Reversible Binding of Kynurenine to Lens Proteins: Potential Protection by Glutathione in Young Lenses

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PURPOSE. Human ultraviolet light (UV) filters, such as kynurenine (Kyn), readily deaminate to reactive unsaturated ketones that covalently modify proteins in older human lenses. The aim of this study was to examine in vitro rates of formation and decomposition of the three major Kyn-amino acid adducts and possible consequences for the lens.

METHODS. The t-Boc-protected Kyn-His, Kyn-Lys, and Kyn-Cys adducts and Kyn-Cys were synthesized from the corresponding amino acids and Kyn. Calf lens proteins were modified with Kyn by incubation at pH 7. Stability and competition studies of the adducts were conducted under physiological conditions. Kyn-amino acids and their decomposition products were quantified using HPLC.

RESULTS. At physiological pH, Kyn-Cys adducts formed more rapidly than either Lys or His adducts, but they also decomposed readily. By contrast, His adducts were stable. Cysteine (Cys) residues in β-crystallins were major sites of modification. The Kyn moiety, initially bound to Cys residues, was found to transfer to other amino acids. Glutathione promoted the breakdown of Kyn-Cys.

CONCLUSIONS. These data may help explain why proteins in young lenses are not modified by UV filters in situ. The initial phase of the modification of proteins in the human lens by UV filters may be a dynamic process. In lenses, Cys residues of crystallins modify preferentially, but these adducts also decompose to release deaminated Kyn. This can then potentially react with other amino acids. Glutathione, which is present in high concentrations in the lenses of young people, may play a vital role in keeping proteins free from modification by intercepting reactive deaminated kynurenines formed by the spontaneous breakdown of free UV filters, promoting the decomposition of Kyn-Cys residues, and sequestering the unsaturated ketones once they are released from modified proteins. (Invest Ophthalmol Vis Sci. 2007;48:3705–3713) DOI:10.1167/iovs.06-1061

The human lens becomes increasingly colored and fluorescent with age,1–3 particularly in the nuclear region, because of the accumulation of posttranslational modifications on proteins. This result, in part, from the covalent attachment of the ultraviolet light (UV) filters 3-hydroxykynurenine glucoside (3-OHKG), kynurenine (Kyn), and 3-hydroxykynurenine (3-OH-Kyn) to lens proteins.1–6

In the normal human lens, levels of protein-bound UV filters increase in an age-dependent manner, with a significant increase in the levels of modification occurring after middle age.4–6 A corresponding decrease with age in the levels of free UV filters in lenses is also observed, with 3-OHKG, 3-OH-Kyn, and Kyn all declining by approximately 12% per decade.2

UV filters are intrinsically unstable under physiological conditions and undergo side chain deamination to yield α,β-unsaturated ketones.2,7 These can react with nucleophilic species in the lens through Michael addition. Reaction with protein side chains is particularly favored because the lens has the highest protein concentration of any human tissue.8 Approximately 90% of the proteins are crystallins,9 making these major targets for modification.

Around middle age in humans, a barrier forms in the lens9,10 that hinders the diffusion of low molecular-weight species, such as the antioxidant glutathione, from the cortex into the nucleus and traps small molecules, including UV filters, in the lens nucleus. In proteins in the nuclei of older lenses, this results in greater susceptibility to modification by compounds such as deaminated UV filters.

Protein turnover in the human lens is negligible11; hence, modifications accumulate over a person’s lifetime12 that may affect protein tertiary structure and predispose the lens to age-related nuclear cataract. For example, model studies show that proteins to which Kyn is attached become oxidized after exposure to wavelengths of light that penetrate the cornea.13

The present study investigated the rate of formation of Kyn-amino acids and the stability of these adducts under physiological conditions in vitro. These data point to an important role for protein cysteine (Cys) residues in the initial phase of modification by UV filters and suggest that glutathione may play a key role in preventing posttranslational modification of lens proteins.

