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Stability of the βB2B3 crystallin heterodimer to increased oxidation by radical probe and ion mobility mass spectrometry

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ABSTRACT

Ion mobility mass spectrometry was employed to study the structure of the βB2B3-crystallin heterodimer following oxidation through its increased exposure to hydroxyl radicals. The results demonstrate that the heterodimer can withstand limited oxidation through the incorporation of up to some 10 oxygen atoms per subunit protein without any appreciable change to its average collision cross section and thus conformation. These results are in accord with the oxidation levels and timescales applicable to radical probe mass spectrometry (RP-MS) based protein footprinting experiments. Following prolonged exposure, the heterodimer is increasingly degraded through cleavage of the backbone of the subunit crystallins rather than denaturation such that heterodimeric structures with altered conformations and ion mobilities were not detected. However, evidence from measurements of oxidation levels within peptide segments, suggest the presence of some aggregated structure involving C-terminal domain segments of βB3 crystallin across residues 115–126 and 152–166. The results demonstrate, for the first time, the ability of ion mobility in conjunction with RP-MS to investigate the stability of protein complexes to, and the onset of, free radical based oxidative damage that has important implications in cataractogenesis.

1. Introduction

β-Crystallins are water-soluble proteins in the lens of the eye of humans and other vertebrates that play a vital role in helping it to retain its transparency that is important to vision (Slingsby and Clout, 1999; Hejtmancik et al., 2004). They possess different N and C-terminal extensions that have been implicated in promoting self-association and interactions (Trinkl et al., 1994) with other crystallins to form a wide range of oligomers (Bax et al., 1990). These complexes help the subunit proteins to shield themselves from oxidative degradation and damage when exposed to reactive oxygen species produced in large part from the exposure of the eye to ultraviolet light (Soustov et al., 2008). Long-term oxidative damage reduces the transparency of the lens and leads in humans to the development of cataract, a leading cause of blindness worldwide (Srivastava, 1988; Davies, 1990). Inherited mutations in genes that encode β-crystallins have also been associated with the development of human cataract (Lou et al., 2009). Oxidized β-crystallins have been found to be prevalent in aged cataractous lenses and lead to the formation of insoluble, light-scattering protein aggregates (Spector, 1995; Moreau and King, 2012). Initial low molecular weight dimers appear to be the precursors of the higher mass aggregates that are induced upon protein oxidation (Shang et al., 1994).

βB2-crystallin is the most predominant of the β-crystallins and also the most versatile in terms of its ability to interact with other crystallins and self-associate into dimers. Dimerization is an energetically favoured, first step in the formation of β-crystallin complexes (Hejtmancik et al., 1997; Lampi et al., 2006). Two basic β-crystallins, so-called βB2 and βB3 crystallin, have been shown to either self-associate, or associate with one another, to form homodimers and heterodimers (Slingsby and Bateman, 1994). The βB2B3 heterodimer has been detected by mass spectrometry under non-denaturing conditions (Diemer et al., 2009) and this prompted its structure to be probed through the application of radical probe mass spectrometry (RP-MS) (Downard et al., 2011).

Pioneered in the late 1990s (Maleknia et al., 1999, 2001; Maleknia and Downard, 2001, 2007, 2014; Takamoto and Chance, 2006; Konermann et al., 2008), RP-MS involves the exposure of proteins or their complexes to high fluxes of hydroxyl, and other oxygen containing, radicals on low millisecond timescales to effect

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their limited oxidation. The level of oxidation at reactive amino acid residue side chains (Maleknia et al., 1999, 2004), under low oxidation conditions, is commensurate with their accessibility to the bulk solvent providing a means with which to probe the surface of a protein, or establish the degree of shielding conferred to that surface when it binds to another macromolecule.

Previous studies revealed that both the βB2B2 homodimer and the βB2B3 heterodimer were present in a βB_L (beta light) crystallin mixture extracted from bovine lens containing both low molecular weight βB2 and βB3 crystallins (Diemer et al., 2009; Downard et al., 2011). No evidence for a βB3 monomer or βB3B3 homodimer was observed. Thus all βB3 crystallin was determined to preferentially associate with βB2 crystallin (Downard et al., 2011).

A subsequent model for the structure of the βB2B3 heterodimer was constructed through the application of ion mobility mass spectrometry (IM-MS) and homology modeling (Downard et al., 2011). IM-MS provides a measure of the average collision cross section (CCS) of proteins and their complexes on the basis of the time ions take to travel through a high-pressure drift region of an ion mobility mass spectrometer during which they are subjected to multiple collisions with an inert nitrogen gas (Scarff et al., 2008). A close correlation has been found to exist between the CCS of protein complexes and those calculated from X-ray crystallographic data (Jurneczko and Barran, 2011).

We have previously reported on the ability to study the onset of oxidative damage (Shum et al., 2005) by RP-MS and its impact on the interaction of proteins (Issa and Downard, 2006) through their exposure to hydroxyl radicals produced within an electrical discharge source. The time of exposure can be adjusted in this source by varying the flow rate of the protein solution through the discharge needle (Maleknia et al., 1999). At high flow rates proteins and their complexes are oxidized to a limited extent, in accord with the millisecond exposures employed within synchrotron radiolysis experiments, and retain their structure (Wong et al., 2003, 2005). At lower flow rates, proteins are exposed to hydroxyl radicals for longer periods of time resulting in either structural perturbation or oxidative degradation (Shum et al., 2005).

