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# Photochemical and thermal reactions of kynurenines

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**Abstract. Kynurenine and its derivatives are present in the lens of the human eye where they function as the UV filters. Photochemical and thermal reactions of these compounds, which can result in irreversible modification of the lens proteins and play an important role in cataract development, are considered. The bibliography includes 123 references.**

## I. Introduction

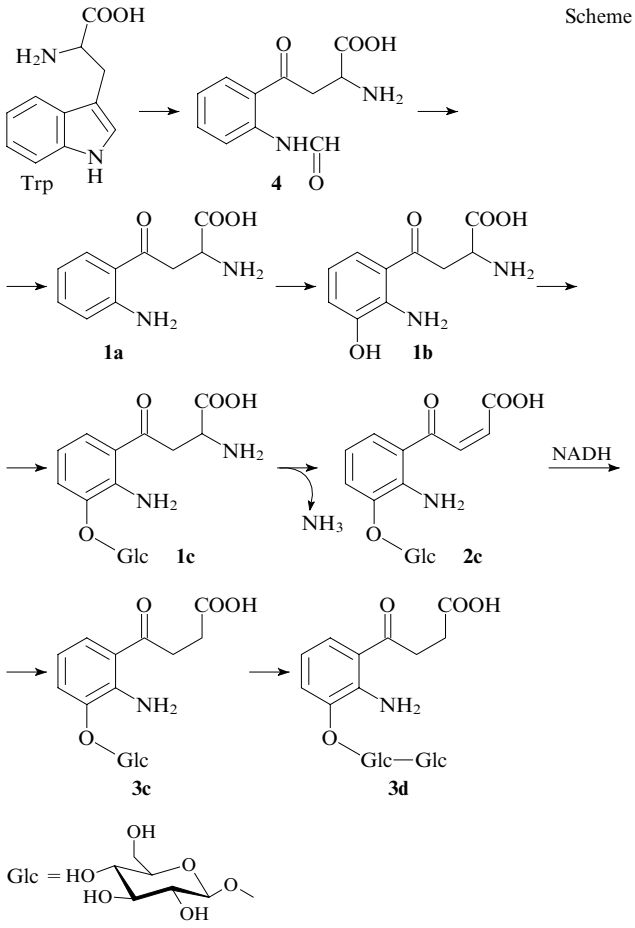
Cataract formation, the clouding of the eye's lens, nowadays is the major cause of blindness. More than half of the Earth population older than 65 develop this disease.[1] In recent years, ophthalmologists have noted that the onset of the disease is earlier, with patients 40 years old not uncommon. Since there is currently no medicine capable of restoring the lens transparency, surgical removal of the cataract is the only available treatment (if traumatic incidents are not considered).

The mechanism of cataract initiation and development remains unclear till now. The so-called senile (age-related) cataract is characterised by oxidation and modification of lens proteins (crystallins) resulting in their colouration, aggregation, cross-linking and loss of solubility.[2–6] There is no protein turnover in the centre of the lens, therefore protein modifications accumulate with age.

The main reason for crystallin modification is the oxidative stress.[4, 7, 8] The colouration of peptide chains and cross-linking suggest that post-translational modification of proteins is caused by reactions with small molecules.

A group of fluorescent low-molecular-weight compounds is present in the human eye; these compounds absorb most of the UV light penetrating the cornea in the region from 300 to 400 nm.[9–14] They are 2-amino-4-(2-aminophenyl)-4-oxobutanoic acid [kynurenine (**1a**)], 2-amino-4-(2-amino-3-hydroxyphenyl)-4-oxobutanoic acid [3-hydroxykynurenine (**1b**)], 2-amino-4-(2-amino-3-hydroxyphenyl)-4-oxobutanoic acid *O*-β-D-glucoside [3-hydroxykynurenine *O*-β-D-glucoside (**1c**)] and 4-(2-amino-3-hydroxyphenyl)-4-oxobutanoic acid *O*-β-D-glucoside (**3c**). These compounds are metabolites of tryptophan (Trp) (Scheme 1). In the lens, they act as UV filters protecting the retina and the lens itself from photo-

Scheme 1




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induced damage and improving visual acuity by eliminating chromatic aberrations. It has been suggested that attachment of these compounds to lens proteins can lead to protein colouration, cross-linking and, eventually, play an important role in the cataract development.

The concentration of the UV filters in the human lens varies significantly from individual to individual. Typically, there is a pronounced tendency for UV filter levels in the lens to decrease with age. As a result, the lenses of older people are less protected from photoinduced damage.[15] The concentration of **1c**, the major UV filter in primate lenses, varies in human lenses from 300 to 700 nmol $g^{-1}$ (for people below 20) to 100 nmol $g^{-1}$ and lower (for people above 80). Similar pattern is found for other UV filters. The average concentrations of kynurenine and some of its derivatives in the human lens are given below.[15]

| Compound | Concentration (nmol $g^{-1}$) in human lens | |
|---|---|---|
| | below 20 | above 80 |
| **1a** | 30 | 5 |
| **1b** | 15 | 5 |
| **3c** | 50 | 25 |

