FEBS Letters 348 (1994) 173–176

FEBS 14215

A new UV-filter compound in human lenses

Roger J.W. Truscott*, Andrew M. Wood, John A. Carver, Margaret M. Sheil, Glen M. Stutchbury, Jiulin Zhu, Greg W. Kilby

Australian Cataract Research Foundation, University of Wollongong, Northfields Avenue, Wollongong, N.S.W. 2522, Australia

Received 3 June 1994

Abstract

A new UV-filter compound, 4-(2-amino-3-hydroxyphenyl)-4-oxobutanoic acid *O*-glucoside, has been identified in human lenses. The structure suggests that it is derived biosynthetically from tryptophan. Quantification studies on the new compound show that it is the second-most abundant UV-filter compound in the lens with an absorption and fluorescence spectrum similar to that of 3-hydroxykynurenine glucoside.

Key words: Ultraviolet light; Photooxidation; Cataract; Lens; Tryptophan; Filter

1. Introduction

The lenses of many vertebrates contain chemicals which act as UV-filters. In primates, the major component is 3-hydroxykynurenine glucoside (3OHKG) [1] although smaller amounts of kynurenine (Kyn) and 3-hydroxykynurenine (3OHKyn) are also present [2,3]. All of these are derived biosynthetically from tryptophan [2,4] and act to remove light in the 300–400 nm region of the spectrum. Light of wavelengths less than 300 nm does not pass through the cornea.

The function of such compounds may be to protect the lens and retina from UV-induced photodamage or to reduce chromatic aberration, thus sharpening the image on the retina.

The role of UV light in the genesis of senile cataract is still controversial; however, the importance of this factor cannot be considered in isolation. Data on the identification, biosynthesis and breakdown of endogenous human lens UV-filter substances are of obvious importance.

In addition to this aspect, we have also proposed that reactive tryptophan-derived metabolites may be implicated both in the normal age-dependent colouration of the human lens and in crystallin modification during the development of senile cataract [5,6]. In this paper we describe the structural elucidation of a major new UV-filter compound present in human lenses.

2. Materials and methods

Human lenses were obtained from those removed during cataract surgery and also from donor eyes used for corneal grafting.

Lenses were homogenised in 80% ethanol (0.5 ml/lens) and centrifuged (12,000 × *g*, 20 min). The pellet was re-extracted and the combined supernatants lyophilised. The residue was redissolved in water and injected onto a RP-HPLC system.

The HPLC system consisted of a K35D pump (ICI Instruments), a Rheodyne 7125 sample injector and a Knauer Variable Wavelength Monitor. Analytical separations were performed on a 250 mm × 4.6 mm Spherisorb S5ODS2 GoldPak column (Activon Scientific Products Co. Pty. Ltd., Sydney, Australia). The mobile phase was 20 mM sodium acetate/acetic acid buffer pH 4.50 [7] at a flow rate of 0.6 ml · min^{-1}. After 60 min the mobile phase was changed to 20% methanol.

NMR spectra were acquired at 400 MHz and 25°C on a Varian Unity 400 NMR spectrometer. The sample was dissolved in 0.7 ml of D_2O and spectra were referenced to the residual solvent resonance at 4.8 ppm. Two dimensional ^1H NMR experiments (COSY, TQF COSY, TOCSY and NOESY) were acquired with the standard Varian pulse sequences. The removal of the residual solvent resonance was accomplished by transmitter presaturation.

Mass spectrometry experiments were conducted on a VG Quattro mass spectrometer with a hexapole collision cell (VG Biotech, Altrincham, UK). For electrospray ionization (ES-MS) the sample was dissolved in 50% aqueous acetonitrile. Either 1% formic acid (for positive ion MS) or 1% ammonia (for negative ion MS) was added to these solutions. For MS/MS experiments, argon collision gas was used at a pressure corresponding to 30–50% transmission of the incident ion beam. The collision energy was 250 eV.