MATERIALS AND METHODS

Materials

Ultrapure water (Milli-Q; Millipore, Bellerica, MA) was used in the preparation of all solutions. Amino acids (N-acetyl-L-His, N-acetyl-L-Lys, N-acetyl-L-Cys, and Cys), DL-kynurenine and DL-kynurenone (Kyn) sulfate salt, HCl (6 M, sequencing grade), glutathione, guanidine HCl, phenol, thioglycolic acid, trifluoroacetic acid (TFA), formic acid, and sodium hydroxide were all purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO). Protein concentrations (MWt cutoff, 10,000 Da) were purchased (Vivaspin; Vivascience Sartorius ATG, Göttingen, Germany). All organic solvents were HPLC grade (Ajax, Auburn, NSW, Australia).

Rate of Formation of Kyn-Amino Acids

N-acetyl (tert-butyloxy)carbonyl amino acids were used to confine chemical reactions to the amino acid side chains and therefore to serve...
as a model for the amino acid residues in proteins. DL-Kyn (5 mM) and either N-α-t-Boc-L-His, N-α-t-Boc-L-Lys, N-α-t-Boc-L-Cys, or L-Cys (75 mM) were dissolved in 200 mM phosphate buffer (pH 7.2). For these and subsequent incubations, each vial was bubbled with argon, sealed, wrapped in foil, and incubated at 35°C. Triplicate samples (70 μL) were taken at the indicated times and diluted with aqueous 0.05% (vol/vol) TFA (70 μL).

HPLC Measurement of Kyn-Amino Acid Formation
Reverse-phase (RP)-HPLC was performed on an HPLC system with a diode array detector (SPD-M10AVP, Shimadzu, Kyoto, Japan). For analytical scale separation, an analytical C18 column (100 Å, 5 μm, 4.6 mm × 250 mm; Microsorb-MV, Varian, Palo Alto, CA) was used under the following mobile-phase conditions: solvent A (0.05% vol/vol TFA) for 5 minutes, followed by a linear gradient of 0% to 40% solvent B (80% acetonitrile/H2O, 0.05% vol/vol TFA) over 50 minutes, then a linear gradient of 40% to 0% solvent B over 5 minutes at a flow rate of 1 mL/min.

Stability of Kyn-Amino Acid Adducts
The t-Boc-protected Kyn-amino acid adducts Kyn-t-Boc-His and Kyn-t-Boc-Lys and the sulfone of Kyn-t-Boc-Cys were prepared as previously described,3 dissolved (at 1.0–2.5 mM) in 200 mM phosphate buffer, pH 7.2, and incubated. Triplicate samples were taken every 20 hours for RP-HPLC.

Incubation of Kyn-Cys in the Presence of Excess N-α-t-Boc-L-His
Kyn-Cys (1.0 mg) was dissolved in 2.0 mL of 200 mM phosphate buffer (pH 7.2), with a 20-fold molar excess of N-α-t-Boc-L-His. Aliquots (100 μL) were removed every 12 hours and analyzed by RP-HPLC.

Modification of Calf Lens Proteins with Kyn
Proteins were extracted from bovine lenses as described previously.14 Calf lens protein (CLP; 10 mg/mL) and DL-Kyn sulfate (2 mg/mL) were dissolved in 200 mM phosphate buffer, pH 7.2. Chloroform (100 μL) was added as an antibacterial agent. After 14 days of incubation, modified CLP was dialyzed against 1 mM phosphate buffer (pH 6) at 4°C and lyophilized.

Effect of Glutathione on CLP Modification
CLP (100 mg/mL) and DL-Kyn sulfate (2 mM) were dissolved in chelating resin (Chelex; Bio-Rad, Hercules, CA)-treated phosphate buffer (200 mM), in the absence and presence of glutathione (2 mM). Chloroform (10 μL) was added as an antibacterial agent. The solution was placed under argon, sealed, and incubated at 35°C for 4 days. Modified protein was isolated from the reaction mixture with the use of a centrifugal filter unit (Centricron, with YM-3 membrane; Millipore). Aliquots of the modified protein (200 μL) were loaded onto units (Centricron; Millipore) and washed with ultrapure water (1 mL; Milli-Q: Millipore), followed by 6 M guanidine HCl (1 mL). The centrifugal filter unit (Centricron; Millipore) was inverted and spun at 1000g for 3 minutes to remove the protein from the membrane. UV-Vis spectra of the modified proteins were obtained on a spectrophotometer (UV-1700; Shimadzu) to determine levels of modification using absorbance at 360 nm.

