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UV filter decomposition. A study of reactions of 4-(2-aminophenyl)-4-oxocrotonic acid with amino acids and antioxidants present in the human lens

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Abstract

Deamination of UV filters, such as kynurenine (KN), in the human lens results in protein modification. Thermal reactions of the product of kynurenine deamination, 4-(2-aminophenyl)-4-oxocrotonic acid (CKA), with amino acids (histidine, lysine, methionine, tryptophan, tyrosine, cysteine) and antioxidants (ascorbate, NADH, glutathione reduced) were studied. The rate constants of the reactions under physiological conditions were measured. The rate constants of CKA addition to cysteine $k_{Cys} = 36 \pm 4\ M^{-1}\ s^{-1}$ and to glutathione $k_{GSH} = 2.1 \pm 0.2\ M^{-1}\ s^{-1}$ are 4–5 orders of magnitude higher than the rate constants of CKA reactions with the other amino acids and antioxidants. The Arrhenius parameters for k_{Cys} and k_{GSH} were determined: $A_{GSH} = (1.8 \pm 0.7) \times 10^5\ M^{-1}\ s^{-1}$, $E_{GSH} = 29.2 \pm 5.6\ kJ\ mol^{-1}$, $A_{Cys} = (2.7 \pm 0.9) \times 10^8\ M^{-1}\ s^{-1}$, $E_{Cys} = 40.4 \pm 5.7\ kJ\ mol^{-1}$. The large difference in frequency factors for k_{Cys} and k_{GSH} is attributed to steric hindrance, peculiar to the bulky GSH molecule.

Keywords: kynurenine; UV filter compounds; cataract; deamination; lens protein modification

1. Introduction

Enzymatic transformations of tryptophan (Trp) in the primate lens result in the formation of low molecular weight compounds, playing the role of UV filters: kynurenine (KN), 3-hydroxykynurenine (3OHKN), 3-hydroxykynurenine *O*-β-D-glucoside (3OHKG), and 4-(2-amino-3-hydroxyphenyl)-4-oxobutanoic acid glucoside (AHBG) (van Heyningen, 1973; Wood and Truscott, 1993, 1994; Truscott et al., 1994). These compounds possess an absorption band between 300 and 400 nm. They are characterized by short fluorescence time and low quantum yields of fluorescence, triplet state formation, and active forms of oxygen generation (Dillon and Atherton, 1990; Dillon et al., 1990; Krishna et al., 1991; Tsentalovich et al., 2005). Due to such photochemical properties, these compounds protect the retina and the lens itself from the UV-light induced damage.

With aging, the concentration of UV filter compounds in human lenses decreases (Bova et al., 2001), and the lens proteins become colored, fluorescent, and insoluble (Lerman and Borkman, 1976; Yu et al., 1979). Such processes may contribute to the subsequent development of age-related nuclear (ARN) cataract—a disease which worldwide is the major cause of blindness. ARN cataract is characterized by oxidation, coloration, insolubilization, and cross-linking of proteins in the lens (Dilley and Pirie, 1975; Truscott and Augusteyn, 1977a,b; Harding, 1991). There is no protein turnover in the center of the lens, so the proteins in the lens nuclei are as

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old as the individual, and the protein modifications are accumulating with age. It has been proposed that the modification of the lens proteins is associated with the covalent binding of the UV filter compounds to amino acid residues of proteins (Truscott, 2003, 2005). The linkage of UV filter compound to proteins may proceed either photochemically (Dillon, 1983, 1984; Tomoda et al., 1990; Ellozy et al., 1994a,b; Roberts et al., 2001), or via thermal reactions (Aquilina et al., 1997; Aquilina and Truscott, 2000; Korlimbinis et al., 2006; Taylor et al., 2002a,b; Garner et al., 2000; Malina and Martin, 1995, 1996). It has been shown that three of the UV filter compounds—KN, 3OHKN and 3OHKG—are unstable under physiological conditions, undergoing the reactions of deamination and decarboxylation (Taylor et al., 2002a; Tsentalovich et al., 2006). The rate constant of the former reaction is about 20 fold greater than that of the latter (Tsentalovich et al., 2006), and at 37 °C and pH 7 the rate constant of KN deamination is approximately $1.5 \times 10^{-7}\ s^{-1}$. The deamination product 4-(2-aminophenyl)-4-oxocrotonic acid, or carboxyketoalkene (CKA), is highly reactive species. It can undergo cyclization, yielding kynurenine yellow (KN yellow) (Taylor et al., 2002a), or react with nucleophylic amino acid residues of proteins—cysteine (Cys), histidine (His) and lysine (Lys) (Aquilina and Truscott, 2000, 2002; Vazquez et al., 2002). Antioxidants present in lenses can prevent the reactions of CKA with proteins via reactions of reduction or addition (Garner et al., 1999, 2000; Taylor et al., 2002a,b). In these reactions, new chromophores such as AHBG and glutathione−kynurenine adduct (GSH−KN) are formed (Scheme 1).

