Acta Chemica Scandinavica A 31 (1977) 281—286

On the Mechanism of Radical Formation during UV Irradiation of Polar Solutions of Aromatic Molecules at 77 K

JOHAN MOAN

Norsk Hydro's Institute for Cancer Research, The Norwegian Radium Hospital, Montebello, Oslo 3, Norway

Frozen polar solutions of the aromatic amino acids L-tryptophan and L-tyrosine have been exposed to UV-light of wavelengths 254 and 310 nm. The kinetics of radical formation and triplet formation and decay have been studied by ESR methods. A significant fraction of the ejected electrons recombine with their mother ions. When an electron scavenger is present this recombination is prevented, whereby the total radical yield increases. In the absence of scavenger the recombination probability increases with increasing irradiation time.

Furthermore, it is concluded that monophotonic processes do not contribute significantly to radical formation. The radical formation mechanism is biphotonic ionization and the intermediate in all cases studied, except possibly one, is the triplet state. The exception is L-tryptophan irradiated at 310 nm, where the intermediate seems to have shorter lifetime than the triplet state.

Numerous investigations of the mechanisms of photoionization of aromatic molecules have been published. Most authors seem to agree that the ionization process in rigid glasses at low temperatures is biphotonic with the triplet state as an intermediate. However, some authors [1,2] conclude from their data that monophotonic processes also contribute, notably at the shorter wavelengths. This seems probable since in most cases solvated electrons seem to be formed in monophotonic processes in liquid solutions. If molecules in the triplet state are the only intermediates, the rate of radical production should be proportional to the number of molecules in the triplet state. Hélène *et al.*[3] have investigated this in the case of nucleic acid derivatives in an alcohol glass, and their data indicate that there is no proportionality. Thus we found it worthwhile to investigate this problem more thoroughly.

Model

It can easily be shown [4] that the number N_s of biphotonic ionization events should follow the equation:

$$N_s = \frac{K_1 C_s \varepsilon_M I^2}{k+aI}\left[t - \frac{1}{k+aI}\left(1-e^{-(k+aI)t}\right)\right] \qquad (1)$$

where K_1 is a constant, I is the intensity of the UV light, t is the time after onset of the light, k is the rate constant of decay of the intermediate, ε_M is the extinction coefficient of the intermediate (related to natural logarithms), C_s is the quantum efficiency for permanent ionization of the intermediate; *i.e.* ionization that results in radicals which are longlived enough to be registered.

$$a = C_s \varepsilon_M + C_M \varepsilon_0 \qquad (2)$$

where ε_0 is the extinction coefficient of the ground state and C_M is the quantum efficiency for production of the intermediate state. For a clarifying scheme of the biphotonic ionization model see Fig. 1 in Ref. 4. Eqn. (1) is correct only when a small fraction of the molecules in the ground state is affected. The number of molecules in the metastable state should follow the equation:

$$N_M = \frac{K_1 I}{k+aI}\left(1-e^{-(k+aI)t}\right) \qquad (3)$$

If monophotonic processes contribute, eqn. (1) will take the form:

$$N_s = \frac{K_1 C_s \varepsilon_M I^2}{k+aI}\left[t - \frac{1}{k+aI}(1-e^{-(k+aI)t})\right] + K_2 It \quad (4)$$

where K_2 is a constant.

Eqn. (1) describes a curve that approaches a straight line as t increases. This line intercepts the t-axis in

$$t_0 = 1/(k+aI) \quad (5)$$

As $t \to 0$ the slope of the curve approaches 0 while the curve described by eqn. (4) has a slope that approaches $K_2 I$ as $t \to 0$. Thus by measuring the ionization yield accurately for short irradiation times it can be determined whether monophotonic processes contribute. Furthermore, if the triplet state is the only intermediate the rise time τ_R defined by the equation $N_M = \text{const}\ (1-e^{-t/\tau_R})$ should be equal to t_0. τ_M can easily be measured by either ESR or phosphorescence methods. If monophotonic processes contribute t_0 should be smaller than τ_R according to eqn. (4). Generally, if intermediates with a lifetime different from that of the triplet state are involved t_0 should be different from τ_R.