It is possible that the age-dependent decrease in UV filter concentrations in lenses is caused by the formation of the internal barrier between the lens cortex and nucleus, which occurs in middle age and prevents the diffusion of small molecules to and from the central region of the lens.[7,16,17] The barrier formation is likely to be caused by both the decrease in penetrability of the cell membranes and the disruption of the cell packing at the border cortex/nucleus. The existence of such a barrier slows down the movement of antioxidants to the lens centre, exposing the lens nucleus to additional oxidative stress. Indeed, the concentration of reduced glutathione (GSH), one of the main antioxidants in the human lens,[18] significantly decreases with age.[15,19] A significant decrease in the GSH concentration has been revealed in the cataractous lenses of humans[20,21] and experimental animals.[22–24]

It is known that GSH levels can substantially influence the oxidation processes.[25] Therefore, a decrease in the glutathione concentration, especially in the central region of the lens, can play an important role in the process of protein oxidation and cataract development. Besides, the unstable molecules (such as kynurenine and its derivatives), once penetrated into the lens nucleus, in the presence of the barrier reside there much longer and have more time to undergo chemical reactions, including the addition to the lens proteins. It has been shown that proteins extracted from human lenses contain a significant amount of kynurenines covalently bound to amino acid residues, in the first place to histidine and lysine; the concentration of protein-bound kynurenines increases with age markedly.[26,27]

Thus, the reactions of kynurenine and its derivatives can lead to significant modifications of the lens proteins, which in turn can cause cataract development. Establishing the detailed mechanisms of these reactions may help to clarify the molecular mechanisms of the cataract formation and to develop medicines for its prevention and treatment. The present review is devoted to the analysis of the literature data on thermal and photochemical reactions of kynurenine and its derivatives, primarily, the reactions related to the processes occurring in the eye's lens.

## II. Photochemical properties and reactions of kynurenines

UV radiation presents a risk factor for the majority of biological tissues, including the eye's lens.[28–37] The UV irradiation of proteins extracted from lenses results in the active generation of reactive oxygen species[38,39] and a shift of the absorption and fluorescence spectra of proteins to longer wavelengths.[40,41] The formation of radicals registered by optical methods[42,43] and ESR spectroscopy[44] is observed under UV irradiation of lens homogenates. Since most of the UV light reaching the lens is absorbed by kynurenines, it is natural to suppose that the modification of the lens proteins occurs due to the photochemical reactions involving kynurenines.[40,42,45–50]

Early studies[51] demonstrated that the majority of kynurenines are rather weak photosensitisers. It has been reported that the yields of singlet oxygen and superoxide $O_2^{-\bullet}$ are extremely low on UV irradiation of the oxygen-saturated aqueous kynurenine solutions.[51] Compounds **1b** and **1c** possess very short fluorescence lifetimes ($\tau_{\mathbf{1b}} < 0.01$ ns and $\tau_{\mathbf{1c}} = 0.032$ ns) and low fluorescence quantum yields ($\Phi_{\mathbf{1b}} < 0.001$, $\Phi_{\mathbf{1c}} = 0.035$).[43] The only exception is 2-amino-4-(2-formamidophenyl)-4-oxobutanoic acid [*N*-formylkynurenine (**4**), see Scheme 1] and its derivatives, which demonstrate noticeable photoactivity.[52–54]

The formation of the triplet state **4**($T_1$) with an absorption maximum at 440 nm and of a radical with an absorption maximum at 465 nm was found upon photolysis of aqueous solutions of compound **4**.[52–54] Oxygen quenches[54] the triplet state of *N*-formylkynurenine (**4**) with the rate constant $2 \times 10^9$ litre $mol^{-1}$ $s^{-1}$, the quantum yield of the singlet oxygen formation[51] is 0.17. Compound **4** in the triplet state also reacts with biological substrates such as tryptophan, tyrosine, cysteamine, adenosine, guanosine[54] with rate constants ranging from $3 \times 10^7$ to $3 \times 10^9$ litre $mol^{-1}$ $s^{-1}$.

One should notice that *N*-formylkynurenine (**4**) is an intermediate product of the enzymatic synthesis of kynurenine from tryptophan (Scheme 1), and its concentration in the human lens is extremely low. Kynurenines **1a–c**, the levels of which in the lens are fairly high, were considered for a long time to be photochemically inert. It was assumed that the energy of light absorbed by these compounds was directed into benign channels,[42,43,51] and ultimately converted into heat.

An analysis of photostability of kynurenine (**1a**)[55] has demonstrated that UV irradiation of aqueous kynurenine solutions under aerobic conditions results in kynurenine decay and the formation of products with absorption bands in the region of 400–500 nm. The quantum yield for photodegradation of compound **1a** upon irradiation by UV light in the range of 300–400 nm is $2 \times 10^{-5}$ under anaerobic conditions and $1.1 \times 10^{-4}$ (Ref. 56) in the presence of oxygen. In the presence of the antioxidant cysteine, the rate of the photodegradation decreases, which points to the oxidative character of photodegradation.[55]

It was established that photoexcited kynurenines reduce oxygen to form superoxide $O_2^{-\bullet}$, as well as cytochrome *c* and nitromethane.[55,57–59] Kynurenine (**1a**) may also play the role of photooxidant with respect to such compounds as

cysteine, sodium azide and ascorbate, EDTA, and NADH.[55] In the presence of oxygen, the rate of oxidation of these compounds significantly increases. The mechanisms of these reactions remain unknown. Reszka *et al.*[55] suppose that the photolysis products are more potent photosensitisers than kynurenine itself.