UV-visible absorption spectra were recorded on a Shimadzu UV-265 recording spectrophotometer. Fluorescence spectra were recorded on an Hitachi F-4500 fluorescence spectrometer. Digestion of lens extracts was carried out using 1.0 mg · ml^{-1} (7IU) β-glucosidase (Sigma) for 3 h at 37°C. Lens incubations with radioactive tryptophan were carried out as described in [2].

3. Results

The HPLC trace of a human lens extract is shown in Fig. 1a. Detection was at 365 nm, a wavelength chosen to enable detection of lenticular UV-filter compounds. Using the initial acetate buffer system, three peaks were observed which correspond to 3OHKyn (peak 1), 3OHKG (peak 2) and Kyn (peak 3) [2].

When the HPLC eluant was changed to 20% methanol, two additional peaks (peaks 4 and 5) were eluted (Fig. 1a). This paper describes the identification of the component (Unknown 1) corresponding to peak 4.

* Corresponding author. Fax: (61) (42) 214 287.

SSDI 0014-5793(94)00601-Q

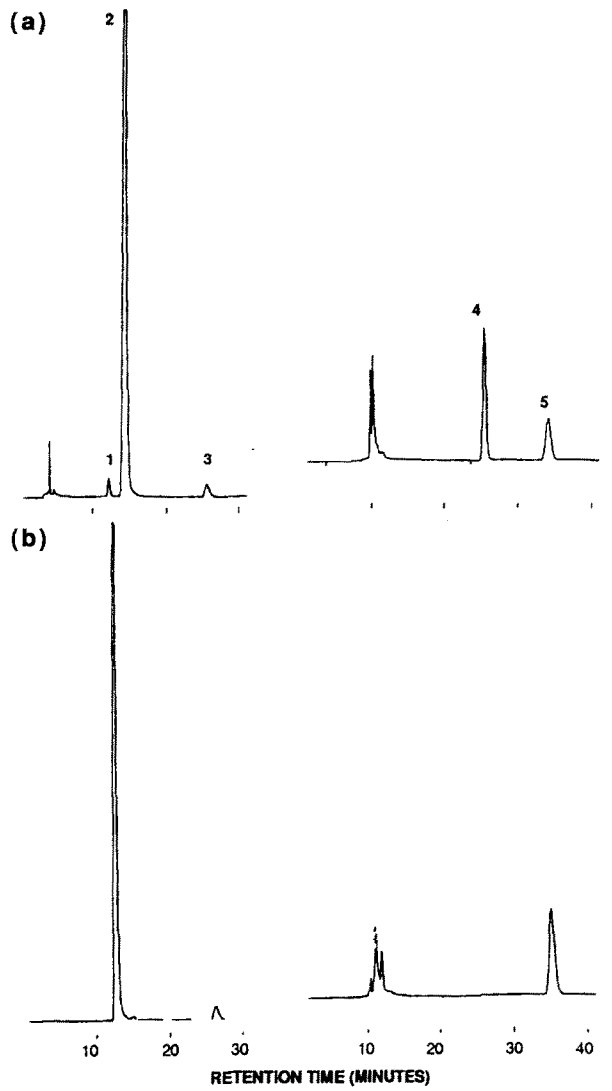

Fig. 1. (a) An extract of a human lens chromatographed by HPLC using acetate buffer (left) followed by 20% methanol (right). UV-detection was at 365 nm. **1** = 3-hydroxykynurenine; **2** = 3-hydroxykynurenine glucoside; **3** = kynurenine; **4** = Unknown 1; **5** = Unknown 2. (b) HPLC profile of an extract from the same lens following treatment with β-glucosidase.

The UV-visible spectrum of Unknown 1, following its collection from HPLC, closely resembled that of 3OHKG (Fig. 2), suggesting a similar chromophoric structure. That Unknown 1 was a glucoside was demonstrated initially by incubating a portion of the lens extract with β-glucosidase [4] and rechromatographing (Fig. 1b). After treatment with the enzyme, 3OHKG (peak 2) was hydrolysed to 3OHKyn and concurrently the retention time of Unknown 1 shifted to that of the second unknown peak.