Preparation of Human Lens Proteins
Human tissue was handled in accordance with the tenets of the Declaration of Helsinki. Normal human lenses were obtained from the NSW Lions Eye Bank (Sydney, NSW Australia). Nuclei were obtained with a cork borer (5 mm), and the ends (1 mm) were removed. Nuclei were homogenized in 500 μL of 6 M guanidine HCl and dialyzed overnight against 100 mM sodium acetate/acetate acid buffer, pH 4, and lyophilized.

Acid Hydrolysis of Kyn-Modified Proteins and Human Lens Proteins
Kyn-modified lens proteins (4–8 mg) or human lens proteins (5 mg) were hydrolyzed with 6 M HCl for 24 hours at 110°C, then analyzed by HPLC as described previously.6

Rate of Formation of Kyn-Amino Acid Adducts on Proteins
CLP (10 mg/mL) was incubated with Kyn (2 mg/mL) in 200 mM phosphate buffer, pH 7.2, under argon at 37°C. Aliquots (10 mg protein) were removed every 2 days and modified protein filtered (6000g, 50 minutes, 10°C) in a concentrator (Vivaspun; Vivascience Sartorius AG) and washed three times with 1 mL water. Lyophilized protein (4–7 mg) was acid hydrolyzed and analyzed by RP-HPLC. The filtrate was also analyzed by RP-HPLC using conditions for measuring the formation of Kyn-amino acid adduct.

Stability of Protein-Bound Kyn
CLP (5 mg/mL, covalently modified with Kyn at pH 7.2) was incubated in 200 mM phosphate buffer, pH 7.2, at 37°C. Aliquots (7–10 mg protein) were filtered with a concentrator (Vivaspun; Vivascience Sartorius AG) and washed with water (1 mL), and the protein was lyophilized. Protein samples were acid hydrolyzed and analyzed by RP-HPLC.

Tryptic Digestion
CLP (1.0 mg) was modified with Kyn in 50 mM ammonium bicarbonate buffer, pH 9.0, for 48 hours and then digested with trypsin (1 mg/mL) in 0.1 M morpholino ethane sulfonic acid (MES) buffer, pH 6.0, for 48 hours at 37°C at a substrate/enzyme ratio of 50:1 (Cys-Kyn is stable at pH 6.0).

Incubation of Kyn-t-Boc-Cys with Excess Glutathione
Kyn-t-Boc-Cys (1 mM) was dissolved in 100 mM phosphate buffer, pH 7.2, containing glutathione (5, 20, or 40 mM). Chloroform (20 μL) was added. Aliquots were taken every 24 hours for RP-HPLC.

RESULTS
Reactivity of Amino Acids with α,β-Unsaturated Ketone Derived from Kyn
At neutral pH, the side chain of Kyn undergoes deamination and gives an α,β-unsaturated ketone that reacts with amino acid nucleophiles.7 The aim of this study was to investigate the rate of reaction of the N-α-t-Boc (tert-butyloxycarbonyl) nucleophilic amino acids N-α-t-Boc-L-His, N-α-t-Boc-L-Lys, and N-α-t-Boc-L-Cys with Kyn under physiological conditions (pH 7.2, 37°C). N-α-t-Boc-derivatives were chosen to limit the reaction of Kyn to the amino acid side chains. Incubation of Kyn with these amino acid derivatives, at pH 7.2, yielded all three Kyn-amino acid adducts. The data obtained (Fig. 1) indicate that N-α-t-Boc-L-Cys was the best nucleophile, followed by N-α-t-Boc-L-His and then by N-α-t-Boc-L-Lys (Fig. 1), with the apparent rates of reaction 6.0 (±0.8) × 10–6, 2.0 (±1) × 10–7, and 1.6 (±0.93) × 10–6 μmol/min, respectively. These figures do not take into account any possible decomposition under the reaction conditions.