According to (1) and (3) the following equation is correct when $t \gg t_0$:

$$\frac{dN_s}{dt} = C_s \varepsilon_M I N_M \quad (6)$$

Thus, in processes where the triplet state is the only intermediate the rate of radical production is proportional to the number of molecules in the triplet state, provided C_s, the quantum efficiency of radical production by the second quantum, is constant. I and ε_M are supposed to be constant. If C_s changes with the irradiation time because of a change in the probability of recombination of shallowly trapped electrons τ_R will also change according to the equation:

$$\tau_R = t_0 = \frac{1}{k+(C_s \varepsilon_M + C_M \varepsilon_0)I} \quad (7)$$

EXPERIMENTAL

The solvent was a mixture of ethylene glycol and water (EG/H_2O) 1 : 1 by volume. To this solvent tyrosine or tryptophan of analytical grade from Sigma Chem. Co. was added, usually in a concentration of 2.5×10^{-2} M. CCl_3COONa of analytical grade from Merck was used as an electron scavenger. 100 µl of sample solution was transferred to quartz ESR tubes and cooled to 77 K by immersion in liquid N_2. The samples were exposed to the light from a 200 W high pressure mercury lamp fitted to a Bausch &

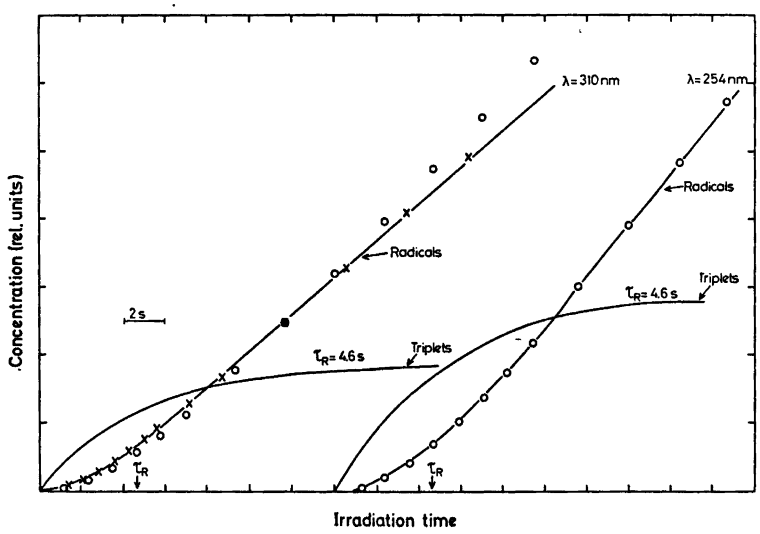

Fig. 1. The yield of radicals and triplets as a function of the exposure time in 2.5×10^{-2} M solution of L-tryptophan in EG/H_2O containing 0.1 M CCl_3COONa as an electron scavenger. The full lines are the experimental curves. O represents curves described by eqn. 1, under the assumption that $t_0 = \tau_R = 4.6$ s. × represents a similar curve with $t_0 = 2.9$ s. The light intensity at 310 nm was adjusted to make τ_R equal at the two wavelengths.

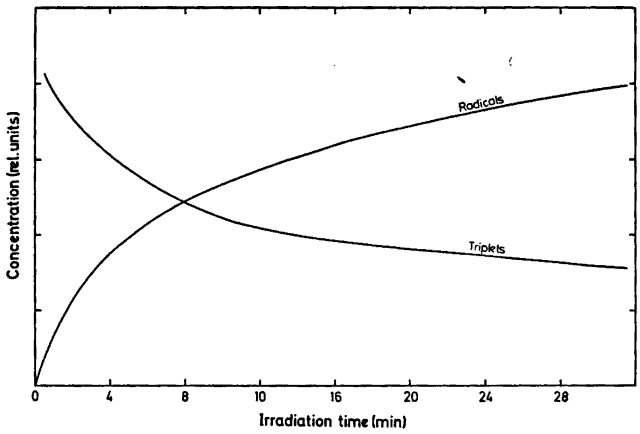

Fig. 2. The yield of radicals and triplets as a function of the exposure time on a longer timescale than in Fig. 1. The wavelength of the UV-light was 310 nm, but similar results were found also at 254 nm.

