.M. Downard (1999) *Rapid Commun. Mass Spectrom.* 13: 2352.

(2) S.D. Maleknia, C.Y. Ralston, M.D. Brenowitz, K.M. Downard, M.R. Chance (2001) *Anal Biochem.*, 289: 103-15.

(3) S.D. Maleknia, K.M. Downard (2001) *Mass Spectrom. Rev.* 20: 388-401.

(4) J.W.H. Wong, S.D. Maleknia, K.M. Downard (2003) *Anal. Chem.* 75: 1557-1563.

(5) S.D. Maleknia, J.W.H. Wong, K.M. Downard (2004) *Photochem. Photobiol. Sci.* 3: 741-748.