

A new mechanism of the photochemical oxidation of tryptophan sensitised with the uranyl ion

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It has been found that tryptophan oxidation sensitised with the uranyl ion occurs as a chain reaction of $\text{Trp}^{+\bullet}$ cation radicals with O_2 .

The photooxidation of tryptophan (Trp) used to receive much attention¹ since, along with other aromatic amino acids, such as tyrosine, phenylalanine, and cystine, it is one of the main chromophores determining the photochemical reactions of proteins. The currently accepted mechanism² involves ion-radical photooxidation of Trp in the reaction of its cation radical ($\text{Trp}^{+\bullet}$) with the oxygen superoxide ion ($\text{O}_2^{\bullet-}$). The main steps of tryptophan photooxidation are:³ formation of a $\text{Trp}^{+\bullet}$ cation radical and a hydrated electron (e_{aq}) upon photolysis followed by its reaction with oxygen to give an $\text{O}_2^{\bullet-}$ superoxide ion. The reaction of $\text{O}_2^{\bullet-}$ with $\text{Trp}^{+\bullet}$ via an intermediate dioxetane (D) gives *N*-formylkynurenin (FK).

In an electronically excited state, UO_2^{2+} and Trp become strong oxidising and reducing species, respectively, and can initiate intracomplex photoinduced electron transfer (PET).^{4,5} As noted previously,⁶ the rate of Trp photooxidation increases if uranyl is selectively excited; this leads to the assumption that a chain reaction of $\text{Trp}^{+\bullet}$ cation radicals with oxygen is possible.⁷

This paper deals with a study of the mechanisms of Trp photooxidation in complexes with UO_2^{2+} upon selective excitation of the latter.[†]

By determining the yield of Trp photooxidation, it is possible to determine, which mechanism, namely, reaction of $\text{Trp}^{+\bullet}$ with $\text{O}_2^{\bullet-}$ superoxide ion ($\phi \leq 1$) or chain reaction of cation radicals with O_2 ($\phi > 1$), occurs upon selective excitation of the electron donor (Trp) and acceptor (UO_2^{2+}). The quantum yield of photooxidation of free Trp [the primary photochemical act is $\text{Trp}^* \rightarrow \text{Trp}^{+\bullet} + e_{\text{aq}}$ ($\Delta\lambda_{\text{excit.}} = 240\text{--}400\text{ nm}$)] that we determined in the substrate conversion range from 5 to 20 mol% is 1.0, in agreement with other data: $\phi = 0.97$ and 1.14 at $c(\text{Trp}) = 10^{-3}$ and $10^{-2}\text{ mol dm}^{-3}$, respectively.¹

[†] The quantum yield of Trp photooxidation was determined according to a published procedure.¹³ Aerated Trp solutions ($0.01\text{ mol dm}^{-3}\text{ HClO}_4$) kept at constant temperature (298 K) were irradiated in a quartz cell using a DKSSh-1000 xenon lamp (LOS-2 illuminator) through UFS-5 ($\Delta\lambda_{\text{excit.}} = 240\text{--}400\text{ nm}$) and SZS-17 optical filters ($\lambda_{\text{excit.}} > 320\text{ nm}$). The amount of absorbed light was determined using a ferrioxalate dosimeter prepared by a reported procedure;¹⁴ its quantum yield and fraction of absorbed light vary insignificantly in the irradiation wavelength range studied.¹³ Since wide-band optical filters were used for irradiation rather than a monochromatic light source, the emission spectrum of the lamp and the transmittance spectrum of the optical filter were taken into account in determinations of the absorbed light intensity. The concentration of Trp that underwent the reaction was monitored spectrophotometrically using a Specord M-40 spectrophotometer. Analytical grade D-tryptophan was recrystallised from twice-distilled water and dried *in vacuo*. Analytical grade $\text{UO}_2(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ was used without preliminary purification. The concentrations of Trp and UO_2^{2+} were $10^{-4}\text{ mol dm}^{-3}$.

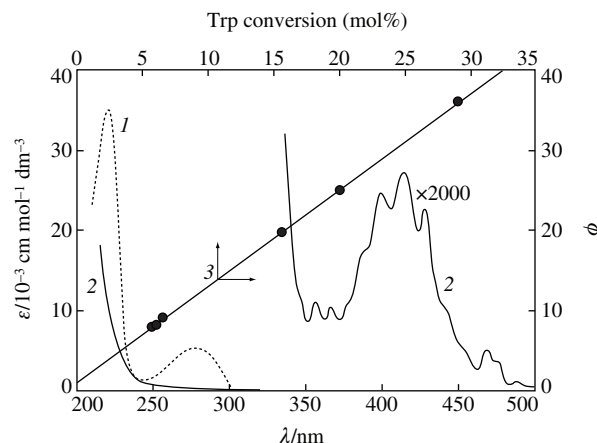
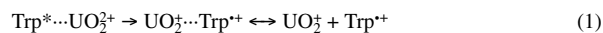


Figure 1 Absorption spectra: (1) Trp; (2) UO_2^{2+} . Plot of ϕ vs. degree of conversion for Trp photooxidation (3) [$c(\text{Trp}) = 10^{-4}\text{ mol dm}^{-3}$, $c(\text{UO}_2^{2+}) = 10^{-4}\text{ mol dm}^{-3}$, $l = 1\text{ cm}$, 0.01 M HClO_4 , 298 K].