Stability of Kyn-Amino Acid Adducts
Kyn-t-Boc-adducts were incubated at pH 7.2 under argon at 37°C. After 80 hours, 28% and 46% decreases were seen in the levels of Kyn-t-Boc-Cys and Kyn-t-Boc-Lys,15 respectively (data not shown), whereas the levels of Kyn-t-Boc-His remained

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unchanged. In Cys and Lys adducts, a major decomposition product—eluting at 32 minutes—was deaminated Kyn (m/z 192, which coeluted with an authentic standard in HPLC).

Incubation of Kyn-Cys under analogous conditions resulted in a 70% decrease in the levels of the adduct over 80 hours (Fig. 2). Therefore, the t-Boc adduct was twofold to threefold more stable than Kyn-Cys under these conditions. These data show that protection of the α-amino moiety with a t-Boc group significantly increased the stability of the Kyn-Cys adduct at pH 7.2, suggesting that the α-amino group of Cys might have assisted the deprotonation necessary for the formation of deaminated Kyn7 or that the bulky t-Boc group might have obstructed the access of basic species (e.g., hydroxyl ion) to the Kyn side chain.

To understand the mechanism of breakdown of Kyn-Cys, we used HPLC/MS to examine the major colored (360-nm absorbing) products generated during the incubation of Kyn-Cys. Deaminated Kyn increased significantly in the first 20 hours and remained relatively constant (Fig. 3). Three other products (eluting at 26, 45, and 51 minutes) were also observed to form slowly, at much lower levels than deaminated Kyn. The peak at 26 minutes coeluted with synthetic Kyn yellow and had an identical absorbance maximum (372 nm).16 The peak, eluting at 51 minutes, was analyzed by nanospray tandem mass spectrometry and was found to have a molecular ion of m/z 385, which gave product ions of m/z 192, 174, and 136 in consistent with a dimer of deaminated Kyn, and the product was analogous to that of a Kyn dimer formed during the decomposition of Kyn (Scheme 1). The compound that eluted at 45 minutes was not identified.

**Transfer of the Kyn Moiety: Incubation of Kyn-Cys with Excess N-α-t-Boc-L-His**

Given that Kyn-Cys was unstable at neutral pH, it underwent deamination, and that Kyn-t-Boc-His was stable under these conditions,15 possible transfer of the Kyn moiety from Cys to His was investigated. Kyn-Cys was incubated with a 20-fold molar excess of N-α-t-Boc-L-His, at pH 7.2. As shown in Figure 5, Kyn-Cys rapidly decreased in concentration, with an 82% loss after 24 hours and a corresponding formation of deaminated Kyn (data not shown). Deaminated Kyn then decreased in concentration as it reacted with t-Boc-His. Most deaminated Kyn reacted with N-α-t-Boc-L-His; after 48 hours, the level of Kyn-t-Boc-His (confirmed by ESI-MS/MS) corresponded to 75% of the original level of Kyn-Cys.

**Glutathione Promotes Decomposition of the Kyn-Cys Adduct**

Because t-Boc-His appeared to promote the breakdown of Kyn-Cys, we hypothesized that glutathione might do the same. We used Kyn-t-Boc-Cys in these experiments to more closely mimic the Kyn adduct in proteins. Inclusion of glutathione resulted in a concentration-dependent increase in the rate of decomposition of the Cys adduct (Fig. 6). Formation of the glutathione adduct of Kyn accounted for most of the loss of Kyn-t-Boc-Cys (Fig. 6).

