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Protein-bound and free UV filters in cataract lenses. The concentration of UV filters is much lower than in normal lenses

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Abstract

In human cataract lenses the UV filters, 3-hydroxykynurenine glucoside (3OHKG) and kynurenine (Kyn) were found to be covalently bound to proteins and the levels in the nucleus were much higher than in the cortex. The levels of the bound UV filters in cataract nuclei were much lower than those in age-matched normal lenses. 3-Hydroxykynurenine could not be detected in cataract lenses. As with normal lenses, protein-bound 3OHKG in cataract lenses was found at the highest levels followed by Kyn. Free UV filter concentrations were also markedly reduced in cataract lenses. This feature may well contribute to the lower protein-bound levels; however, there was no clear relationship between free and bound UV filter contents when individual lenses were examined. We propose that since cysteine is a major site for UV filter binding, the well-documented oxidation of protein sulfhydryl groups during the progression of nuclear cataract may account, in part, for the pronounced decrease in bound UV filters in cataract lenses.
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Keywords: cataract; lens; proteins

1. Introduction

Human lenses synthesize 3-hydroxykynurenine glucoside (3OHKG) as a UV filter from the amino acid tryptophan (Trp) (van Heyningen, 1971). The immediate precursors, kynurenine (Kyn) and 3-hydroxykynurenine (3OHKyn) are also present in lenses at measurable levels.

All three Trp metabolites are unstable at physiological pH and deaminate to produce α,β-unsaturated ketones that are subject to nucleophilic attack (Taylor et al., 2002a). In lenses that contain sufficient glutathione (GSH) (Bova et al., 2001) or cysteine (Cys), (Bova et al., 2001; Dickerson and Lou, 1997), the thiol groups of these antioxidants react with the unsaturated ketones (Garner et al., 1999) before they bind to proteins (Taylor et al., 2002b). It is likely that the UV filter adducts thus formed, can diffuse out of the lens as has been found for other UV filters in cultured human lenses (Wood and Truscott, 1993). In the lens, once the barrier to diffusion forms at middle age, (Moffat et al., 1999; Sweeney and Truscott, 1998; Truscott, 2000) the nucleus becomes a partially uncoupled region in which metabolites spend a longer time than in the young lens. Thus decomposition of intrinsically unstable molecules is favoured in older lens nuclei. Coupled with a diminished flux of GSH from the cortex, this results in increased covalent binding of UV filters, and other reactive small molecules, to nuclear proteins after middle age (Hood et al., 1999; Korlimbinis and Truscott, 2006; Vazquez et al., 2002).

In this study we examined the levels of all three bound UV filters in cataract lens proteins using a novel assay system in which lens proteins were incubated with excess GSH at pH 9.5 (Korlimbinis and Truscott, 2006). Under these conditions, the UV filters that are attached to proteins are released, and the GSH adducts thus formed can be quantified by HPLC.

It has been proposed that the color of human age-related nuclear (ARN) cataract lenses may be due to the oxidation

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of bound compounds such as UV filters (Aquilina and Truscott, 2000, 2001; Garner et al., 2000; Hood et al., 1999; Korlimbinis and Truscott, 2006). If this were true, it would be expected that protein-bound UV filters, particularly compounds like 3OHKyn that are susceptible to oxidation, might be lower in cataract lenses. It was therefore of interest to compare the levels of bound, and free, UV filters in individual cataract lenses and to compare these data with those obtained from normal lenses. The implications of this for the genesis of ARN cataract are discussed.

2. Materials and methods

2.1. Materials

Milli-Q® water (purified to 18.2 MΩ/cm^2) was used in the preparation of all solutions. All organic solvents were HPLC grade (Ajax, Auburn, NSW, Australia). 3OHKyn, reduced GSH, trifluoroacetic acid (TFA), and guanidine HCl were obtained from Sigma–Aldrich Chemical Co. (St. Louis, MO, U.S.A.). ARN cataract lenses were obtained from K.T. Sheth Eye Hospital, Rajkot, Gujarat, India. Lenses were removed and the Tenets of the Declaration of Helsinki for dealing with human samples were followed. Ethics approval was obtained from the University of Sydney and the University of Wollongong. Twenty human cataract lenses in the age range 55–101 years were examined. Data were compared with that from 15 normal human lenses above age 50 (age range from 50 to 83) (Korlimbinis et al., 2007). All lenses were stored at −80 °C in Australia, and at −20 °C in India, prior to shipment to Australia. Each lens was separated into the nucleus and cortex using a cork borer (5 mm), and the ends (∼1 mm) of the nuclear core were removed. Ten dark (Type III/IV Pirie classification, Pirie, 1968) and 10 light (Type I/II Pirie classification, Pirie, 1968) cataract lenses, covering the age range 55–101 years, were analyzed.