Lomb grating monochromator. The irradiation took place in the cavity of the ESR spectrometer and the concentration of radicals or molecules in the triplet state could be measured during the irradiation. The light intensities at 254 and 310 nm at full slit widths were, respectively, 10^{-10} and 10^{-9} Einstein/mm² s. The band width was 30 nm.

The ESR spectrometer was an X-band instrument with reflection cavity, operating at 9200 MHz.

RESULTS AND DISCUSSION

In the following experiment we found it necessary to add an electron scavenger since the trapped electrons are readily bleached by the UV light. 0.1 M CCl_3COONa is sufficient to reduce the yield of trapped electrons to less than 5 % of that found in the absence of scavengers. The scavenger radicals were unbleachable by the UV-doses used in this work. This was confirmed by exposing an X-irradiated sample of 0.1 M CCl_3COONa in EG/H_2O (which contained the same radicals as an UV irradiated sample) to UV light. It was confirmed that UV irradiation of 0.1 M CCl_3COONa without tyrosine and tryptophan only gave insignificant amounts of radicals, which in no case is of importance for the present results. It was also checked that the presence of oxygen had no influence on the results. Fig. 1 shows the yield *vs.* time curves. It can be seen that the slope of the curve approaches 0 as $t \rightarrow 0$. These curves can be very accurately reproduced, and it is found that at $t = 15$ s, monophotonic processes contribute less than 5 % to the radical production at both wavelengths. Similar results were found also for tyrosine. Corresponding results were obtained in 0.5 M H_2SO_4, except for tryptophan at 254 nm, where the radical production is so slow that curves cannot be meas-

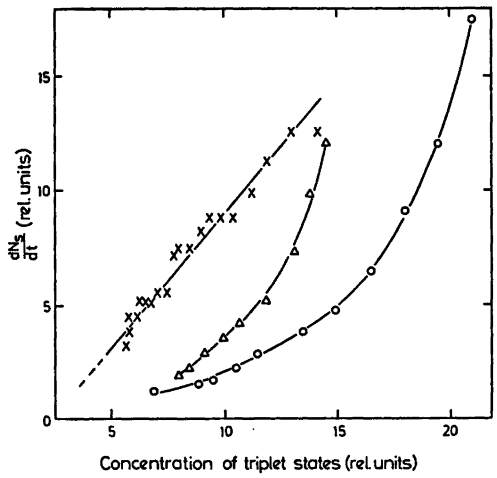

Fig. 3. The rate dN_s/dt of radical production as a function of the concentration of triplet states. ○ 2.5×10^{-2} M L-tryptophan in EG/H_2O containing 0.1 M CCl_3COONa irradiated at 310 nm. △, 3×10^{-2} M L-tyrosine in 0.5 M H_2SO_4 310 nm. ×, data from a work of Hélène *et al.*[3] for UV irradiation of 10^{-3} M purine in ethanol.

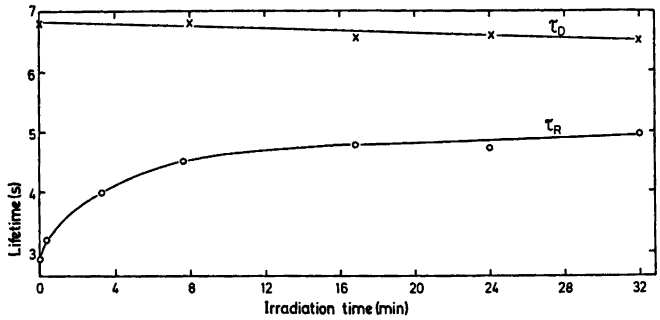

Fig. 4. The rise and decay lifetime of the triplet state of tryptophan as a function of the exposure time at 310 nm. The system is the same as that described under Fig. 1.

ured with sufficient accuracy. Thus in all cases the radical formation goes *via* a metastable intermediate.