**Formation of Kyn-Amino Acid Adducts in Proteins**

The rate of formation of Kyn-amino acid adducts in crystallins under physiological conditions (pH 7.2, 37°C) was then investigated. Kyn was incubated with CLP in phosphate buffer at pH 7.2. Modification of CLP occurred primarily at Cys residues (Fig. 7A). The rate of formation of Kyn-Cys under these conditions corresponded to 1.1 (±0.13) × 10−7 mol Kyn-Cys/(mol protein·min). The rate of formation of Kyn-His was significantly lower, 4.3 (±0.69) × 10−9 mol Kyn-His/(mol protein·min). Determination of the rate of formation of Kyn-Lys was hindered by a peak that coeluted on HPLC. Kyn-Lys could only be quantified after 10 days of incubation (approximately 0.07 mol Kyn-Lys/mmol Kyn).

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**FIGURE 1.** Adduct formation after incubation of Kyn with a 25-fold molar excess of N-α-t-Boc-L-His, N-α-t-Boc-L-Lys, or N-α-t-Boc-L-Cys. Incubations were performed in phosphate buffer, pH 7.2, at 37°C, with aliquots analyzed by RP-HPLC. Single analyses were performed at each time point.

**FIGURE 2.** Stability of Kyn-t-Boc-Cys and Kyn-Cys in phosphate buffer, pH 7.2, at 37°C. Samples were removed every 20 hours and analyzed by RP-HPLC. Plotted are mean ± SD values (n=2). Inset: structures of Kyn-t-Boc-Cys and Kyn-Cys.
mol/mol protein) using a second HPLC step. The identity of all adducts was confirmed by MS/MS.

Progressive yellowing of lens proteins was observed during the incubation of CLP with Kyn under these conditions. Because Cys was the major site of modification, this appeared to have been caused largely by products arising from Cys residues. The yellowing was consistent with previous findings, suggesting that Kyn may contribute to age-related coloration of the human lens.5

Ultrafiltrate from the incubation of Kyn with CLP was analyzed by RP-HPLC to determine the major Kyn-derived species formed in the presence of protein. A doublet peak, eluting at 41 to 42 minutes (HPLC profile not shown), had an elution time similar to that of an authentic standard of Kyn dimer.7 Mass spectrometry confirmed this identification, with a molecular ion at $m/z$ 400 and characteristic fragment ions at $m/z$ 383 (loss of NH3), 265 (loss of NH2PhCOCH3), and 192 (loss of Kyn).7 Deaminated Kyn was also identified in the filtrate. Concentrations of both species increased steadily with incubation time (Fig. 7B). A similar trend was observed previously in which the incubation of 3 mM Kyn at pH 7, in the absence of protein, led to the formation of 1.25 mM Kyn dimer and approximately 0.3 mM deaminated Kyn after 7 days.7

Sites of Modification

Major sites of Kyn modification on CLP were identified by tryptic digestion at pH 6 (Kyn-Cys is stable at pH 6), HPLC separation of the peptides, and MS/MS analysis. Data are summarized in Table 1. Peptides derived from β-crystallins were found to predominate.

Long-term Incubation of Kyn-Modified Lens Proteins

Incubation of Kyn-Cys with N-α-t-Boc-L-His provided evidence of transfer of the deaminated Kyn moiety from one amino acid to another (Fig. 5). It was hypothesized that prolonged incubation of Kyn-modified lens proteins, which contain predominantly Kyn-Cys residues, may yield Kyn-His, as a dominant modification. To test this hypothesis, CLPs were modified with Kyn (pH 7.2) for a period of 14 days and then were purified from unbound Kyn by exhaustive dialysis against 1 mM phosphate buffer, pH 6.0. The solution was readjusted to pH 7.2, and then the solution was incubated at 37°C. Aliquots of protein were removed at intervals, purified, and hydrolyzed in the presence of antioxidants.