2.2. Reversed-phase HPLC (RP-HPLC)

RP-HPLC was performed on a Shimadzu system (Kyoto, Japan). For analytical scale separations, a Phenomenex column (Jupiter, 5 μm, C18, 300 Å, 250 × 4.6 mm) was used with the following mobile phase conditions: solvent A (aqueous 0.1% v/v TFA) for 5 min followed by a linear gradient of 0–50% solvent B (80% v/v acetonitrile/H_2O, 0.1% v/v TFA) over 20 min, followed by a linear gradient of 50–100% B over 15 min and re-equilibration in the aqueous phase for 15 min. The flow rate was 0.5 mL/min and with detection at 360 nm.

2.3. Extraction of free UV filters from lenses

Each nucleus and cortex was homogenized (Bova et al., 2001) with 100% ethanol (300 μL) and centrifuged (10 000*g*, 10 °C, 20 min). The precipitate was further extracted with 80% ethanol and then centrifuged (10 000*g*, 10 °C, 20 min). The supernatant was collected, and combined with that from the first extraction. The ethanol extracts for each of the nuclei and cortices were dried down and resuspended in 0.1% (v/v) TFA and analyzed by HPLC for levels of free UV filters.

2.4. Quantification of protein-bound UV filters

The lyophilised protein from each extracted cortex and nucleus was weighed, then dissolved in 6 M guanidine HCl, pH 5.5 (2 mL) and dialyzed overnight against 0.1 M sodium acetate/acetic acid buffer pH 4, with several changes to ensure the removal of all non-covalently bound material. The lyophilised protein (∼15 mg) and reduced GSH (100 mg) were dissolved in 6 M guanidine HCl (0.4 mL) and 50 mM Na_2CO_3/$NaHCO_3$ buffer, pH 9.5 (1.1 mL). The pH was readjusted to 9.5 with 6 M NaOH, and the tube was sealed, and incubated for 4 h at 37 °C. The final concentration of GSH was ∼200 mM to ensure the trapping of all released UV filter compounds. After adjusting the pH to less than 5 with acetic acid, the solution was centrifuged (6000*g*, 4 °C, 60 min) in a Vivaspin concentrator (10 000 MW cut off) to separate the non-covalently bound material (filtrate) from the protein. The filtrate was examined by HPLC. Standard curves were used for quantification of UV filters. Values are expressed per mg of protein on the basis of the weight of dried lens tissue following dialysis. In order to check the efficiency of extraction, four nuclei and cortices from normal lenses and four similar tissues from dark cataract lenses were extracted separately using the protocol outlined above. The protein from the first extraction was then re-extracted as described above. In the second extract levels of Kyn and 3OHKyn were below the levels of detection; however, 3OHKG could be quantified. Of the total bound 3OHKG, 9.8 ± 4.2% was present in the second extract of normal lenses, and 9.9 ± 5.9% in cataract lenses. In the third extract the levels were too low to quantify.

2.5. Statistical analysis

Statistical analysis was performed using JMP 5.1 Software. A one-way ANOVA analysis of variance was employed to compare the data points obtained for the normal lenses with each of the dark and light cataract lenses. $P < 0.01$ was considered significant.

3. Results

The major aim of this investigation was to quantify protein-bound UV filters in the nucleus and cortex of individual cataractous lenses and to compare the values with those from normal lenses. In addition, we compared the levels of protein-bound UV filters with the levels of free UV filters. Previous studies have shown that UV filters covalently attach to Cys, His and Lys residues of proteins (Aquilina and Truscott, 2000, 2001; Garner et al., 2000). Acid hydrolysis had been used previously to quantify Kyn bound to His and Lys in normal and cataractous lens proteins, (Vazquez et al., 2002, 2004); however, this technique cannot be used for protein-bound 3OHKG, since acid is known to cleave the glucose moiety.

In this study we used a newly developed method for determining the levels of protein-bound UV filters in the lens cortex and nucleus (Korlimbinis and Truscott, 2006). The method involves incubating the proteins with excess GSH at pH 9.5. At this pH, protein-bound UV filters are hydrolysed and the α,β-unsaturated ketones corresponding to each UV filter are released from the protein. In the presence of excess GSH, each ketone that is released, forms its corresponding glutathionyl UV filter adduct (Fig. 1) which can then be separated by HPLC. All three adducts are diastereoisomers and elute as doublet peaks. Using this method the levels of the three UV filters bound to proteins of individual lenses can be determined (Korlimbinis and Truscott, 2006).

In a sample of nuclear protein from a cataract lens treated in this way, the GSH adducts were well separated (Fig. 1A). Large peaks corresponding to Kyn and 3OHKG were observed in the protein samples from both lens cortex and nucleus (Fig. 1). A doublet due to the GSH adduct of 3OHKyn was detected in the nuclear proteins of normal lenses (Korlimbinis et al., 2007); however, this peak was absent in the cataract samples (Fig. 1). Free UV filters do not interfere with the assay of protein-bound UV filters (Korlimbinis et al., 2007) and >90% of the bound UV filters were released using this extraction procedure for both normal and cataract lenses.

