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Deaminated UV filter 3-hydroxykynurenine *O*-β-D-glucoside is found in cataractous human lenses

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ABSTRACT

Analysis of UV filter levels in 48 cataractous human lenses was performed with the use of HPLC. A new chromophore with the absorption maximum at 410 nm and molecular mass of 369 Da was detected and assigned as deaminated 3-hydroxykynurenine *O*-β-D-glucoside (3OHCKAG). Cataractous lenses are characterized by the wide range of the UV filter concentrations and remarkably lower levels of UV filters and glutathione than published for the normal lenses. No correlation between the lens age and the level of UV filters has been found in cataractous lenses.

1. Introduction

The human eye lens contains several low molecular weight tryptophan-derived compounds, which absorb UV light in 300–400 nm region of the spectrum, protecting the lens and retina from UV-induced photodamage. The most abundant UV filter compounds are 3-hydroxykynurenine *O*-β-D-glucoside (3OHKG), 4-(2-amino-3-hydroxyphenyl)-4-oxobutanoic acid glucoside (AHBG), and glutathionyl-3-hydroxykynurenine glucoside (GSH–3OHKG), followed by kynurenine (KN) and 3-hydroxykynurenine (3OHKN) (Truscott et al., 1994; van Heyningen, 1973; Wood and Truscott, 1993, 1994). The concentrations of the UV filter compounds vary with age. In young lenses, the levels of 3OHKG, KN and 3OHKN are rather high, whereas GSH–3OHKG is practically absent (Bova et al., 2001). With age, the concentration of the 3OHKG, KN, and 3OHKN decays by approximately 12% per decade, while the concentration of GSH–3OHKG increases, and in old lenses it becomes as high as the concentration of the most abundant UV filter, 3OHKG (Bova et al., 2001). The concentration of every particular UV filter in the lens is determined by the dynamic equilibrium of UV filter formation, transformation and decay (Scheme 1). The first four steps in Scheme 1 are enzymatic reactions. The transformation of tryptophan into *N*-formylkynurenine is catalyzed by indoleamine 2,3-dioxygenase, the hydrolysis of NFK yields KN, the hydroxylation and glycosylation of the later produce 3OHKN and 3OHKG (Moroni, 1999; van Heyningen, 1973). Three of the UV filter compounds, 3OHKG, KN, and 3OHKN, can undergo deamination, which is the key reaction in UV filter evolution. At physiological conditions, the deamination occurs spontaneously (Taylor et al., 2002a; Tsentalovich et al., 2006). The resulting deaminated kynurenines—carboxyketoalkenes (CKA, 3OHCKA, and 3OHCKAG for deaminated KN, 3OHKN, and 3OHKG, respectively)—are highly reactive species. *In vitro* studies demonstrated that CKA readily reacts with

Abbreviations: UV, ultraviolet; HPLC, high-performance liquid chromatography; LC/MS, liquid chromatography/mass spectrometry; ESI, electrospray ionization; APCI, atmospheric pressure chemical ionization; KN, kynurenine; 3OHKN, 3-hydroxykynurenine; NFK, N-formylkynurenine; 3OHKG, 3-hydroxykynurenine *O*-β-D-glucoside; AHBG, 4-(2-amino-3-hydroxyphenyl)-4-oxobutanoic acid *O*-β-D-glucoside; AHBDG, 4-(2-amino-3-hydroxyphenyl)-4-oxobutanoic acid *O*-β-D-diglucoside; AHA, 4-(2-aminophenyl)-4-oxobutanoic acid; AHB, 4-(2-amino-3-hydroxyphenyl)-4-oxobutanoic acid; NADH, β-nicotinamide adenine dinucleotide; GSH, glutathione reduced; Cys, cysteine; His, histidine; Lys, lysine; Cys-3OHKG, cysteinyl-3-hydroxykynurenine *O*-β-D-glucoside; GSH–3OHKG, glutathionyl-3-hydroxykynurenine *O*-β-D-glucoside; CKA, carboxyketoalkene, deaminated kynurenine, 4-(2-aminophenyl)-4-oxocrotonic acid; 3OHCKA, deaminated 3-hydroxykynurenine, 4-(2-amino-3-hydroxyphenyl)-4-oxocrotonic acid; 3OHCKAG, deaminated 3-hydroxykynurenine *O*-β-D-glucoside, 4-(2-amino-3-hydroxyphenyl)-4-oxocrotonic acid *O*-β-D-glucoside; ARN, age-related nuclear.