During the first 6 days of incubation, the concentration of Kyn-Cys decreased by 54% and then plateaued (Fig. 8). In
agreement with the proposed transfer of the deaminated Kyn moiety, the levels of Kyn-His increased by 38% after 14 days of incubation. Despite this, Kyn-Cys remained the dominant modification. Therefore, although the initial time points obtained from incubation of Kyn-modified CLP mimicked those observed from the amino acid studies, later ones did not. Thus, significant Kyn-Cys levels were observed in the CLP even after extended incubation, and Kyn-His increased only slowly, despite deaminated Kyn in the solution. These findings suggest an important role for protein structure and solvent accessibility in determining the final outcome of the modification. In agreement with this, significant levels of Kyn-Cys were found in proteins isolated from older normal human lenses (22 pmol/mg protein, 76 years; 26 pmol/mg protein, 73 years).

**DISCUSSION**

This study has revealed the dynamic nature of the interaction of the human lens UV filter compound Kyn with proteins. Cys, His, and Lys all reacted with Kyn, but their rates of reaction varied significantly, as did the stabilities of the products. Cys was the most reactive, but the covalent adduct was unstable. Its stability was also influenced markedly by the concentration of glutathione (Fig. 6).

In the lens, the role of glutathione appears to be crucial. Glutathione can inhibit the reaction of proteins with UV filters in three ways: by intercepting the initial reactive product formed by deamination of the kynurenine\(^7\); by promoting decomposition of protein Kyn-Cys adducts once formed (Fig. 8) and by covalently linking to the deaminated UV filters released from the modified protein so that they can diffuse from the lens.

It is therefore possible to rationalize the observation that young human lenses have little coloration, despite free UV filters being present in higher concentrations than in older lenses.\(^2\) Glutathione levels in young lenses are high. When deamination of a UV filter takes place, the \(\alpha,\beta\)-unsaturated ketone formed will predominantly react with glutathione before it can bind to proteins.\(^2,17\) To test whether glutathione at physiological concentrations could prevent covalent modification by UV filters, CLP was incubated with Kyn in phosphate buffer, pH 7.2, in the presence and absence of 2 mM glutathione. Inclusion of glutathione prevented this linear increase (data not shown).

The concentration of protein thiol groups, even in young lenses, is higher than that of glutathione, but it is unknown what percentage of these Cys residues are solvent exposed. If some UV filter decomposition molecules do bind to crystallins, the binding will most likely be to Cys residues. The Kyn-Cys

**SCHEME 1.** Proposed mechanism of the formation of the Kyn dimer. Deaminated Kyn dimer (Fig. 4) is thought to have been formed in an analogous manner.

**FIGURE 4.** (A) Mass spectrum of the compound present in the HPLC profile (Fig. 3A) with a retention time of 51 minutes. (B) MS/MS spectrum of m/z 383 showing the loss of water (18 Da) from the dimer of deaminated Kyn, to give an ion of m/z 365. Characteristic Kyn fragments included m/z 192 (deaminated Kyn) and m/z 136. Inset: structure of the deaminated Kyn dimer.
Adducts will decompose at pH 7, but the rate is increased considerably by high glutathione. In addition, because the barrier to diffusion between the cortex and the nucleus has not yet formed, the average residence time for UV filters in the lens center, and therefore the time available for deamination, is greatly reduced. After middle age, when glutathione levels decrease and the barrier forms, net covalent modification of proteins in the nucleus is observed.

The present investigation aimed to explore some aspects of the kinetics of Kyn-amino acid formation and the relative stabilities of Kyn-amino acid adducts under physiological conditions. Studies using t-Boc-protected amino acids, as a model for His, Lys, and Cys residues in proteins, revealed that Cys reacts with Kyn threefold to fourfold faster than His or Lys (Fig. 1). Cys was also more rapidly modified in CLP (Fig. 7). These results are consistent with those of a previous study that examined the reactivity of a-crystallin with Kyn in which the single Cys of a-crystallin was found to be the initial site of modification. Thiol compounds are known to be good nucleophiles. The same relative order of nucleophilicity of Cys, His, and Lys residues at physiological pH has been shown in other systems. For example, 4-hydroxynonenal, an oxidation product of lipids, reacts with proteins at nucleophilic sites through Michael addition.