The UV-visible and fluorescence spectra of Unknown 1, together with that of 3OHKG, are shown in Fig. 2. The spectral characteristics of Unknown 1 and 3OHKG are clearly very similar.

When Unknown 1 was examined by TLC it was found to be fluorescent but, unlike 3OHKG, it did not stain with ninhydrin. This finding suggested that the amino group in the side chain was absent or blocked. Preliminary evidence that the amino group may be missing came from mass spectrometric (MS) investigations. A protonated molecular ion at *m/z* 372 was observed in the positive ion electrospray mass spectrum and a deprotonated molecular ion at *m/z* 370 in the negative ion electrospray spectrum. These data indicate a molecular weight of 371, which is 15 less than that of 3OHKG, and is consistent with the molecular mass of 3OHKG without the α-amino group. The mass spectrometry and NMR data described below are consistent with the structure of Unknown 1 as shown in Fig. 3.

A number of MS/MS experiments were performed on the initial isolates of the unknown. These supported the proposed structure; for example, three intense fragment peaks were observed in the MS/MS spectrum of the $[M-H]^-$ ion (*m/z* 370). These correspond to loss of (a) the glucose (*m/z* 208), (b) the glucose plus the carboxyl group from the side chain (*m/z* 164) and (c) the glucose plus the entire side chain (*m/z* 107).

In order to confirm the structure of the unknown, several hundred human lenses were extracted and approximately 5 mg of the compound purified by HPLC, and subjected to characterisation by one- and two-dimensional NMR spectroscopy. Three isolated spin systems were observed in the NMR spectrum. Firstly, three single proton resonances (δ 6.8t, 7.35d, and 7.75d) were present. Such a pattern is typical of a 3OHKyn derivative [8]. Secondly, an isolated $-CH_2-CH_2-$group (δ 2.6t and 3.3t) and thirdly resonances from a glucose moiety were also noted. The assignments for these spin systems were obtained from a combination of 2D correlation experiments. The assignments of the first two of the above spin systems was readily achieved from a COSY spectrum. However, because of the overlapping nature of the seven proton resonances from the glucose group, additional correlation experiments were required to facilitate their assignment. A TOCSY experiment with a mixing time of 85 ms enabled the connectivities to be traced out from the anomeric proton (H-1′) through to the two H-6′ protons [9]. The identity of the three-spin H-5′, H-6′ system of glucose was confirmed by the appearance of strong cross-peaks between these resonances in a triple-quantum-filtered COSY experiment [9].

The three spin systems were linked together via a NOESY experiment (mixing time 300 ms). Strong nOes were observed across the glycosidic linkage from H-1′ to H-4 of the aromatic ring and from the other aromatic doublet (H-6) to the downfield (δ 3.3)-CH_2-resonance. These nOes are consistent with the structure in Fig. 3. The name of this compound is 4-(2-amino-3-hydroxyphenyl)-4-oxobutanoic acid *O*-glucoside (AHBG).