Fig. 1 also shows the rise kinetics of the triplet state from which τ_R may be determined. It can be seen that at 254 nm there is excellent agreement between the experimental curve and the curve described by eqn. (1) on the assumption that $\tau_R = t_0$. The same was found for tyrosine at both wavelengths (254 and 310 nm). In the case of tryptophan irradiated with 310 nm light, however, it seems that $t_0 < \tau_R$ (Fig. 1). This may indicate that an intermediate other than the triplet state is involved. This state could be a "semiionized" state of the same type as that proposed by Ottolenghi [5] to explain biphotonic ionization of aromatic molecules.

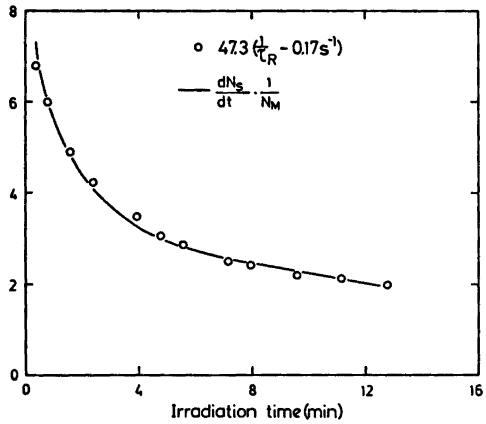

Fig. 5. The full line represents data from Fig. 2. (dN_s/dt) $(1/N_M)$ is plotted as a function of t. The points represent the curve 47.3 $(1/\tau_R - 0.17$ s$^{-1})$ *i.e.* C_s from eqn. 7 with $k + C_M \varepsilon_0 I = 0.17$ s^{-1}. τ_R is taken from Fig. 4.

Tryptophan has at least two electronic transitions in the wavelength region 250 – 305 nm.[6] In principle, therefore, it is possible that one of these transitions populate the "semi-ionized" state while the other does not.

At extended irradiation times the concentration of molecules in the triplet state decreases as shown on Fig. 2. Similar observations were made by Neubacher and Walla [7] for sensitized ethanol photolysis. The reason for this decrease could be (a) that the accumulation of radicals in the sample causes a quenching of the excited states of tryptophan or (b) that the irradiation results in a destruction of tryptophan molecules. Alternative (a) can be ruled out since X-irradiation of the sample with doses giving considerably larger amounts of radicals than UV irradiation (the same type of radicals are found in the two cases) has no effect on the concentration or lifetime of triplet states induced by UV light. Thus it seems that the UV irradiation causes a permanent destruction of tryptophan molecules.

Provided that the radical production results from triplet absorption and that the constants in eqns. (1) and (3) do not change with the UV dose, one should expect eqn. (6) to be correct for extended irradiation times. (It should be remembered that the same relationship will exist if another metastable state is involved alone or in addition to the triplet state. The number of molecules in such a state will be proportional to the number of molecules in the ground state and therefore also to the number of molecules in the triplet state). Fig. 2 shows that dN_s/dt decreases with t but dN_s/dt is not proportional to N_M as demonstrated in Fig. 3.

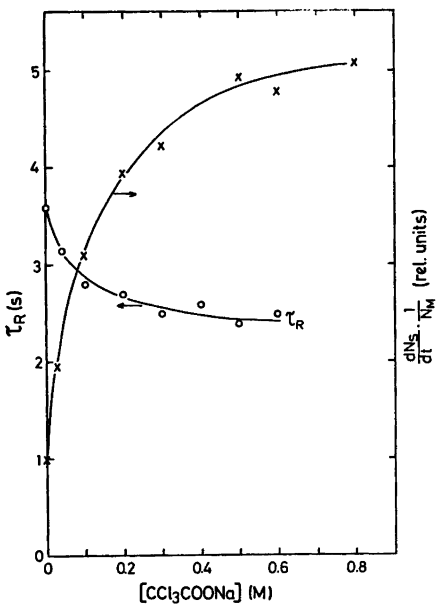

Fig. 6. Rise lifetime of the triplet state (τ_R) and (dN_s/dt) $(1/N_M)$ as functions of the scavenger concentration. The system is 2.5×10^{-2} M L-tryptophan in EG/H_2O irradiated at 310 nm. The decay lifetime was practically constant (6.7 s) up to 0.6 M. Similar results were found at 254 nm.