3.1. Protein-bound UV filters

3.1.1. 3-Hydroxykynurenine glucoside

3.1.1.1. Nucleus. The levels of bound 3OHKG found in both the light and dark cataract lenses showed no clear correlation with age (Fig. 2Ai and ii). The mean levels of bound 3OHKG in the nuclei of dark and light cataract lenses were 153 and 277 pmol/mg, respectively (Table 1). The difference in the levels of protein-bound 3OHKG in the nuclei of normal lenses compared to those in both types of cataract lenses was statistically significant ($P < 0.0001$). Although in the nuclei of light cataract lenses all values were greater than 200 pmol/mg of protein and in the dark cataract lenses all were less than 240 pmol/mg of protein (Fig. 2A), comparison of the levels between the cataract lens groups showed that the differences were not statistically significant.

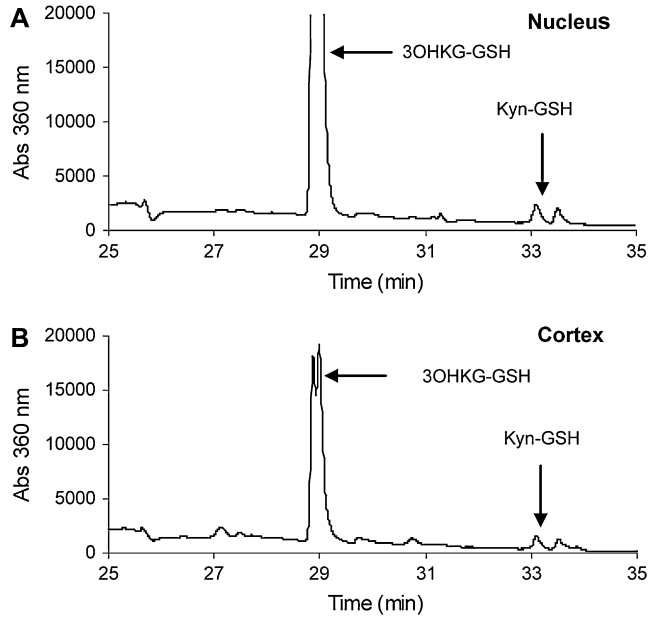

Fig. 1. HPLC chromatogram of GSH-conjugated UV filters released from human light cataract lens proteins. Lens proteins were incubated with excess GSH at pH 9.5 for 4 h. (A) Cataract 67-year-old nucleus; (B) cataract 67-year-old cortex.

3.1.1.2. Cortex. The cortical levels of bound 3OHKG in both the light and dark cataract lenses also showed no clear relationship with age (Fig. 2Bi and ii). Bound 3OHKG levels were consistently lower than those in the nucleus from the same lenses (Fig. 2A). The average amount of bound 3OHKG in dark cataract lenses was 53 pmol/mg of protein, and the average amount in light cataract lenses was 45 pmol/mg protein (Table 1). This difference was not statistically significant and nor were the levels in the normal lenses compared to those in cataract lenses ($P = 0.77$).

3.1.2. Kynurenine

3.1.2.1. Nucleus. The levels of protein-bound Kyn in nuclei of both the light and dark cataract lenses showed no clear correlation with age (Fig. 3Ai and ii). There was greater scatter noted in the Kyn content of the light lenses. The average amount of bound Kyn in dark cataract lenses was 6 pmol/mg protein, and the average amount in light cataract lenses was 12 pmol/mg protein (Table 1). Levels in the normal lenses compared to the levels in cataract lenses were statistically significant ($P < 0.0001$). There was no statistically significant difference between the Kyn levels in the two different grades of cataract.

3.1.2.2. Cortex. The levels of protein-bound Kyn in cortices of the dark cataract lenses also showed no clear correlation with age, but the levels seen in the light cataract lenses appeared to vary more with the two highest values observed in the oldest two lenses (Fig. 3B). The average amount of bound Kyn in dark cataract lenses was 2 pmol/mg protein, and the average amount in light cataract lenses was 4 pmol/mg protein (Table 1). Levels in the normal lenses compared to the levels in cataract lenses showed that they were not statistically significant ($P = 0.10$).

3.1.3. 3-Hydroxykynurenine

Bound 3OHKyn was not detected in any of the cataract lens proteins examined.

