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Characterisation of a novel UV filter in the lens of the thirteen-lined ground squirrel (*Ictidomys tridecemlineatus*)

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ABSTRACT

Structural analysis of a novel UV filter present in the lens of the thirteen-lined ground squirrel has shown that it is related in structure to *N*-acetyl-3-hydroxykynurenine. This finding is consistent with the fact that the squirrel lenses also contain high levels of this tryptophan metabolite. Analysis of both NMR and mass spectrometric data suggested that the novel UV filter compound forms by condensation of proline with *N*-acetyl-3-hydroxykynurenine. Its absorption maximum at 340 nm is more than 20 nm lower than that of the kynurenines and it may therefore assist in filtering the more damaging shorter wavelengths of UVA.

1. Introduction

The lenses of humans, and other animals, contain low molecular weight compounds that act as UV filters by absorbing light in the 300–400 nm range (Heyningen, 1973; Cooper and Robson, 1969; Thorpe and Douglas, 1993). In primates these are tryptophan-derived kynurenines that prevent UV-induced photodamage to the lens and retina (Zigman and Paxhia, 1988; Dillon et al., 1990). Since UV light has frequently been suggested to be involved in human cataract (Taylor, 1999; Balasubramanian, 2005) it is ideal, in order to study it experimentally, to have an animal model whose lenses contain UV filters similar to those of humans. Commonly used animal models, such as rats and mice, are poor choices for such UV irradiation as they can see UV light and presumably lack UV filters in their lenses (Truscott, 2005; Jacobs et al., 1991).

Due to the similarity of the UV filters present in the thirteen-lined ground (TLG) squirrel (*Ictidomys tridecemlineatus*) – formerly classified as *Spermophilus tridecemlineatus* (Hains et al., 2006) to those found in humans, this species could act as an appropriate animal model for investigating the effects of UV radiation on cataract, and other ocular diseases, thought to involve exposure to light. It is of interest that squirrel lenses contain *N*-acetylated derivatives of kynurenine and 3-hydroxykynurenine, whereas in primates, the comparable UV filters have free alpha amino groups. Since spontaneous loss of the alpha amino group and formation of reactive enones (Taylor et al., 2002) is a key process involved in binding of UV filters to human crystallins in older lenses (Korlimbinis and Truscott, 2006; Parker et al., 2007; Korlimbinis et al., 2007), squirrel lenses should not be subject to these same posttranslational modifications.

Previous work by Hains et al. (Hains et al., 2006) showed that *N*-acetyl-3-hydroxykynurenine (*N*-acetyl-3OHKyn) and *N*-acetylkynurenine (*N*-acetyl-Kyn) were the major UV filters found in the lens of the TLG squirrel. An additional UV filter, whose structure remained to be elucidated, was also detected. This paper documents its characterisation and structural assignment.

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2. Materials and methods

2.1. Lens extraction

Adult thirteen-lined ground squirrels were obtained from TLS Research (Bloomington, IL, USA) with ethical approval from the Oakland University Institutional Animal Care and Use Committee, protocol #04081. The gender of the animals was not determined. Since the animals were wild-trapped, their exact ages were not known. The supplier estimated ages of 1–3 years. Following euthanasia of the animals, lenses (27 mg wet wt. each) were removed from the eyes. UV filters were extracted by homogenising each lens in 300 μL of 100% (v/v) ethanol, leaving at room temperature for 1 h, centrifuging for 10 min at 12 000× *g* and re-extracting two times in 300 μL 80% (v/v) ethanol and treated as above. The supernatant was observed to be pale yellow in colour, and the precipitated protein was white. The supernatants were pooled and lyophilised. The dried extracts were then couriered to Australia.

2.2. HPLC purification

Combined extracts from 18 TLG squirrel lenses were dissolved in water (0.1% TFA) and injected into a Prominence HPLC system (Shimadzu Corporation, Japan) equipped with a UV–visible detector (SPD-20A) and a fraction collector. Purification was achieved using a Kinetex™ 100 mm × 4.6 mm × 2.6 μm, 100 Å C18 column (Phenomenex Australia) at ambient temperature and the eluent was monitored at 360 nm and 280 nm. The gradient was 100% water (0.1% TFA) to 50% acetonitrile (0.1% TFA) over 50 min with a flow rate of 1 mL/min.

2.3. UPLC analysis

UPLC analyses were performed using an ACQUITY UPLC System fitted with an ACUITY UPLC BEH 100 mm × 2.1 mm × 1.7 μm C18 column (Waters, Milford, MA). The gradient was 100% water (0.1% TFA) to 100% acetonitrile (0.1% TFA) with a flow rate of 200 μL/min over 3 min.