Recently [60] it was discovered that photolysis of aqueous solutions of kynurenine (**1a**) leads to the formation of a triplet state **1a**($T_1$). The quantum yield for this process ($\Phi_T$) strongly depends on pH: in acidic media (pH 0.1) it is close to unity, while in neutral solution the value of $\Phi_T$ is 0.018. Very likely, this difference is determined by the different properties of the protonated and deprotonated forms of singlet excited kynurenine: the main channel for decay of the protonated form is the intersystem crossing to the triplet state, while for the deprotonated form other processes (fluorescence, radiationless transitions) are the major pathways. In the same paper, spectra of triplet kynurenine in neutral and acidic media were obtained, and the value of the acid-base equilibrium constant ($pK_a = 4.7$) for the triplet state was measured.

Further investigations have shown that under intense laser irradiation of solutions of kynurenine (**1a**) and its 3-hydroxy derivative **1b**, double-quantum photoionisation takes place.[61] The triplet state, which absorbs the second quantum of light and decays yielding a radical cation and a solvated electron, is a precursor for photoionisation. The ejected electron can add to a molecule of the starting material with the rate constant $2.0 \times 10^{10}$ litre mol$^{-1}$ s$^{-1}$ to form a radical anion.[61,62] The spectra obtained for the triplet states and radicals are presented in Figs 1 and 2, and the main spectroscopic features of these intermediates are listed in Tabl. 1.

From a biological viewpoint, an essential question is the reactivity of the kynurenine triplet state toward molecules present in the eye's lens: antioxidants, proteins, amino acids. Triplet kynurenine quenching by amino acids and antioxidants was studied,[63] and the quenching rate constants are listed in Tabl. 2. It was revealed that the quenching proceeds by electron transfer from the quencher molecule to the triplet kynurenine molecule. The highest values of the quenching rate constant were obtained for tryptophan and tyrosine, as well as for ascorbate anion,

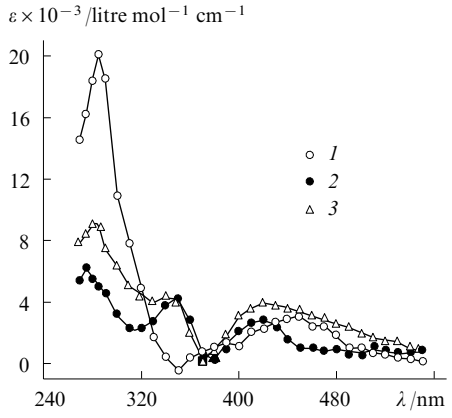



**Figure 1.** Electronic absorption spectra of intermediates formed in the photolysis of kynurenine (**1a**) in a neutral buffer (pH 7.0).
Radical anion (*1*), radical cation (*2*), triplet (*3*).

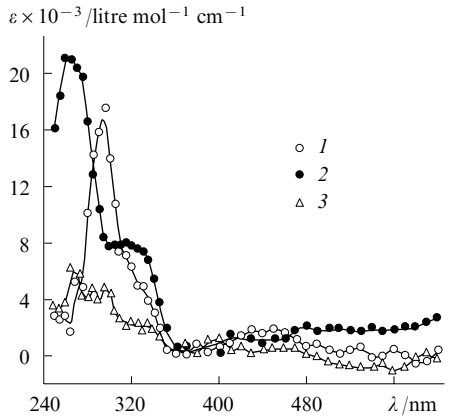



**Figure 2.** Electronic absorption spectra of intermediates formed in the photolysis of 3-hydroxykynurenine (**1b**) in a neutral buffer (pH 7.0).
Radical anion (*1*), radical cation (*2*), triplet (*3*).

**Table 1**. Spectral features of kynurenines and their intermediates.

| Compound | State | $\lambda_{max}$ /nm | $\varepsilon_{max}$ /litre mol$^{-1}$ cm$^{-1}$ |
|---|---|---|---|
| **1a** | ground state | 225 | 22000 |
| | | 260 | 7200 |
| | | 360 | 4500 |
| | radical cation | 275 | 6100 |
| | | 350 | 4100 |
| | | 420 | 2800 |
| | radical anion | 285 | 20000 |
| | | 450 | 3100 |
| | triplet state | 280 | 9100 |
| | | 340 | 4430 |
| | | 430 | 3700 |
| **1b** | ground state | 230 | 19000 |
| | | 270 | 8000 |
| | | 370 | 4300 |
| | radical cation | 260 | 21200 |
| | | 315 | 8000 |
| | radical anion | 295 | 17600 |
| | | 450 | 2000 |
| | triplet state | 265 | — |
| | | 295 | — |