3.2. Free UV filters

The mean levels of free UV filters determined in this study are summarised in Table 1. The values for normal lenses are comparable to the levels reported previously by Bova et al. (2001) and for cataract lenses by Streete et al. (2004). Data

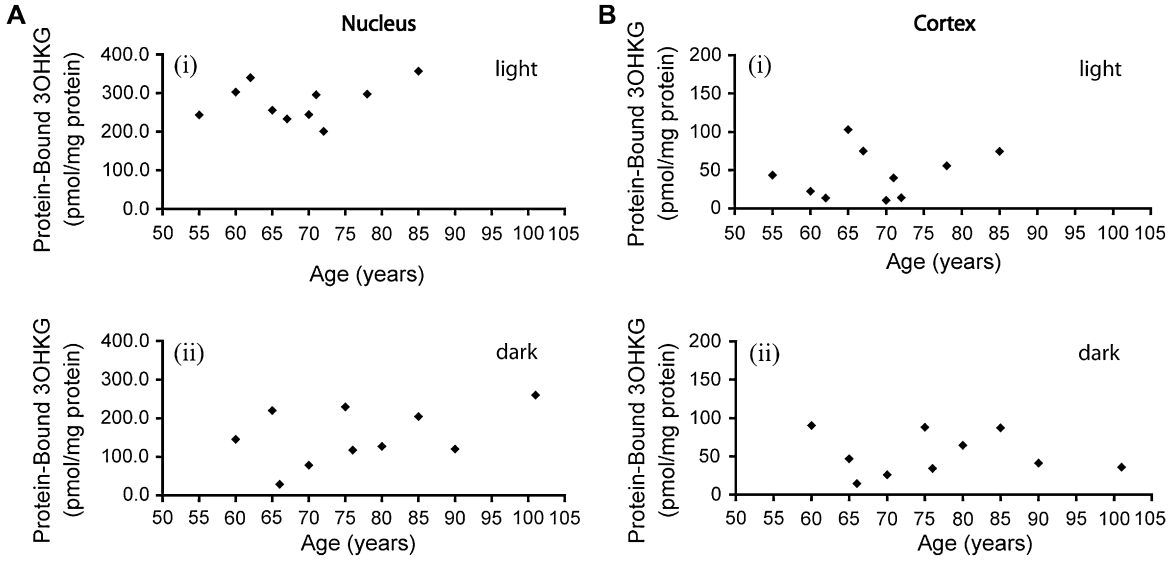

Fig. 2. Protein-bound 3OHKG in cataract lenses. The concentration of bound 3OHKG in the (A) Nucleus; (i) light cataract lenses, (ii) dark cataract lenses. (B) Cortex; (i) light cataract lenses, (ii) dark cataract lenses. Normals: by comparison the values for normal lenses over 50 were nucleus 1307 ± 89 and cortex 56 ± 10 pmol/mg protein (mean ± standard deviation).

for cataract lenses as a function of age have not previously been published. As shown in Fig. 4Ai and ii, the 3OHKG concentration in both the light and dark cataract lens group was typically quite constant as a function of age. The mean value in light lenses was twice that in the dark cataract lenses (Table 1). A similar pattern was observed with Kyn (Fig. 4Bi and ii). Again, as was found for 3OHKG, the values in the nucleus were constant irrespective of age and the free Kyn in the light cataract lenses was approximately double that of the dark lenses.

In order to determine if there was any relationship between the concentrations of free and bound UV filters in cataract lenses, their levels were compared and the results for 3OHKG are shown in Fig. 5. The results for Kyn are not shown but the relationship between free and bound was comparable to that for 3OHKG. As can be seen, there was no clear relationship between the levels of free 3OHKG and corresponding protein-bound concentrations. This finding suggests that the environment of the lens, for example, the conformation of the proteins and/or the availability of reducing agents such as GSH, Cys and NADH, that can intercept the reactive intermediates, may be of greater importance than simply the amount of free UV filter available for deamination.

Table 1
Average concentration of protein-bound and free UV filters in human cataract lenses over age 50

UV filter	Concentration (pmol/mg protein)		
	Light cataract	Dark cataract	Normal
Protein-bound			
3OHKG (N)	277 ± 109	153 ± 109	1307 ± 89
3OHKG (C)	45 ± 12	53 ± 12	56 ± 10
Kyn (N)	12 ± 4	6 ± 4	37 ± 3
Kyn (C)	4 ± 1	2 ± 1	2 ± 1
3OHKyn (N)	N.D.	N.D.	9 ± 3
3OHKyn (C)	N.D.	N.D.	N.D.
	Concentration (pmol/mg tissue)		
Free levels			
3OHKG (N)	82 ± 19	31 ± 18	534 ± 25
3OHKG (C)	40 ± 7	35 ± 7	557 ± 10
Kyn (N)	4 ± 1	2 ± 1	16 ± 1
Kyn (C)	2 ± 1	1 ± 1	6 ± 1
3OHKyn (N)	1 ± 1	1 ± 1	3 ± 1
3OHKyn (C)	1 ± 1	1 ± 1	1 ± 1

Levels in normal human lenses have been listed for comparison.
N.D., not detected.
Dark cataract, $n = 10$.
Light cataract, $n = 10$.
C, cortex; N, nucleus.
Normal lenses over 50 years old, $n = 15$ (protein-bound), $n = 5$ (free levels).
Values shown are mean ± standard deviation (S.D.).