In this study, the impact of increased levels of oxidation on the global structure of the βB2B3 heterodimer was studied by ion mobility mass spectrometry. The impact of that oxidation on the structure of the complex was also examined at a local structural level following proteolytic digestion of the subunit proteins.

2. Materials and methods

2.1. β_L-Crystallin stock

β_L-Crystallin extracted from bovine lens was purchased from Sigma–Aldrich (North Ryde, Sydney, Australia) and used without further purification. It comprises the βB2 and βB3 crystallin proteins with sequences reported in the UniProt database (P02522 and P19141 respectively) in which the N-terminal methionine residues are removed and the N-terminus of each is acetylated.

2.2. Oxidation of β_L-crystallin solutions using an electrical discharge

Solutions of β_L-crystallin (30 μM) in a combination 50 mM ammonium acetate/5 mM ammonium bicarbonate buffer (pH 7) were infused into an electrospray needle assembly at between 5 and 30 μl/min with the needle voltage held at between 6.5 and 7 kV at 30–60 μA by means of a Harvard Apparatus (model 11) syringe pump (Holliston, MA, USA). High purity oxygen was used as a sheath gas and held at a pressure of 250 kPa corresponding to an approximate flow rate of 10 l/min. A visible discharge was observed at the needle tip under low light conditions. The oxidized sample was collected by condensation of the electrosprayed droplets into a grounded aluminum-capped Eppendorf tube and the sample was reconstituted back into the same volume of a solution of 50 mM ammonium acetate.

2.3. SDS–PAGE of oxidized β_L-crystallin solutions

Between 5 and 11 μg of oxidized β_L-crystallin was loaded onto a polyacrylamide gel (5% stacking, 12% resolving) and the gel run in 25 mM Tris, 192 mM glycine with 0.1% sodium dodecyl sulfate using a Biorad Powerpac model 300 power supply.

2.4. Ion mobility mass spectrometry of unoxidized and oxidized βB2B3-crystallin under non-denaturing and denaturing conditions

Nanospray ESI mass spectra and ion mobility spectra were acquired on a Waters Corporation (Manchester, UK) SYNAPT G2 quadrupole ion mobility time-of-flight (Q-IM-TOF) mass spectrometer (Pringle et al., 2007). β_L-Crystallin at between 30 μM in 50 mM ammonium acetate buffer (pH 6.8) was electrosprayed from 8-μm internal diameter HUMANIX nanospray tips (Hiroshima, Japan). A capillary voltage of between 0.7 and 0.9 kV and a sample cone voltage of 20–30 V were typically applied. Ions were transmitted orthogonally through the desolvation interface and quadrupole regions of the instrument, and trapped in an initial traveling-wave (T-wave) ion guide. A second T-wave ion guide was maintained at 3 mbar with nitrogen gas to separate ions on the basis of their conformation and size. The separation of ions according to their mobility can be controlled in this region by altering the maximum voltage (wave height) and velocity of the traveling waves of electric field potentials. Ion mobility measurements were optimized in these experiments with a wave height of 40 V and a velocity of 800 ms^{-1}. Ions were finally directed from a third T-wave ion guide into an orthogonal acceleration time-of-flight mass analyzer. Measured drift times are converted into average collision cross sections (CCS) by using the calibration with the proteins of known CCS values (Ruotolo et al., 2008). All data analysis and processing was performed with MassLynx v4.1 (Waters Corporation, Manchester, UK).

Mass spectra for denatured β_L-crystallin were acquired on the same mass spectrometer following the addition of a 1% formic acid solution and acetonitrile to solutions of unoxidized and oxidized β_L-crystallin at a 1:1:1 ratio by volume.

2.5. MALDI FT-ICR mass spectrometry of the tryptic products of unoxidized and oxidized β_L-crystallin

The unoxidized and oxidized β_L-crystallin was digested with trypsin (Promega, Alexandria, NSW, Australia) and the mass spectra recorded by high resolution FT-ICR mass spectrometry. The digested samples were diluted 1:5 with a MALDI matrix solution (containing 2 mg/ml α-cyano-4-hydroxycinnaminic acid, in 60% acetonitrile, 0.1% TFA) and spotted onto a MALDI target (MTP AnchorChip TM 400/384 TF). Mass spectra were acquired on a 7T Bruker APEX-Qe instrument (Bruker Daltonics, Billerica, MA, USA) in the positive ion mode as previously described (Schwahn et al., 2009). Spectra were averaged from ions accumulated ions from 100 to 200 laser shots per scan. Spectra were recorded for 1 M data points using a broadband excitation. The acquisition mass range was set to m/z 404–4000. A mass resolution of over 100,000 (FWHM) at m/z 1296 was typically achieved. An external mass calibration was applied using a mixture of peptides comprising angiotensin I, adrenocorticotropic hormone residues 18–39, and a synthetic hemagglutinin peptide. Mass spectra were processed using the Bruker Data Analysis v3.4 software (Billerica, MA, USA) software. Mass accuracies of better than 1 ppm were routinely

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