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Oxindolealanine in age-related human cataracts

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Abstract

The present study was performed in order to obtain structural and quantitative information regarding the modifications that take place in the human lens as a result of tryptophan oxidation. In particular, the early tryptophan oxidation product, oxindolealanine (OIA) has been detected in lyophilized and hydrolyzed cataractous lenses by mass spectrometry. OIA was confirmed in human cataract samples by observing its ion (*m*/*z* 221), fragmentation pattern and absorption spectrum. Quantitative results indicate that there are differences in the amounts of OIA in the nucleus versus the cortex in human cataractous lenses. Expressed as a ratio to the level of phenylalanine (Phe), the nucleus has more than one and a half times greater levels of OIA as compared to the cortex [nucleus $= (3.7 \pm 0.7) \times 10^{-2}$ versus cortex $= (2.3 \pm 0.3) \times 10^{-2}$]. Furthermore, the average value for the OIA/Phe ratio in the calf lens (controls) was $(0.8 \pm 0.2) \times 10^{-2}$ as compared to $(3.7 \pm 0.7) \times 10^{-2}$ in human cataractous lens nucleus ($p < 0.05$). The quantitative results correspond to a 4.6-fold increase of OIA in human cataractous lenses. In a separate series of experiments using HPLC with photodiode array (PDA) detection only, the differences in OIA levels in cataract nucleus versus cortex and cataracts versus controls closely matched the LC/MS data. The results suggest that OIA levels are elevated in human cataractous lenses thus providing further evidence to implicate tryptophan oxidation in this process.

Keywords: tryptophan; oxindolealanine; lens cataracts; α-crystallin; photo-oxidation; oxidation; mass spectrometry; HPLC

1. Introduction

During cataract development, human lens proteins undergo numerous post-translational modifications making cataractogenesis a mechanistically complicated process (Young, 1991; Harding, 1991). Previous studies have suggested a number of reaction pathways involved in damage of lens crystallins including formation of reactive oxygen species (Varma et al., 1991), photochemical processes (Ellozy et al., 1994a,b; Gaillard et al., 2000), enzymatic processes (Kamei, 1998) and non-enzymatic processes such as glycosylation (Liang and Rossi, 1990) and nitration (Paik and Dillon, 2000). The resulting structural modifications that have been reported include oxidation of methionine residues (Lund et al., 1996), deamidation of glutamine and asparagine residues (Lund et al., 1996; Lampi et al., 1998; Ma et al., 1998; Miesbauer et al., 1994), formation of disulfide bonds (Lund et al., 1996; Ma et al., 1998; Miesbauer et al., 1994), and cleavage at the C- and N-terminal ends of the crystallins (Lund et al., 1996; Lampi et al., 1998; Ma et al., 1998; Miesbauer et al., 1994; Hanson et al., 2000; Smith et al., 1991). Such modifications are believed to contribute to the progressive aggregation and insolubilization of human lens crystallins (Bron et al., 2000; Truscott, 2005).

Oxidative stress, both light mediated and thermal, is considered to be a major factor in both the aging of the lens and

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cataractogenesis (Ellozy et al., 1994a,b; Garland et al., 1988). Several lines of evidence suggest that oxidative damage to lens proteins plays an important role in the formation of nuclear cataract, including mechanisms related to post-vitrectomy cataract (Barbazetto et al., 2004) and hyperbaric oxygen induced cataract (Palmquist et al., 1984). In addition, high levels of anti-oxidant compounds located in the lens suggest that oxygen may play a deleterious role (Giblin, 2000). With regard to cataractogenesis, lens protein oxidation increases during cataract formation and is concentrated in the lens protein fraction containing the greatest amount of damage, the insoluble fraction. The oxidation is also found to be greater in the nucleus than in the cortex (Young, 1991; Harding, 1991).

Tryptophan, cysteine, and methionine residues are present in lens crystallins and are susceptible to oxidation (Harding, 1991). Of these three amino acids, however, tryptophan can play a key role in lens damage by virtue of its ability to absorb UV light; it absorbs approximately 97% of the high energy UV-B radiation (295–320 nm) that is incident on the lens (Young, 1991). Tryptophan oxidation and photo-oxidation have been suspected to play a role in cataractogenesis for many years and several tryptophan oxidation products have been associated with the insoluble colored proteins from the nucleus of cataractous lenses. These products include kynurenine and its derivatives (Van Heyningen, 1973; Dilley and Pirie, 1974), oxindolealanine (Dillon et al., 1984), and anthranilic acid (Truscott and Augusteyn, 1977a,b; Garcia-Castineiras et al., 1978). Oxindolealanine, which is formed from oxidation of the indole ring of tryptophan prior to ring cleavage, is believed to represent an early intermediate in the degradative pathway of tryptophan-to-kynurenine and its derivatives (Dillon, 1984). The precise mechanisms involved in the formation of such modified compounds remain unknown, although several possibilities are hydroxylation (Finley et al., 1997; Finley et al., 1998a,b; Fu et al., 1998), photo-oxidation (Dillon et al., 1999), deamidation (Takemoto and Boyle, 2000), and non-enzymatic nitration (Paik and Dillon, 2000). In contrast, no appreciable change in phenylalanine content has been associated with either aging or cataractogenesis (Takemoto and Azari, 1976; Ortwerth and Olesen, 1992).