4. Discussion

In this study we compared the levels of free and protein-bound UV filters in individual cataract lenses of known ages. The cataract lenses were divided into two groups based on nuclear color: light cataract (Type I/II lenses in the Pirie classification system, Pirie, 1968) and dark cataract (Type III/IV lenses). A consistent finding was that the amounts of the UV filters bound to nuclear proteins from cataract lenses were always much lower than comparable age-matched normal lenses (Table 1). In addition, the levels of UV filters attached to the nuclear proteins from light cataract lenses were higher than those from dark cataract lenses. To illustrate using lenses aged >50, the mean bound 3OHKG levels (pmol/mg) in the nucleus were: normal (1307), light cataract (277), dark cataract (153). Bound Kyn levels were: normal (37), light cataract (12), dark cataract (6). In the case of bound 3OHKyn, the

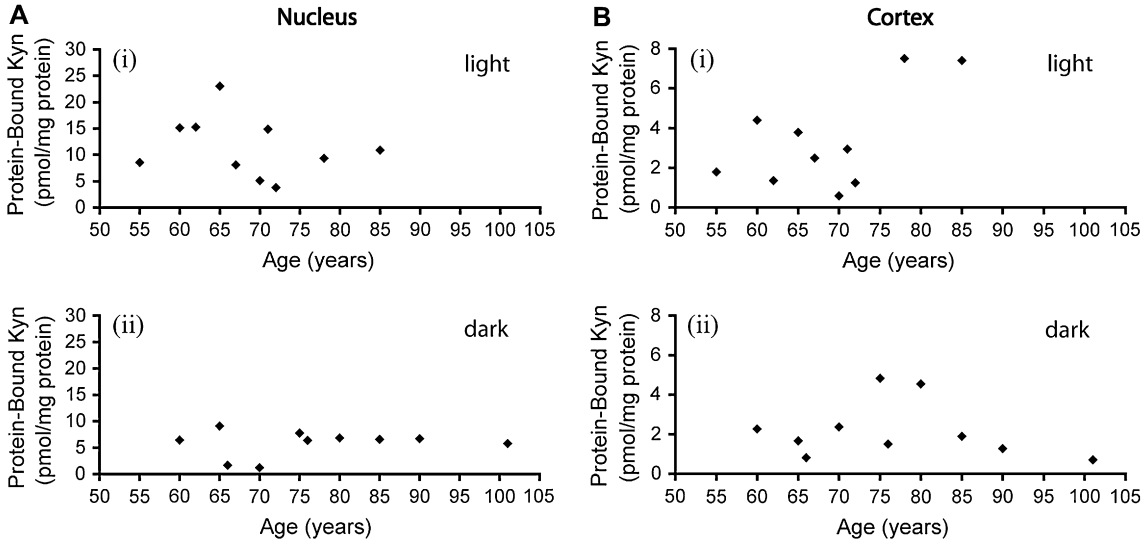

Fig. 3. Protein-bound Kyn in cataract lenses. The concentration of bound Kyn in the (A) Nucleus; (i) light cataract lenses, (ii) dark cataract lenses. (B) Cortex; (i) light cataract lenses, (ii) dark cataract lenses. Normals: by comparison the values for normal lenses over 50 were nucleus 37 ± 3 and cortex 2 ± 1 pmol/mg protein (mean ± standard deviation).

levels in both light cataract and dark cataract lenses were below the limits of detection. The data were analyzed using ANOVA one-way analysis of variants. Levels in cataract lenses were significantly different from normals for the nuclear contents of protein-bound 3OHKG and Kyn. The light and dark cataract lenses displayed different mean values; however, these were not statistically significant.

While the amounts of UV filters bound to nuclear proteins from cataract lenses were very different, being on average six (Kyn), or nine times (3OHKG) lower than normals for dark lenses, the values in the cortices of normal and cataract lenses were much the same. In this study normal lenses were sourced from Australia and the cataract lenses from India. Because cataract lenses were stored at −20 °C in India, sometimes for several weeks prior to transport and analysis in Australia, we cannot be certain that this did not affect the UV filter levels. If this were in fact the case, we might expect that free/bound UV filter levels in all lens regions would be affected. The finding that the cortical levels of both protein-bound 3OHKG, and protein-bound Kyn, were the same as in normals (3OHKG: normal 56, dark cataract 53 pmol/mg; Kyn: normal 2, dark cataract 2 pmol/mg), suggests that, for bound UV filters, this issue may not be a problem. However, free UV filters may have been affected, and without access to normal Indian lenses, it is not possible to prove that the differences we observed between cataract and normal samples is not simply a consequence of the geographical origin of the lenses.