**Table 2**. Rate constants $k_q$ for the triplet kynurenine quenching by amino acids and antioxidants.

| Amino acid or antioxidant | $k_q$ /litre mol$^{-1}$ s$^{-1}$ |
|---|---|
| L-Ascorbate | $(8.5 \pm 1.2) \times 10^8$ |
| L-Glutathione reduced | $< 10^6$ |
| *N*-Acetyl-L-tryptophan | $(3.3 \pm 0.6) \times 10^8$ |
| L-Tryptophan | $(2.6 \pm 0.7) \times 10^8$ |
| *N*-Acetyl-L-tyrosine | $(6.7 \pm 1.5) \times 10^7$ |
| L-Cysteine | $< 10^6$ |
| *N*-Acetyl-L-histidine | $< 10^6$ |
| *N*-Acetyl -L-methionine | $< 10^6$ |

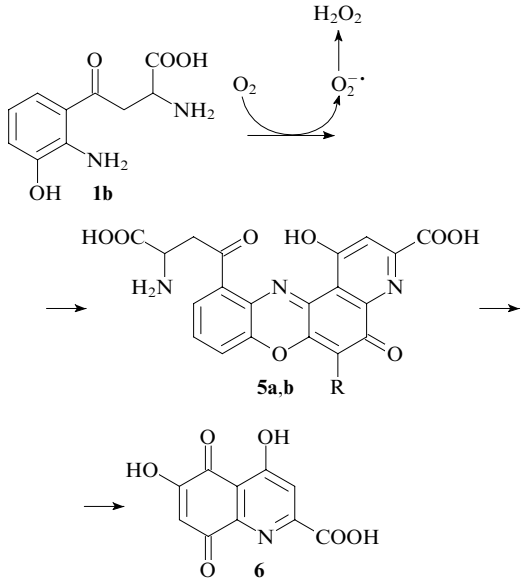
**Note**. The rate constant for the triplet kynurenine quenching by oxygen is equal to $(2.1 \pm 0.5) \times 10^9$ litre mol$^{-1}$ s$^{-1}$.

which is an antioxidant present in the human and animal lenses in high concentrations.[64] Consequently, the amino acid residues tryptophan and tyrosine in the lens proteins are the most vulnerable against oxidation induced by photoexcited UV filter molecules, and ascorbate plays an important role in the protection of the lens proteins against photoinduced modifications.

## III. Thermal reactions of kynurenines

### 1. Autoxidation of 3-hydroxykynurenine

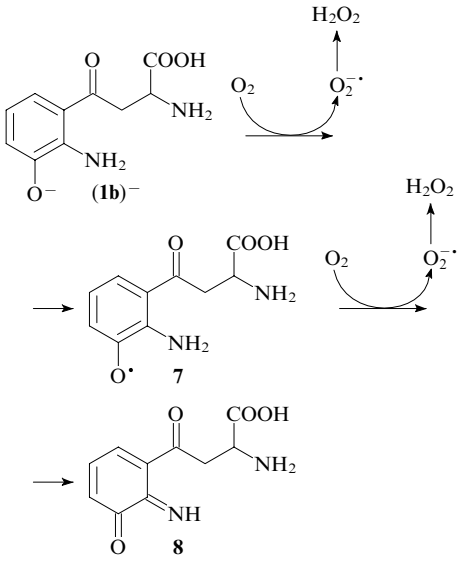
3-Hydroxykynurenine is a neurotoxic compound,[65] it plays a significant role in the development of some severe diseases such as Huntington's and Parkinson's diseases.[66–68] This compound comprises an *o*-aminophenol moiety, and under aerobic conditions it is readily oxidised.[69–71] Autoxidation of 3-hydroxykynurenine (**1b**) results in the formation of numerous coloured products[71] including xanthommatin (**5a**), hydroxyxanthommatin (**5b**) and 2-carboxy-4,6-dihydroxyquinoline-5,8-quinone (**6**).




R = H (**5a**), OH (**5b**).

It was found that incubation of lens proteins in the presence of compound **1b** under aerobic conditions results in the formation of oxidised forms of **1b** bound to proteins.[72,73] Unlike UV filters, the oxidation products of **1b** absorb light in the visible region of the optical spectrum, therefore their presence in the lens may colour proteins yellow, which is one of the main indicators of cataract development. It was also revealed that the formation of hydrogen peroxide and hydroxyl radicals occurs in the autoxidation of 3-hydroxykynurenine (**1b**),[71,74–76] which explains the toxicity of this compound.

It was suggested that hydrogen peroxide is formed due to oxygen reduction by the phenoxide anion $(\mathbf{1b})^-$ to superoxide $O_2^{-\cdot}$ followed by superoxide dismutation into $H_2O_2$.[71]




The resulting semiquinone radical **7** and/or quinone imine **8** are involved in condensation reactions yielding compounds **5a** and **5b**.

It was shown[77–79] that the incubation of lens proteins with 3-hydroxykynurenine (**1b**) under aerobic conditions results in the irreversible oxidation of amino acid residues in proteins (primarily, methionine and tryptophan residues[79]) and protein colouration. Interactions of 3-hydroxykynurenine (**1b**) with copper and iron, as well as with α-crystallins, the most important proteins in the lens, were also studied.[80] It was demonstrated that compound **1b** reduces Cu(II) and Fe(III) to Cu(I) and Fe(II), generates hydrogen peroxide and favours cross-linking in molecules of α-crystallins in a copper-dependent manner.