The environment of Kyn appeared to have a marked effect on its stability. Thus, Kyn-t-Boc-Cys was more stable than Kyn-Cys, with the t-Boc on the α-amino group enhancing adduct stability by approximately threefold. The increased stability of Kyn-t-Boc-Cys might have resulted from the t-Boc group providing steric hindrance, the free α-amino group of Cys participating in base-catalyzed hydrolysis of Kyn-Cys, or both. Incubation in the presence of t-Boc-His also increased the rate of breakdown of Kyn-Cys (Fig. 5; see Fig. 2).

Decomposition of Kyn-Cys was accompanied by the release of deaminated Kyn. This unsaturated ketone appears to be involved in three further reaction pathways: intramolecular condensation of the aromatic amino group of deaminated Kyn with the unsaturated side chain, leading to the formation of Kyn yellow; reaction with the thiol group of Cys to re-form the Kyn-Cys adduct; or reaction with another molecule of deaminated Kyn, through intermolecular nucleophilic addition of the aromatic amino group to form a dimer of deaminated Kyn (Fig. 3). In the human lens, whose absorbance in the 360- to 370-nm region increases with age, formation of a dimer of deaminated Kyn is likely to be a minor reaction compared with the reaction of deaminated Kyn with lens proteins because His, Cys, and Lys side chains are present in higher concentrations than Kyn.

With t-Boc amino acids, it was demonstrated that the deaminated Kyn moiety can transfer from one amino acid to another. For example, incubation of Kyn-Cys with excess t-Boc-His at pH 7.2 resulted in a loss of Kyn-Cys and a corresponding increase in Kyn-t-Boc-His because of the release of deaminated Kyn from the breakdown of Kyn-Cys and its subsequent reaction with t-Boc-His (Fig. 5).

Formation of Kyn-amino acid adducts on lens proteins was also examined. Bovine crystallins were used because of their considerable sequence homology with human proteins. Bovine lenses do not contain UV filter compounds; therefore, the resultant CLPs are not modified by these compounds. Reaction of Kyn with CLP at pH 7.2 resulted in the formation of colored adducts.
TABLE 1. Sites of UV Filter Modification on Crystallins

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<th>Sequence</th>
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<tr>
<td>(R)PVLÇAHNSDR(K)</td>
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<td>βA1(T17)</td>
</tr>
<tr>
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<tr>
<td>(K)ÇLFEGAN(FK)</td>
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Kyn modification is indicated by underlined and bolded residues. Peptides were obtained after tryptic digestion (pH 6) of Kyn-modified lens proteins.

Lys is the most abundant (α, 7.8%; β, 5.6%; γ, 7.3%) followed by His (α, 4.6%; β, 3.5%; γ, 3.1%) and then by Cys (α, 0.3%; β, 1.9%; γ, 3.4%). Therefore, the pattern of modification observed is not simply a function of amino acid abundance. Local environment and solvent accessibility of nucleophilic residues in the proteins are likely to be major determinants.

The present findings suggest that, in the lens, Cys residues are the most reactive, binding to deaminated Kyn at a faster rate than that of His or Lys. It is proposed that the Kyn moiety attaches preferentially to Cys residues in lens proteins. However, after the attachment of Kyn to Cys, a proportion of this protein-bound Kyn is lost because of the intrinsic instability of the Kyn-Cys adduct (Scheme 2). The extent of this decomposition may be influenced by the degree of solvent exposure and the environment of the Cys residues. This decomposition is accelerated by glutathione in a concentration-dependent manner (Fig. 6). These studies emphasize the importance of maintaining high glutathione levels in the lens if posttranslational modification by UV filters is to be minimized. It is unknown whether processes that involve reactions of thiols may facilitate the oxidation of Cys residues that accompany age-related nuclear cataract.

In conclusion, the levels of Kyn attached to proteins reflect the relative nucleophilicity of amino acid residues and the stability of the Kyn-amino acid adducts and their environment.
These factors, together with the local concentration of glutathione in the lens, appear to determine the final degree of crystallin modification by this UV filter. Studies are under way with other UV filter compounds.

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**References**