Thus, CKA is the key intermediate product of the KN thermal decomposition, and its reactivity is important for understanding the metabolism of UV filter compounds in the human lens. The goals of the present work were to separate CKA as an individual compound and to study its reactions with common amino acids and antioxidants present in the human lens.

2. Materials and methods

2.1. Materials

D,L-Kynurenine (KN), *N*-acetyl-L-tryptophan (Trp), *N*-acetyl-L-tyrosine (Tyr), *N*-acetyl-L-histidine (His), D,L-lysine (Lys), D,L-cysteine (Cys), *N*-acetyl-L-methionine (Met), L-ascorbic acid, β-nicotinamide adenine dinucleotide disodium salt reduced (NADH), L-glutathione reduced (GSH) and trifluoroacetic acid were purchased from the Sigma/Aldrich and used as received. H_2O was doubly distilled. Organic solvents (HPLC grade) were purchased from Cryochrom, Russia and used as received.

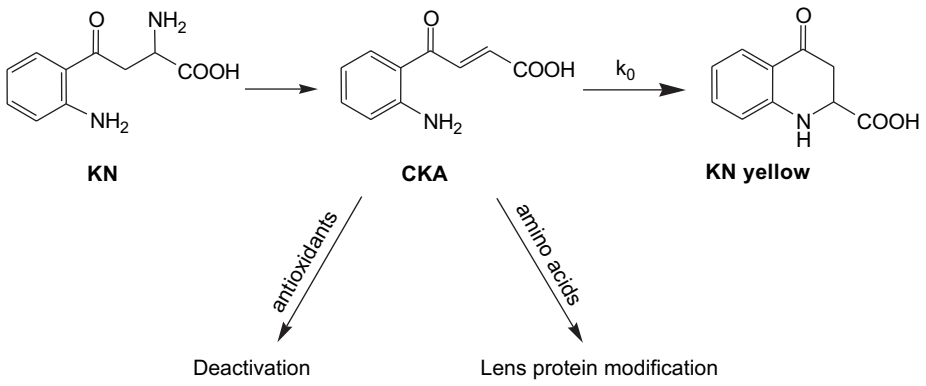
2.2. Incubation

For CKA synthesis, aqueous KN solution (5 mM) was prepared without use of buffers. pH value was adjusted to pH 8.3 by drop-wise addition of NaOH and controlled by an Orion Research pH-meter with a glass electrode before and after incubation. The solution (volume 20 ml) was placed in a glass vial, bubbled with argon, capped, sealed with parafilm, and incubated in a water thermostat at 70 °C.

Aqueous CKA solutions (typically, the concentration of CKA was about 0.5 mM) were prepared using 0.1 M phosphate buffer pH 7.0. The solutions (volume 0.5 ml) placed in glass vials were bubbled with argon, capped, sealed with parafilm, and incubated in a water thermostat at 37 °C. For every incubation time an individual vial was used. All vials were placed in the thermostat simultaneously and removed at various intervals. After incubation 0.25 mM of *N*-acetyl-L-tryptophan was added to every sample, which was used as an internal standard for HPLC. The samples were resealed, stored at 4 °C and then analyzed by HPLC.

2.3. HPLC spectrometry

HPLC separation of the products of KN incubation was performed with the use of an Agilent LC 1200 chromatograph equipped with an automatic gradient pump and a multiple wavelength UV-Vis detector. Separation was performed on a 9.4 × 250 mm ZORBAX Eclipse XBD-C18 Semi-Preparative column using an acetonitrile/0.05% (v/v) TFA in H_2O gradient. The acetonitrile percentage in the gradient was 0−30% (0−2 min), 30−55% (2−32 min), 55−100% (32−34 min), 100% (34−40 min). The flow rate was 0.9 ml/min, and the detection was performed simultaneously at five wavelengths—254, 290, 315, 360 and 410 nm.



Scheme 1. CKA formation and decay under physiological conditions.