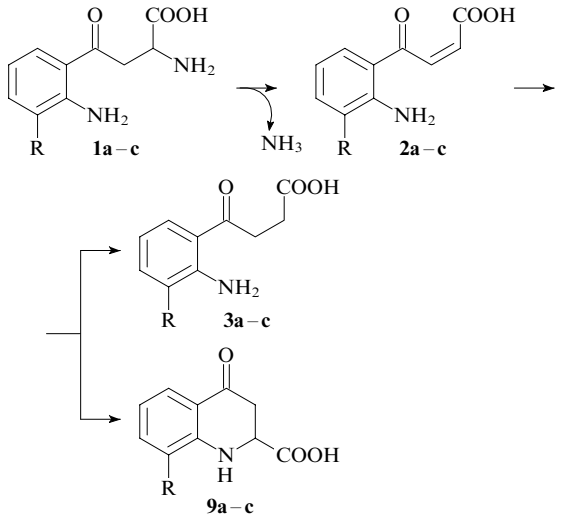
3-Hydroxykynurenine (**1b**) is characterised by the lifetime of approximately 3 h at 35 °C and pH 7 in an oxygen-saturated aqueous solution.[71] Taking the solubility of oxygen in water equal to 1 mmol litre$^{-1}$, one can estimate[81] the rate constant for the reaction of **1b** with oxygen as $k_{O_2} \approx 0.1$ litre mol$^{-1}$ s$^{-1}$. Apparently, in biological media the oxidation rate will be determined by the free oxygen concentration. Oxygen enters the lens *via* diffusion from the surrounding humour, and a significant part of oxygen is consumed in mitochondria for ATP production. The partial oxygen pressure $P_{O_2}$ in the aqueous humour is about 38 mmHg,[82] while in the lens it is much lower.[5,83] Recent studies have shown that $P_{O_2}$ falls below 2 mmHg in the lens nucleus.[84,85] One can assume that under such anaerobic conditions the time scale for the autoxidation of compound **1b** will be at least several weeks. However, the situation becomes much worse if the internal lens cells come into the contact with oxygen-saturated liquids (for example, during lens surgery). In this case, autoxidation of hydroxykynurenine (**1b**) is significantly accelerated. The extremely high percentage (from 50% to 80%) of post-surgery cataract development[86–88] indirectly confirms the hypothesis that oxidation of **1b** plays an important role in the cataract development.

## 2. Deamination of kynurenines

Synthesis of the UV filters in the human lens starts at the birth, or very soon thereafter. The major UV filters in the lens are tryptophan metabolites (enzymatic reaction products) (see Scheme 1). 3-Hydroxykynurenine glucoside (**1c**) is the main UV filter in primates. In the human lens, the amount of this UV filter also prevails over other kynurenine filters.[9]

Recent studies have shown that kynurenine and its derivatives containing the amino acid side chain are intrinsically unstable and undergo spontaneous side chain deamination (Scheme 2) to form unsaturated oxo acids **2**: 2-amino-4-(2-aminophenyl)-4-oxobut-2-enoic acid (**2a**), 2-amino-4-(2-amino-3-hydroxyphenyl)-4-oxobut-2-enoic acid (**2b**), 2-amino-4-(2-amino-3-hydroxyphenyl)-4-oxobut-2-enoic acid *O*-β-D-glucoside (**2c**). The double bond formed in compounds **2a**–**c** is easily reduced. It has been shown[89] that the formation of a new UV filter, namely, compound **3c**, the second most abundant UV filter, occurs from the major UV filter **1c** by the mechanism described above.

Scheme 2




R = H (**a**), OH (**b**), OGlc (**c**).

Study[89] of a model deamination reaction of D,L-α-amino-β-benzoylpropionic acid, which has the same amino acid side chain as compound **1c**, confirmed that UV filter **3c** is formed from compound **1c** by slow non-enzymatic elimination of ammonia from the side chain, with formation of the corresponding unsaturated oxo acid **2c** followed by the reduction of the double bond. Two other UV filters, *viz.*, **1a** and **1b**, also contain the amino acid side chain, therefore one can expect that similar reactions leading to the synthesis of new UV filters are typical of these compounds as well.

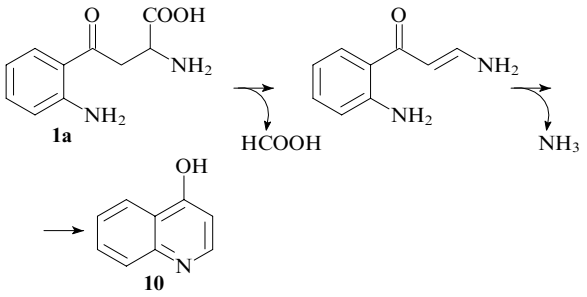
Further studies[56,90] confirmed that under physiological conditions (pH 7, $T = 37$ °C) compounds **1a**–**c** are indeed unstable and undergo deamination and decarboxylation reactions.