The assay system employed here allows quantification of UV filter adducts of Lys and Cys (Korlimbinis and Truscott,

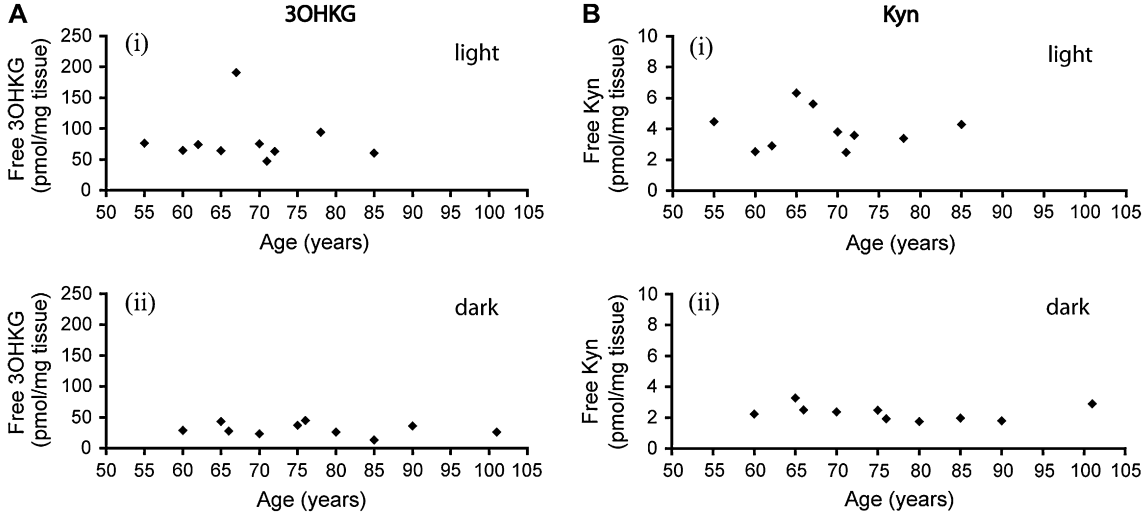

Fig. 4. The concentration of free 3OHKG and free Kyn in the nucleus of cataract lenses. (A) 3OHKG; (i) light cataract lenses, (ii) dark cataract lenses. Normals: by comparison the values for normal lenses over 50 were nucleus 534 ± 25. (B) Kyn; (i) light cataract lenses, (ii) dark cataract lenses. Normals: by comparison the values for normal lenses over 50 were nucleus 16 ± 1 (mean ± standard deviation).

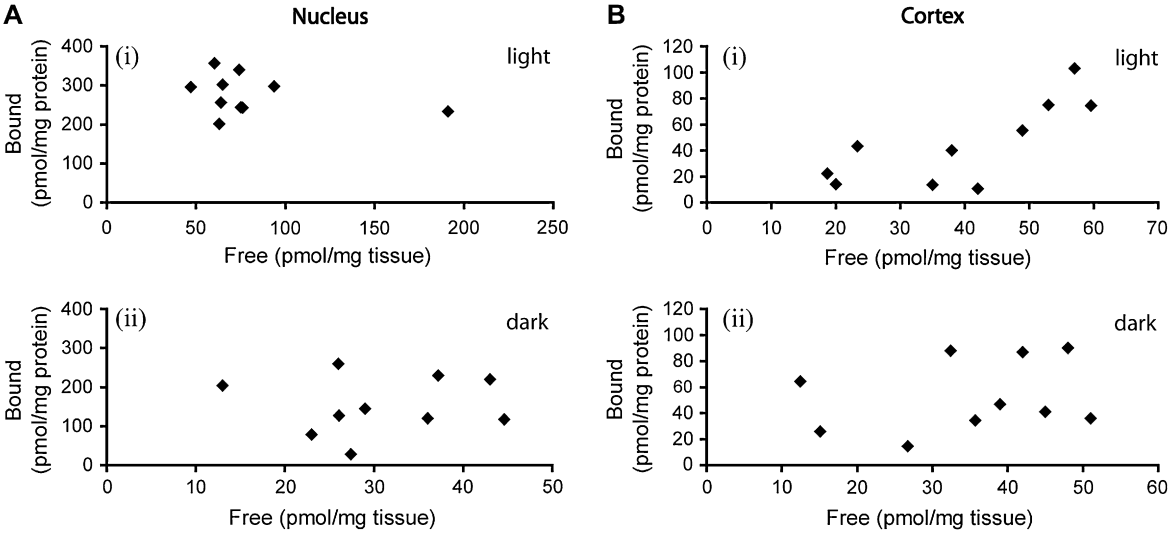

Fig. 5. Relationship between free and bound 3OHKG. (A) Nucleus; (i) light cataract lenses, (ii) dark cataract lenses. (B) Cortex; (i) light cataract lenses, (ii) dark cataract lenses.