The purpose of the present study was to confirm the presence of OIA in cataracts and to examine whether OIA is elevated in human cataractous lenses. Analyses were performed using electrospray ionization-mass spectrometry (ESI-MS). We detected higher levels of OIA in cataractous lenses versus controls (calf lenses) and observe that OIA content is higher in the cataractous lens nucleus as compared to the cortex. In a separate series of experiments using HPLC with photodiode array (PDA) detection only, similar results were obtained using human age-matched controls. From these experimental observations, we hypothesize that the thermal (not photochemical) oxidation of tryptophan by the reactive oxygen species superoxide, is the predominant pathway for the formation of OIA and that increasing concentration of OIA tracks cataract development.

2. Materials and methods

2.1. Materials

HPLC grade methanol (MeOH) (99.99%), L-tryptophan (L-Trp), α-crystallin (from bovine eye lenses), sodium nitrite and trifluoroacetic acid (TFA) were purchased from Sigma–Aldrich (St. Louis, MO). Constant boiling hydrochloric acid (HCl,6 N) was purchased from Pierce (Rockford, IL); 12.1 N HCl and glacial acetic acid (HAc) were purchased from Fisher Scientific (Fairlawn, NJ). Formic acid (HCOOH) was purchased from J.T. Baker Chemical Co. (Phillipsburg, NJ), and spectrophotometric grade dimethyl sulfoxide (DMSO) (99.99%) was purchased from ACROS Organics (Morris Plains, NJ). Human cataractous lenses were obtained from the Chicago Eye Bank (Midwest Eye Banks and Transplantation Centers) for LC/MS experiments and the NY Eye Bank for the HPLC/PDA experiments. Fresh calf eyes (from animals ≤6 months old) were obtained from a slaughterhouse (Brown Packaging Company, South Holland, IL). All organic solvents were HPLC grade and were purchased from Fisher Scientific (Fairlawn, NJ). Water used in the preparation of all solutions was purified by a Millipore Milli-Q Plus PUREpak 2 (Molsheim, France) water purification system to 18.02 $M\Omega\,cm^{-2}$.

2.2. Synthesis of OIA

OIA was synthesized using the method of van de Weert (Van de Weert et al., 1998; Savige and Fontana, 1976). The experimental details of the synthesis are as follows.

2.2.1. Preparation of DMSO:HCl:HAc mixtures

Two different DMSO:HCl:HAc mixtures were prepared by adding 2 mL of 12.1 N hydrochloric acid (HCl) to Eppendorf microtubes with either 2 or 4 mL of glacial acetic acid (HAc). Dimethyl sulfoxide (DMSO, 400 μL) was then added to the microtubes. The solutions were prepared at room temperature. The two mixtures had the following final compositions: 1:5:5 (v/v/v) DMSO:HCl:HAc and 1:5:10 (v/v/v) DMSO:HCl:HAc. The microtubes were tightly sealed and maintained at room temperature for 1 h.

2.2.2. L-Tryptophan (L-Trp) oxidation with DMSO:HCl:HAc

Four solutions were prepared by mixing 100 or 200 μL of either the 1:5:5 (v/v/v) or the 1:5:10 (v/v/v) DMSO:HCl:HAc mixtures with 200 μg of L-tryptophan. The microtubes with reaction mixtures were kept at room temperature for 4 h to 2 days in open tubes.

All of the solutions were analyzed by LC/ESI-MS (ThermoElectron, San Jose, CA) with a Surveyor HPLC/PDA and Synergi Max-RP C12 analytical column. For direct ESI-MS and tandem (MS/MS) mass spectrometry analyses, standard running buffer (49:49:2; H_2O:ACN:HAc) was added to the reaction mixture prior to injection into the mass spectrometer. OIA was identified as the main product of the reaction through identification of characteristic peaks in full and