A key intermediate in the thermal decomposition of UV filter **1** is unsaturated oxo acid **2**, which is highly susceptible to nucleophilic addition. In the absence of nucleophiles, the main reaction of unsaturated oxo acid **2** is cyclisation (see Scheme 2) resulting in the formation of cyclic kynurenine derivatives: 4-oxo-1,2,3,4-tetrahydroquinoline-2-carboxylic acid (**9a**), 8-hydroxy4-oxo-1,2,3,4-tetrahydroquinoline-2-carboxylic acid (**9b**) and 8-hydroxy-4-oxo-1,2,3,4-tetrahydroquinoline-2-carboxylic acid *O*-β-D-glucoside (**9c**) (Refs 56, 90–93).

The synthesis of compound **9a** *in vitro* was first described in the 1960s.[92,94] It was shown that this compound is formed from unsaturated oxo acid **2a** due to an intramolecular Michael addition of the nucleophilic aromatic 2-amino group to the activated double bond C=C of the side chain. In a later study,[90] the formation of cyclic kynurenines **9a**–**c** was observed in the incubation of UV filters **1a**–**c**. The highest yield of the cyclic product was observed for kynurenine (**1a**), and the lowest yield for its derivative **1c**. The authors attributed the decrease in the cyclisation rate in the series **1a** > **1b** > **1c** to an increase in steric hindrance at the *meta*-position of the aromatic ring.

Further studies demonstrated that the yields of cyclic products **9a** and **9b** in their *in vitro* synthesis† from kynurenine (**1a**) and hydroxykynurenine (**1b**) are 26% and 22%, respectively.[95] Kopylova *et al.*[93] reported that the rate constant for the cyclisation of deaminated kynurenine **2a** ($k_{cycl}$) is $(1.6 \pm 0.4) \times 10^{-6}$ s$^{-1}$.

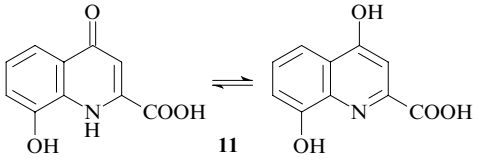
Decarboxylation of kynurenine (**1a**) affords cyclic 4-hydroxyquinoline (**10**).




Since the rate constant for this reaction is approximately 20 times smaller than the rate constant for deamination,[56] one can assume that decarboxylation does not play a significant role in the UV filter transformations.

A study of the temperature dependence of the deamination rate constant[56] gave the activation energy $E_a = 121 \pm 14$ kJ litre mol$^{-1}$ and the frequency factor $\log(A) = 13.6 \pm 0.8$ for the reaction. Extrapolation of the temperature dependence to the physiological temperature of 37 °C gives a value of the deamination rate constant $k_{deam}(37\ ^\circ\text{C}) = 1.5 \times 10^{-7}$ s$^{-1}$. This allows estimation of the lifetime of kynurenines under physiological conditions to be ~2–3 months. In basic solutions the deamination reaction of kynurenine is accelerated.[90,91]

Cyclisation of 2-amino-4-(2-amino-3-hydroxyphenyl)-4-oxobutanoic acid (**1b**) can also lead to 8-hydroxy-4-oxo-1,4-dihydroquinoline-2-carboxylic acid [xanthurenic acid (**11**)].[96]




Two possible mechanisms for the formation of this compound are discussed in the literature, *viz.*, the spontaneous

† It should be noticed that cyclic compounds **9a**–**c** have not been found in either normal or cataractous human lenses.[95]

deamination of 3-hydroxykynurenine (**1b**) followed by cyclisation [96] and direct transformation of compound **1b** into acid **11** under the action of the enzyme kynurenine aminotransferase.[97,98] Xanthurenic acid (**11**) was found in cataractous lenses;[98,99] however, its presence in normal human lenses has not been proven.[100]

Xanthurenic acid is a better chromophore than the kynurenines. It was established that the quantum yield for singlet oxygen formation in the aerobic photolysis of acid **11** in methanol is 0.17, while for kynurine it is only 0.006.[101] Theoretical calculations [102] demonstrate that the reactions of the triplet xanthurenic acid can result in the formation of singlet oxygen, superoxide $O_2^{-\cdot}$ and radical cations and anions of xanthurenic acid. Xanthurenic acid can oxidise proteins and promote their aggregation and denaturation due to either photochemical reactions [103] or thermal addition to the amino acid residues of proteins.[104,105] More detailed data are currently absent regarding the photochemical properties of acid **11** and its analogues and the role they play in cataract development.