Quantification of AHBG in individual human lenses

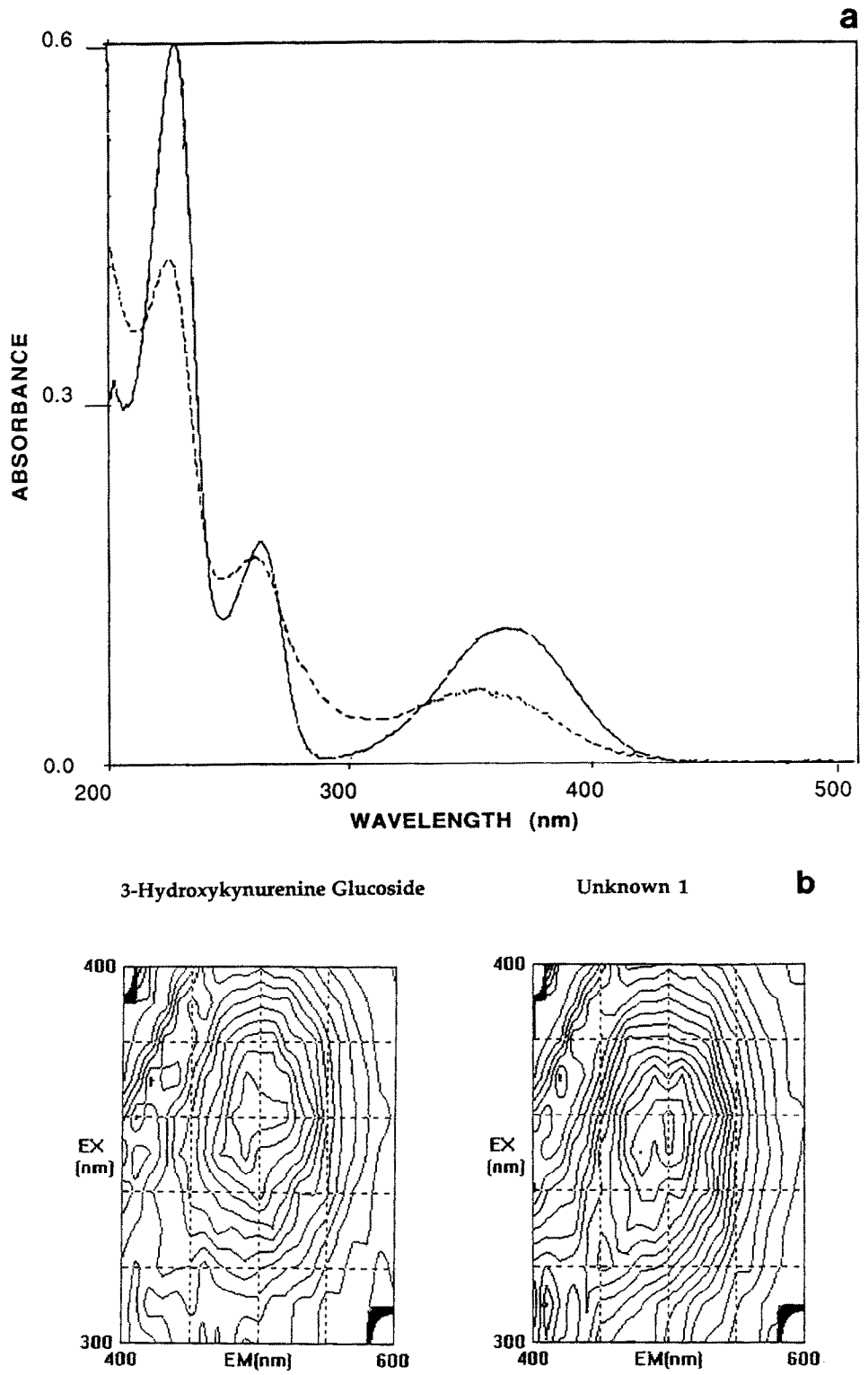

Fig. 2. (a) UV-spectra of 3OHKG (—) [λ_{max} 365, 264 nm] and Unknown 1 (---) [λ_{max} 358, 262 nm]. (b) 3D fluorescence spectra of 3OHKG (Ex 360 nm/Em500 nm) and Unknown 1 (Ex 357 nm/Em500 nm). Compounds were collected from HPLC of a lens extract.

of different ages revealed a strong correlation with that of the concentration of 3OHKG (data not shown). This may suggest a common biosynthetic origin and the structure of AHBG is consistent with that of a metabolite of tryptophan. These studies show that AHBG is, in fact, the second most abundant of the UV-filter compounds present in the lens (0.33–2.61 μmol/g lens protein; $n = 8$). 3OHKG is present in the highest concentration (0.97–

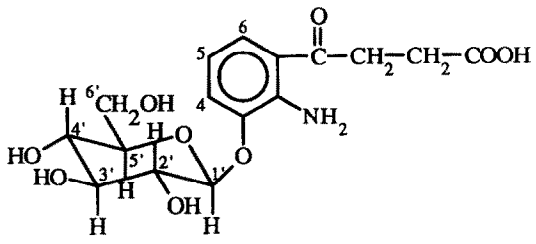

Fig. 3. The structure of Unknown 1.