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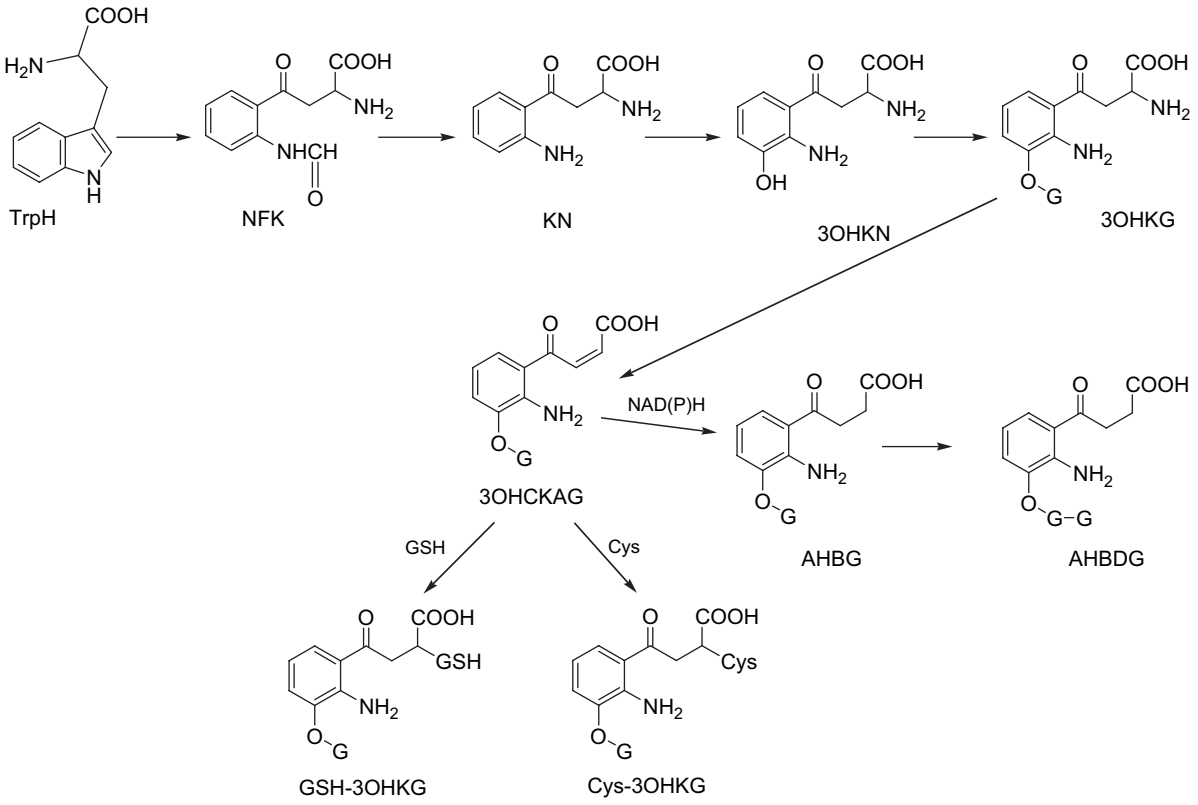

Scheme 1. Evolution of UV filters in the human lens.

antioxidants: NADH reduces CKA to 4-(2-aminophenyl)-4-oxobutanoic acid (AHA), while reduced glutathione adds to CKA forming GSH–CKA adduct (Kopylova et al., 2007; Taylor et al., 2002a,b). CKA may also react with nucleophilic amino acid residues of crystallins—cysteine, histidine and lysine (Aquilina and Truscott, 2000, 2002; Kopylova et al., 2007; Vazquez et al., 2002). The covalent attachment of UV filter compounds to the lens proteins may influence the protein functionality and cause the coloration of crystallins, increasing their susceptibility to the light irradiation. It was suggested that such processes may eventually result in the development of the age-related cataract—a clouding of the natural lens.

Biochemical analysis of normal and cataractous human lenses confirms the reaction chain presented in Scheme 1. Reduced carboxyketoalkenes AHBG, AHA, and 4-(2-amino-3-hydroxyphenyl)-4-oxobutanoic acid (AHB), as well as GSH and Cys adducts to deaminated 3OHKG (GSH–3OHKG and Cys-3OHKG), were found in the protein-free extracts of the human lenses (Bova et al., 1999; Garner et al., 1999; Hains et al., 2006; Hood et al., 1999; Mizdrak et al., 2007). Lens protein modification by kynurenines was revealed in aged human lenses, predominantly at His and Lys sites, and, to a lesser extent, at Cys site (Aquilina and Truscott, 2002; Korlimbinis and Truscott, 2006; Vazquez et al., 2002, 2004). However, the intermediate products of UV filter decomposition, carboxyketoalkenes, have not been detected in the human lens extracts. Here, we report the first observation of deaminated 3OHKG, 3OHCKAG, in the cataractous human lenses.

2. Materials and methods

2.1. Materials and reagents

D,L-Kynurenine (KN), 3-hydroxy-D,L-kynurenine, L-glutathione reduced (GSH), 5,5′-dithio-bis-(2-nitrobenzoic acid), tris-aminomethane and trifluoroacetic acid were purchased from Sigma/Aldrich and used as received. H_2O was doubly distilled. Organic solvents (HPLC grade) were purchased from Cryochrom (Russia) and used as received.

2.2. Synthesis and separation of CKA

CKA synthesis was described earlier (Kopylova et al., 2007). A 5 mM oxygen free aqueous solution of KN (approximately 20 ml, pH 8.3) was incubated for 22 h at 70 °C. The reaction mixture was separated with the use of semi-preparative HPLC. The CKA solutions taken from semi-preparative HPLC were immediately neutralized to pH 7.0 by drop-wise addition of NaOH, and then stored at 4 °C.

2.3. Protein-free lens extracts

Cataractous human lenses (48 lenses in total) were obtained in the Novosibirsk Regional Hospital after surgical removal from patients of the age from 53 to 81 years, the average age being 71 years. The lenses were stored at −72 °C until analysis. The extraction of UV filters was performed by homogenizing the lens in 80% ethanol (0.5 ml/lens). The homogenate was left on ice for 1 h and then centrifuged (15,000 × *g*, 30 min, 4 °C). The pellet was re-extracted with 80% ethanol, the combined supernatants were lyophilized, re-dissolved in 100 μl of water, then analyzed with the use of HPLC and LC/MS methods.

2.4. HPLC and mass spectrometry

HPLC analysis was performed with the use of an Agilent LC 1100 chromatograph equipped with a quaternary pump, an autosampler, and a diode array detector. Chromatographic conditions were: ZORBAX Eclipse XBD-C8 column (4.6 × 150 mm, 5 μm particles);