2.4. High-resolution mass spectrometry (MS) and MS/MS

High mass accuracy analysis and MS/MS fragmentation was performed using a Thermo Scientific LTQ *Orbitrap XL* hybrid FTMS (Fourier Transform Mass Spectrometer) operating in positive ion (electrospray) mode. The orbitrap instrument allows MS/MS^n that enables individual fragment ions to be isolated in the instrument and characterised individually. Prior to analysis the system was calibrated and the resolution was shown to be within an error of 2 ppm.

2.5. NMR spectroscopy

2.5.1. H_2O/D_2O analysis

Samples were prepared in a mixture of 90 μL H_2O and 10 μL D_2O. Spectra were acquired at 25 °C on a Bruker Avance III 800 MHz narrow-bore NMR spectrometer equipped with a high-resolution cryogenic TCI probe-head. Water suppression was achieved using pulsed-field gradients and the WATERGATE sequence. A reduced-volume D_2O-matched (Shigemi) NMR tube (100 μL) was used for all experiments. The following NMR experiments were acquired using standard Bruker pulse sequences: 1D ^1H, 2D ^1H–COSY, 2D ^{13}C–^1H HSQC and ^{13}C–^1H HMBC.

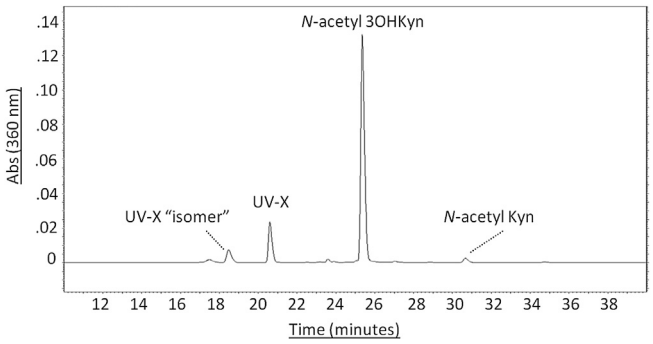

Fig. 1. HPLC trace of the UV filters present in the lens extract of the TLG squirrel. Detection at 360 nm.

2.5.2. DMSO analysis

Samples were dissolved in 100 μL deuterated DMSO-d6 in a reduced-volume DMSO-d6 matched (Shigemi) NMR tube (100 μL). 20% d6-benzene was added and the sample was analysed again.

3. Results

3.1. Purification by semi-preparative HPLC and analysis by FTMS

Previous work by Hains et al. revealed that the major UV filters present in the lens of the thirteen-lined ground squirrel were *N*-

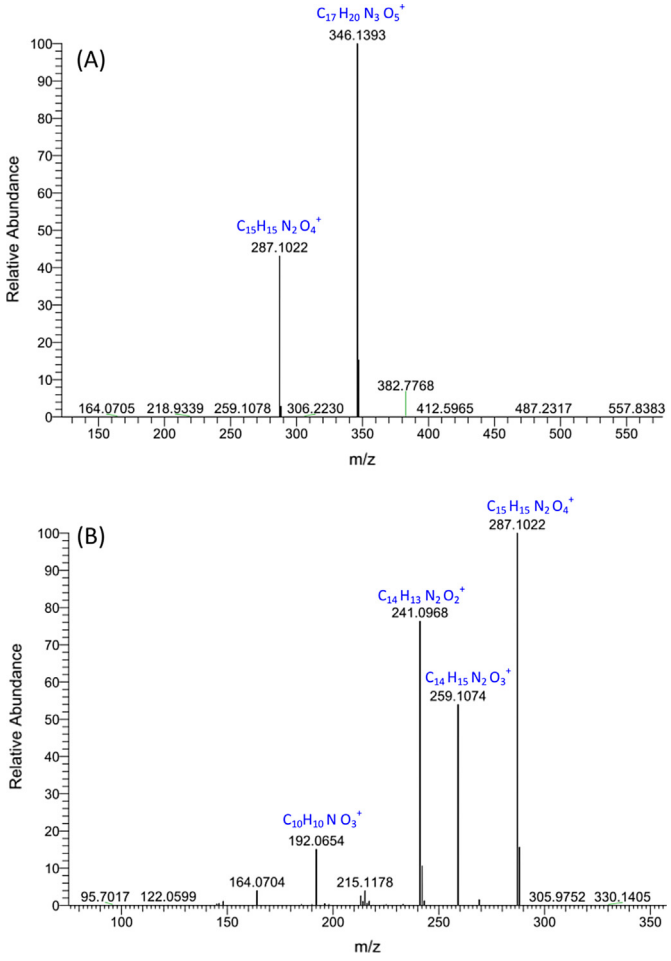

Fig. 2. Analysis of UV–X by mass spectrometry. (A) High resolution MS/MS fragmentation of the *m/z* 346.1393 ion, (B) MS/MS/MS analysis of the *m/z* 287.1022 ion.