2006); however, only a minor percentage of His adducts (~1%) decompose under the conditions used, therefore the amounts of bound UV filters quoted here is an underestimate. Furthermore, values have not been corrected for the recovery of UV filters in the assay (73% Lys–Kyn and 61% Cys–Kyn). Attempts to quantify residual His adducts in the proteins using acid hydrolysis, following the incubation with GSH, were unsuccessful.

There are several possibilities to explain the finding of lower levels of bound UV filters in the nuclei, but not the cortices, of the cataract lenses. One is that these compounds are participating in other reactions in the center of cataract lenses. Another is that bound UV filters are in dynamic equilibrium with free UV filters and that the lower bound levels simply reflect a diminished source of free UV filters. The data in Table 1 showing average values for free versus bound nuclear levels in the three lens groups provide some evidence to support this. In addition, all cataract lenses were shown to contain protein-bound 3OHKG in the largest amount, with lower levels of Kyn, and this reflects the concentrations of the free UV filter compounds in these lenses.

Surprisingly therefore, plots of free UV filter concentrations versus those of bound UV filters in individual cataract lenses (Fig. 5) revealed no clear relationship in the case of either Kyn or 3OHKG. A similar observation has been made for normal lenses (Korlimbinis et al., 2007). The reason for this is unclear, but may be related to multiple pathways present in the lens for scavenging the reactive intermediates. Thus the ultimate amount of a UV filter bound to proteins in the lens is likely to be a reflection of a number of factors. For example, the concentration of each free UV filter coupled with its residence time within different regions of the lens. This latter factor is crucial, since the concentrations of deaminated UV filter intermediates increase steadily as a function of the time of incubation, as has been shown in model experiments in which 3OHKG, Kyn and 3OH-Kyn were incubated at pH 7 (Taylor et al., 2002a). This feature is important for all naturally unstable molecules and is probably responsible for the increase in nuclear levels of protein-bound UV filter compounds after middle age in normal lenses (Korlimbinis and Truscott, 2006). The concentration of scavenger species, in particular GSH (Garner et al., 1999) and cysteine (Hains et al., 2006), is also likely to be crucial since, in model experiments, these compounds efficiently spare lens proteins from modification if the concentration of antioxidant is maintained at high levels (Taylor et al., 2002b). The observation that dark cataract lenses have approximately double the ratio of bound to free 3OHKG compared with normals, and that the light lenses are intermediate (see Table 1), may reflect a loss of these scavenger thiol molecules in cataract lens nuclei.

A third possibility to explain the lower levels of bound UV filters in cataract lenses is that there are fewer protein thiols available in the cataract proteins than in normal lens proteins, and therefore fewer sites for attachment of the UV filters. Model studies indicate that thiol groups react more rapidly with deaminated UV filters than the side chain amino groups of Lys or His (Korlimbinis et al., 2007).

We propose that the reduction in protein thiol content that is associated with nuclear cataract (Truscott and Augusteyn, 1977) is a likely factor that leads to a net decrease in the amount of UV filters that are bound in cataract lenses. Cysteine residues are the preferred sites of binding (Aquilina and Truscott, 2000; Garner et al., 2000) and once these residues have been oxidised in cataract lenses, the deaminated UV filters may undergo as yet undiscovered reactions with proteins. We hypothesise that such reactions may contribute to the variety of colors that are characteristic of human ARN cataract lenses.

5. Conclusion

Levels of protein-bound UV filters are much lower in the nuclei of cataract lenses than in normals. For advanced nuclear cataract lenses the concentration was six to ninefold lower. In

early stage nuclear cataract, the nuclear levels were also decreased, but to a smaller extent. By contrast, no differences were found in the levels of protein-bound UV filters in the cortices of the cataract lenses compared to normal lenses.

The levels of free UV filters also appeared to be significantly lower in cataract lenses. The reason for this is unclear, but it is not due to a decrease in the concentration of the precursor, Trp, since this amino acid is markedly elevated in cataract lenses (Streete et al., 2004).

In advanced cataract lenses, nuclear GSH levels fall to zero, or to very low concentrations (Truscott and Augusteyn, 1977). This decrease in the agent that normally intercepts the reactive UV filter intermediates should act to promote UV filter binding to proteins; however, protein thiol groups also decrease substantially with the development of nuclear cataract. Since we observed a decrease in the overall levels of UV filter attachment to cataract proteins we propose that oxidation of cysteine residues may play a more important role in determining the overall binding to UV filters to lens proteins.