7.22 μmol/g lens protein; $n = 8$). The values for 3OHKG are consistent with those obtained by Bando et al. [10].

4. Discussion

The effectiveness of the UV-filter components present in the human lens can be readily demonstrated. Aphakic individuals can see clearly in a room illuminated by light of 365 nm where those whose eyes contain lenses see nothing at all [11]. Thus, our perception of the world, which is governed to a large extent by our sense of sight, is altered significantly by the presence of these chemicals. Their possible role as UV-protective agents may also be of importance given that the wavelength profile and intensity of UV light impinging upon the earth's surface is being altered as a result of ozone depletion in the atmosphere.

van Heyningen [1] was the first to elucidate the structure of 3OHKG, the major UV-filter compound in human lenses. This compound is made by the lens from the essential amino acid, tryptophan. The activity of this pathway, as judged by the incorporation of radioactivity from tryptophan into 3OHKG in intact cultured lenses, is quite high. Half-lives for lens 3OHKG of between 19 and 110 h were calculated [2].

Surprisingly we have not yet been able to demonstrate incorporation of radioactivity from tryptophan into AHBG. In her pioneering studies on the human lens UV-filter pathway, van Heyningen [4] also reported the presence in the lens of an unknown glucoside which she called F1. F1 was fluorescent, yet appeared to contain no α-amino group. F1 did not become labelled after incubation of lenses in either radioactive tryptophan or glucose. The electrophoretic mobility of AHBG that we have obtained on paper at pH 1.9 suggests that the F1 observed by van Heyningen was, in fact, AHBG. It is not clear why AHBG remains unlabelled following incubation of lenses with radiolabelled tryptophan. This may indicate either that the rate of formation of AHBG is very slow or that tryptophan is not the precursor.

The type of reversed-phase HPLC column used in the analytical separation was found to be important since AHBG did not appear to elute from some of the columns tested. The reason for this is not immediately apparent. We do not yet know the identity of the other UV-filter compound (peak 5) which is eluted by 20% methanol.

Work is underway to discover the biosynthetic pathway leading to the formation of AHBG and also to purify sufficient quantities of Unknown 2 to enable its structural elucidation.

Acknowledgements: The authors would like to thank Dr P. Penfold and Ms. T. Balind who provided lenses obtained from the Lions NSW Eye Bank. The support of AMRAD Corpn. is gratefully acknowledged.

References

[1] van Heyningen, R. (1971) Nature 230, 393–394.
[2] Wood, A.M. and Truscott, R.J.W. (1993) Exp. Eye Res. 56, 317–325.
[3] Wood, A.M. and Truscott, R.J.W. (1994) Vision Res. 34, 1369–1374.
[4] van Heyningen, R. (1973) in: The Human Lens in Relation to Cataract, Ciba Foundation Symp. 19, pp. 151–171, Elsevier, Amsterdam.
[5] Truscott, R.J.W., Pyne, S.G. and Manthey, M.K. (1991) Lens Eye Toxicity Res. 8, 251–257.
[6] Stutchbury, G. and Truscott, R.J.W. (1993) Exp. Eye Res. 57, 149–155.
[7] Elderfield, A.J., Truscott, R.J.W., Gan, I.E.T. and Schier, G.M. (1989) J. Chromatogr. 495, 71–80.
[8] Truscott, R.J.W., Carver, J.A., Thorpe, A. and Douglas R.H. (1992) Exp. Eye Res. 54, 1015–1017.
[9] Homans, S.W. (1994) in: NMR of Macromolecules, A Practical Approach (Roberts, G.C.K. Ed.) Oxford University Press, Oxford.
[10] Bando, M., Nakajima, A. and Satoh, K. (1981) J. Biochem. 89, 103–109.
[11] Wald, G. (1952) J. Opt. Soc. Am. 42, 171–177.