The most important feature of the deamination products of kynurenine derivatives is their ability to add to nucleophilic amino acid residues: histidine, lysine and cysteine. The rate constants for the addition of unsaturated oxo acid (**2a**) to these compounds determined in the incubation of compound **2a** with the amino acids and antioxidants present in the human lens are listed in Tabl. 3.[93] It was found that the rate constants for the addition of **2a** to thiols like cysteine and reduced glutathione are several orders of magnitude higher than to other compounds.[90,93,106] This implies that cysteine residues in proteins are the most susceptible to modification by deaminated UV filters.[107,108]

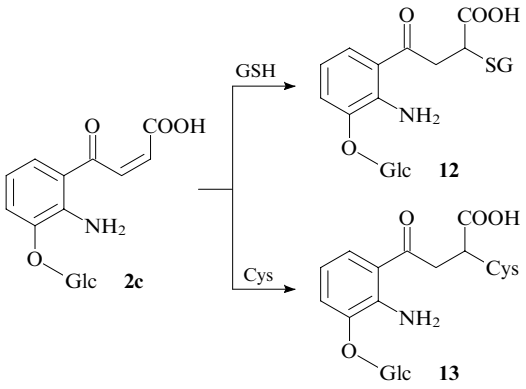
**Table 3**. Rate constants for the reactions of unsaturated oxo acid **2a** with amino acids and antioxidants under physiological conditions ($T = 37$ °C and pH 7.0).

| Second reactant | Type of the reaction | Reaction rate constant /litre mol$^{-1}$ s$^{-1}$ |
|---|---|---|
| – | cyclisation **2a** | $(1.6 \pm 0.4) \times 10^{-6}$ (see [a]) |
| L-Ascorbic acid | addition | $(8.6 \pm 2.4) \times 10^{-5}$ |
| NADH | reduction | $(8.2 \pm 2.5) \times 10^{-5}$ |
| *N*-Acetyl-L-histidine | addition | $(1.2 \pm 0.3) \times 10^{-4}$ |
| D,L-Lysine | " | $(2.3 \pm 0.6) \times 10^{-4}$ |
| *N*-Acetyl-L-methionine | no reaction | – |
| *N*-Acetyl-L-tyrosine | the same | – |
| *N*-Acetyl-L-tryptophan | " | – |
| L-Glutathione | addition | $2.1 \pm 0.2$ |
| D,L-Cysteine | " | $36 \pm 4$ |

[a] Rate constant in s$^{-1}$.

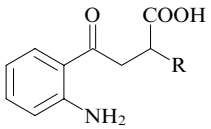
The Arrhenius parameters for the addition reactions of compound **2a** to cysteine and glutathione were determined:[93] $A_{Cys} = (2.7 \pm 0.9) \times 10^8$ litre mol$^{-1}$ s$^{-1}$, $E_a(Cys) = 40.4 \pm 5.7$ kJ litre mol$^{-1}$, $A_{GSH} = (1.8 \pm 0.7) \times 10^5$ litre mol$^{-1}$ s$^{-1}$, $E_a(GSH) = 29.2 \pm 5.6$ kJ litre mol$^{-1}$. A significant difference in the frequency factors $A_{Cys}$ and $A_{GSH}$ suggest the important role of steric factors in the addition reaction, which is why the addition reaction of deaminated kynurenine **2a** to the more bulky glutathione molecule proceeds significantly more slowly than to cysteine. Hence, one can expect that the rate constant for the reaction of unsaturated oxo acids **2** with cysteine residues buried inside the protein globule may be several orders of magnitude smaller than that with free cysteine.

The protection of the lens proteins against modification by deaminated UV filters **2** is provided by glutathione, which can bind reactive molecules such as **2c** with formation of a new UV filter **12**.[109] The reaction of compound **2c** with free cysteine affords yet another UV filter, compound **13**, found in the human lens.[110]




It was established that the reactions of unsaturated oxo acids **2** with the majority of amino acids and antioxidants follow the addition mechanism, NADH being the only exception. In this case, the reaction with **2** results in the reduction of the double bond in molecule **2**.[89,90,93] Apparently, UV filters **3c**, **3d** and their analogues, which are found in the human lens,[15,89,95,111,112] are formed in this reaction.

The formation of adducts of unsaturated oxo acids **2** (for example, **2a**) to glutathione and amino acids (compounds **14**–**17**) is a reversible reaction: the adducts are unstable under physiological conditions and dissociate to the starting compounds.[93,113,114] Among the adducts of compound **2a** to amino acids Cys, Lys and His, the least stable is compound **15**.[113,114]




**14**–**17**

R = GSH (**14**), Cys (**15**), Lys (**16**), His (**17**).

Modification of the lens proteins by deaminated UV filters is a complex dynamic process, in which the same molecule **2** can reversibly add several times to different amino acid residues and/or antioxidants present in the lens. The ability of the organism's defense systems to protect the lens proteins from irreversible changes is determined mostly by the concentration of reduced glutathione (the most important antioxidant in the human eye [18]) inside the lens. It is known that during cataractogenesis, the concentration of GSH in the lens, especially in its central region, significantly decreases.[15,19–24] This noticeably increases the probability of irreversible modification of proteins by deaminated UV filters.