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References

Aquilina, J.A., Truscott, R.J.W., 2000. Cysteine is the initial site of modification of α-crystallin by kynurenine. Biochem. Biophys. Res. Commun. 276, 216–223.

Aquilina, J.A., Truscott, R.J.W., 2001. Kynurenine binds to the peptide binding region of the chaperone αB-crystallin. Biochem. Biophys. Res. Commun. 285, 1107–1113.

Bova, L.M., Sweeney, M.H.J., Jamie, J.F., Truscott, R.J.W., 2001. Major changes in human ocular UV protection with age. Investig. Ophthalmol. Vis. Sci. 42, 200–205.

Dickerson Jr., J.E., Lou, M.F., 1997. Free cysteine levels in normal human lenses. Exp. Eye Res. 65, 451–454.

Garner, B., Vazquez, S., Griffith, R., Lindner, R.A., Carver, J.A., Truscott, R.J.W., 1999. Identification of glutathionyl-3-hydroxykynurenine glucoside as a novel fluorophore associated with aging of the human lens. J. Biol. Chem. 274, 20847–20854.

Garner, B., Shaw, D.C., Lindner, R.A., Carver, J.A., Truscott, R.J.W., 2000. Non-oxidative modification of lens crystallins by kynurenine: a novel post-translational protein modification with possible relevance to ageing and cataract. Biochim. Biophys. Acta 1476, 265–278.

Hains, P.G., Mizdrak, J., Streete, I.M., Jamie, J.F., Truscott, R.J.W., 2006. Identification of the new UV filter compound cysteine-L-3-hydroxykynurenine *O*-β-D-glucoside in human lenses. FEBS Lett. 580, 5071–5076.

van Heyningen, R., 1971. Fluorescent glucoside in the human lens. Nature 230, 393–394.

Hood, B.D., Garner, B., Truscott, R.J.W., 1999. Human lens coloration and aging evidence for crystallin modification by the major ultraviolet filter, 3-hydroxykynurenine *O*-β-D-glucoside. J. Biol. Chem. 274, 32547–32550.

Korlimbinis, A., Truscott, R.J.W., 2006. Identification of 3-hydroxykynurenine bound to proteins in the human lens. A possible role in age-related nuclear cataract. Biochemistry 45, 1950–1960.

Korlimbinis, A., Aquilina, J.A., Truscott, R.J.W., 2007. Protein-bound UV filters in normal human lenses. The concentration of bound UV filters equals that of free UV filters in the centre of older lenses. Investig. Ophthalmol. Vis. Sci. 48, 1718–1723.

Moffat, B.A., Landman, K.A., Truscott, R.J.W., Sweeney, M.H.J., Pope, J.M., 1999. Age-related changes in the kinetics of water transport in normal human lenses. Exp. Eye Res. 69, 663–669.

Pirie, A., 1968. Color and solubility of the proteins of human cataracts. Investig. Ophthalmol. 7, 634–650.

Streete, I.M., Jamie, J.F., Truscott, R.J.W., 2004. Lenticular levels of amino acids and free UV filters differ significantly between normals and cataract patients. Investig. Ophthalmol. Vis. Sci. 45, 4091–4098.

Sweeney, M.H.J., Truscott, R.J.W., 1998. An impediment to glutathione diffusion in older normal human lenses: a possible precondition for nuclear cataract. Exp. Eye Res. 67, 587–595.

Taylor, L.M., Aquilina, J.A., Jamie, J.F., Truscott, R.J.W., 2002a. UV filter instability: consequences for the human lens. Exp. Eye Res. 75, 165–175.

Taylor, L.M., Aquilina, J.A., Jamie, J.F., Truscott, R.J.W., 2002b. Glutathione and NADH, but not ascorbate, protect the lens proteins from modification by UV filters. Exp. Eye Res. 74, 503–511.

Truscott, R.J.W., Augusteyn, R.C., 1977. The state of sulphydryl groups in normal and cataractous human lenses. Exp. Eye Res. 25, 139–148.

Truscott, R.J.W., 2000. Age-related nuclear cataract: a lens transport problem. Ophthalmic Res. 32, 185–194.

Vazquez, S., Aquilina, J.A., Jamie, J.F., Sheil, M.M., Truscott, R.J.W., 2002. Novel protein modification by kynurenine in human lenses. J. Biol. Chem. 277, 4867–4873.

Vazquez, S., Parker, N.R., Sheil, M.M., Truscott, R.J.W., 2004. Protein-bound kynurenine decreases with the progression of age-related nuclear cataract. Investig. Ophthalmol. Vis. Sci. 45, 879–883.

Wood, A.M., Truscott, R.J.W., 1993. UV filters in human lenses: tryptophan catabolism. Exp. Eye Res. 56, 317–325.