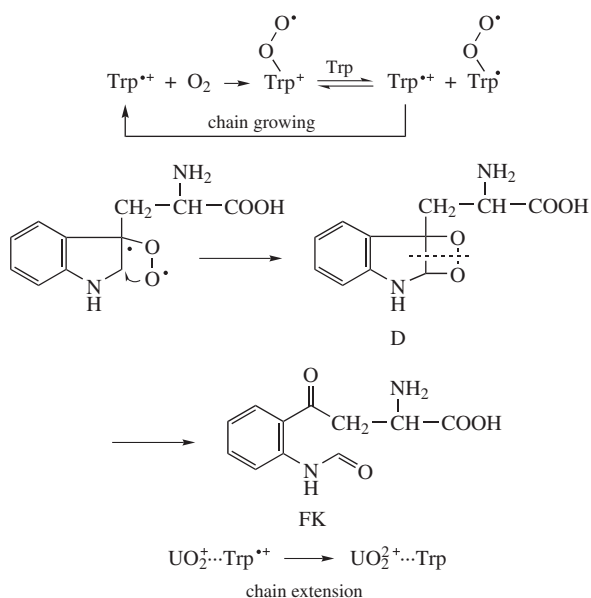
In the presence of uranyl ion, the ϕ of Trp photooxidation upon excitation with light $\Delta\lambda_{\text{excit.}} = 240\text{--}400\text{ nm}$ increases to 1.5 ($\phi > 1$). We failed to strictly fulfill the condition of selectivity of Trp excitation in the $\text{UO}_2^{2+}\cdots\text{Trp}$ complex (Figure 1), since uranyl partially (35%) absorbs light, and the increase in the quantum yield is probably due to the simultaneous occurrence of primary PET photochemical reactions:



On the contrary, the optical excitation selectivity of uranyl ion is possible, since Trp does not absorb light at $\lambda_{\text{excit.}} > 320\text{ nm}$ (Figure 1). The quantum yield of Trp photooxidation sensitised with uranyl ($\lambda_{\text{excit.}} > 320\text{ nm}$) is $\phi = 25$, *i.e.*, higher than unity, which indicates that chain processes occur. Like in equation (1), the cation-radical chain oxidation of Trp photosensitised with uranyl ion is based on the primary PET process. However, after the $\text{UO}_2^+ \cdots \text{Trp}^{+\bullet}$ ion radical pair has formed, secondary photochemical reactions occur according to another mechanism (Scheme 1).

The $\text{Trp}^{+\bullet}$ cation radical adds oxygen to give the $\text{TrpO}_2^{+\bullet}$ cation peroxy radical. The TrpO_2 peroxy biradical is formed in the reaction of $\text{TrpO}_2^{+\bullet}$ with Trp; this reaction also gives a new $\text{Trp}^{+\bullet}$ cation radical (chain growth). The TrpO_2 peroxy biradical undergoes cyclisation to dioxetane D, decomposition of which results in FK.

The difference between the mechanisms of Trp photooxidation is probably due to the specifics of the formation of the coordination sphere of $\text{UO}_2^{2+}\cdots\text{Trp}$ complexes upon selective



Scheme 1

excitation of the metal ion and ligand, which is also inherited by the $\text{UO}_2^{\bullet+}\cdots\text{Trp}^{\bullet+}$ ion-radical pair. It is well known that uranyl forms complexes with amino acids⁸ and UO_2^{2+} excitation is accompanied by an abrupt increase (by a few orders of magnitude) in their stability⁹ owing to an increase in the contribution of $5f$ orbitals to donor–acceptor interactions. For adamantylidene-adamantane, which does not form complexes with UO_2^{2+} , uranyl-photosensitised oxidation^{10–12} occurs with $\varphi = 0.55 < 1$, which rules out the possibility of chain processes. Hence, adamantylideneadamantane-1,2-dioxetane is formed in the reaction of the substrate cation radical with $\text{O}_2^{\bullet-}$ superoxide ion.

It can be assumed that, during selective intracomplex $\text{Trp}\cdots\text{UO}_2^{2+}$ photoexcitation of the amino acid [equation (1)], oxidation occurs in the solvate-separated ion-radical pair $\text{UO}_2^{\bullet+} + \text{Trp}^{\bullet+}$ and the uranyl ion is oxidised to UO_2^{3+} due to the transfer of an electron to oxygen to give $\text{O}_2^{\bullet-}$, which reacts with $\text{Trp}^{\bullet+}$ to give FK. On the contrary, uranyl ion-photosensitised [equation (2)] cation-radical chain oxidation of Trp (Scheme 1) occurs in an intracomplex way and FK remains in the coordination sphere of $\text{UO}_2^{\bullet+}$, as follows from the shifts in the excitation and fluorescence spectra.⁶

However, both mechanisms of Trp photooxidation in the presence of the uranyl ion are not mutually exclusive. In case of cooperative photoexcitation of Trp and UO_2^{2+} ($\lambda_{\text{excit.}} > 220 \text{ nm}$), $\varphi = 2.7$ [by an order smaller than φ (*UO_2^{2+}) but two times higher than φ (*Trp)], which reflects the competition of the two oxidation processes.

The above φ values for Trp photooxidation were obtained at 20 mol% amino acid conversion. However, while the photo-

oxidation quantum yield in the case of selective Trp excitation does not depend on the concentration of the product formed, the φ value increases linearly from 8 to 36 when the Trp conversion changes from 6 to 30 mol% in the case of uranyl excitation (Figure 1).

Thus, uranyl ion-photosensitised oxidation of tryptophan involves the chain reaction (Scheme 1) of $\text{Trp}^{\bullet+}$ cation radicals with $\text{}^3\text{O}_2$ rather than a reaction of the substrate cation radical with $\text{O}_2^{\bullet-}$ superoxide ion.

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