The importance of GSH for neutralising deaminated UV filters **2** was confirmed in our recent studies.[115] For the first time, we experimentally detected deaminated UV filter **2c** in

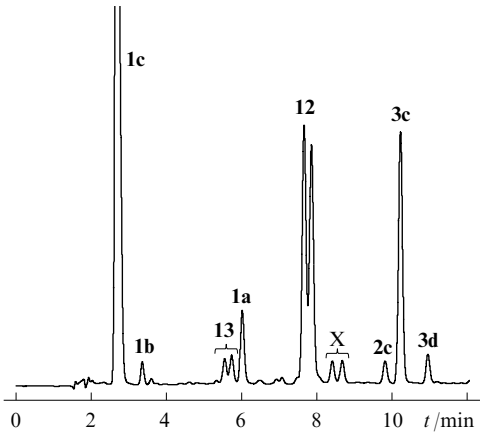



**Figure 3.** Chromatogram of protein-free extracts from the cataractous lens of a 65-years old patient.
X is unknown compound.

protein-free extracts from the cataractous human lenses (Fig. 3). It was established that compound **2c** is observed in lenses with very low natural levels of glutathione (about 0.5 mmol litre$^{-1}$).‡ The addition of glutathione to the extract resulted in disappearance of the signal of compound **2c** from the chromatogram.

The reactions of kynurenine and its derivatives directly with lens proteins have been studied by a number of researchers (see, for example, Refs 26, 77, 79, 116–119). It was established that during the incubation of the lens proteins with kynurenine (**1a**) deaminated kynurenine **2a** adds primarily to the cysteine residues.[116] However, analysis of crystallins extracted from the cataractous human lenses[113, 120, 121] indicated that the most susceptible to modification by UV filters residues are lysine and histidine. This result confirms that the addition of deaminated UV filters to amino acid residues of proteins is a reversible reaction and the stability of the adducts thus formed plays an even larger role than the rate of UV filter addition to a specific residue.

It is important to note that the post-translational modification of the lens proteins by UV filters not only violates their functionality, but also increases the susceptibility of the proteins to photodamage.

Upon irradiation of the modified proteins with UV light, the formation of hydrogen peroxide is observed. In addition, the amount of crystallin-bound tyrosine oxidation products, *viz*., dityrosine and 3,4-dihydroxyphenylalanine, increases upon irradiation.[119, 122] These results show that protein-bound kynurenines are better photosensitisers than free UV filters, and UV irradiation of modified proteins can lead to further damage either due to direct photoreaction or the formation of reactive oxygen species such as singlet oxygen and superoxide.

In order to validate this hypothesis, we have measured[123] the quantum yields of photodecomposition of kynurenine and its adducts to amino acids, as well as the triplet state quantum yield for these compounds, under both aerobic and anaerobic conditions (Tabl. 4). Indeed, the highest quantum yields of the triplet state and anaerobic photodecomposition were observed for the adducts of histidine (**17**) and cysteine (**15**), as well as for the adduct of glutathione to compound **2a** (**14**).

‡ Glutathione level in normal lenses is an order of magnitude higher.[15]

**Table 4**. Photodecomposition quantum yields of different UV filters under anaerobic ($\Phi_{Ar}$) and aerobic ($\Phi_{O_2}$) conditions and triplet state quantum yields ($\Phi_T$).

| Compound | $\Phi_{Ar} \times 10^5$ | $\Phi_{O_2} \times 10^5$ | $\Phi_T \times 10^2$ |
|---|---|---|---|
| **1a** | $1.0 \pm 0.4$ | $3.5 \pm 0.4$ | $1.8 \pm 0.4$ |
| **1b** | $< 1$ | $3.8 \pm 0.8$ | $< 0.5$ |
| **14** | $7.3 \pm 1.6$ | $1.1 \pm 0.2$ | $3.4 \pm 0.7$ |
| **15** | $6.6 \pm 1.3$ | $1.1 \pm 0.2$ | $2.5 \pm 0.6$ |
| **16** | $3.3 \pm 0.6$ | $4.2 \pm 0.8$ | $1.8 \pm 0.4$ |
| **17** | $9.0 \pm 1.8$ | $8.5 \pm 1.1$ | $3.4 \pm 0.6$ |
| **13** | $< 1$ | $9.0 \pm 1.8$ | |

## IV. Conclusion

The material presented here illustrates that both thermal and photochemical reactions of molecular UV filters — kynurenine and its derivatives — can lead to lens protein modification. Autoxidation of 3-hydroxykynurenine (**1b**) in the lens can result in the formation of hydrogen peroxide and hydroxyl radicals, as well as coloured reaction products; however, these processes can occur only in the presence of oxygen, *i.e*., either at the peripheral part of the lens or upon direct contact of the lens with ambient air due to trauma or surgical procedure. Consequently, autoxidation of 3-hydroxykynurenine may play an important role in development of post-traumatic and post-surgical cataracts, but is unlikely to be of significance for the development of the nuclear age-related cataract.

The spontaneous deamination of the 'primary' UV filters **1a**–**c** and the decomposition of the 'secondary' UV filters, the addition products of deaminated kynurenine and its derivatives to glutathione and amino acids, results in the formation of highly reactive unsaturated oxo acids **2** capable of binding to the nucleophilic amino acid residues of proteins. Irradiation of the UV filters gives rise to the formation of triplet states, and the triplet quantum yield for protein-bound UV filters is higher than that for free UV filter molecules.

Protection of lens proteins from photochemical damage and from thermal decomposition of kynurenine and its derivatives is provided by antioxidants, *e.g*., ascorbic acid, which can quench the triplet states of kynurenines, or reduced glutathione, which binds highly reactive molecules **2**. The decrease in concentration of these antioxidants in the eye's lens presents a significant risk factor for cataract development.

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