In this figure is also shown some data for tyrosine in 0.5 M H_2SO_4 and some data from a work of Hélène *et al.*[3] concerning UV irradiation of 10^{-3} M purine in ethanol. Their data show the same tendency though not as pronounced as in the present cases. Analysis of the data of Neubacher and Walla [7] gives the same result. Thus it seems that this is a general trend being observed at both 310 and 254 nm and in different solvents. The following observation provides an explanation. The rise time τ_R of the triplet state increases with the UV-dose delivered to the sample as shown on Fig. 4. The same is true for t_0. The decay lifetime of the triplet state does not change measurably. Therefore, the only quantity which may vary in the expression for the rise time [eqn. (7)] is C_s. (This treatment can easily be adapted to two metastable states). Thus C_s decreases with increasing irradiation time. In systems like the present one a significant fraction of the ejected electrons recombine spontaneously. (See below and Ref. 9). Thus C_s is made up of two terms: $C_s = C_{s,ion}(1 - P_R)$. $C_{s,ion}$ is the initial probability of ionization and P_R is the probability of recombination of an ejected electron. This electron may be in a shallow trap for a short time. According to eqn. (7) $C_s = \frac{1}{\varepsilon_M I}\left(\frac{1}{\tau_R} - k - C_M \varepsilon_0 I\right)$. As demonstrated in Fig. 5 the quantity $(1/\tau_R - 0.17\ s^{-1})$ varies in exactly the same way as $(dN_s/dt)(1/N_M)$. Thus the model is in correspondence with these data, and it seems that the probability of recombination increases with increasing irradiation time. According to this picture the recombination probability should decrease if the concentration of electron scavenger is increased since the scavenger prevents some of the ejected electrons from recombining. This should cause an increase of C_s and hence a decrease of τ_R [eqn. (7)] and an increase of $(dN_s/dt)(1/N_M)$. As shown on Fig. 6 the experimental data are in accordance with this. While the decay lifetime was found to be practically constant over the whole concentration range, the rise lifetime decreased markedly. Furthermore, the quantum efficiency of radical formation by the quanta absorbed by the triplet state increased by a factor of about 5 when the scavenger concentration increased from 0 to 0.8 M. This demonstrates that the scavenger prevents recombination.

A problem remaining to be solved is why C_s decreases with increasing irradiation time. The following is just a tentative explanation. In accordance with our earlier results [9] we find that the total number of radicals in a sample irradiated for 20 s at 310 nm is higher than the number of tryptophan molecules. This means that each tryptophan molecule may give rise to more than one electron. Steen [10] suggested that a tryptophan cation can be neutralized by capturing an electron from the solvent. This seems plausible also in the light of later experiments which indicate that there is almost no tryptophan ion radicals left after UV-irradiation of an ethylene glycol/water glass containing tryptophan.[11] Trapped electrons and ethylene glycol radicals are practically the only radicals observed. If this picture is correct, the majority of the tryptophan molecules in the sample will have solvent and scavenger radicals in their neighbourhood. This may reduce the probability of neutralization and therefore increase the probability of recombina-

tion of the ejected electrons. It is also possible that the proximity of tryptophan molecules runs short of scavenger molecules whereby the recombination probability obviously increases with the exposure time.

REFERENCES

1. Pailthorpe, M. T. and Nicholls, C. H. *Photochem. Photobiol. 15* (1972) 465.
2. Joussot-Dubien, J. and Lesclaux, R. *The Triplet State*, Cambridge Univ. Press, London – New York 1967, p. 197.
3. Hélène, C., Santus, R. and Douzou, P. *Photochem. Photobiol. 3* (1966) 127.
4. Moan, J. and Steen, H. B. *J. Phys. Chem. 75* (1971) 2893.
5. Ottolenghi, M. *Chem. Phys. Lett. 12* (1971) 339.
6. Weber, G. *Biochem. J. 75* (1960) 335.
7. Neubacher, H. and Walla, W. *Radiat. Environ. Biophys. 12* (1975) 51.
8. Kaalhus, O. and Moan, J. *Int. J. Radiat. Phys. Chem. 6* (1975) 222.
9. Moan, J. *Israel J. Chem. 9* (1971) 637.
10. Steen, H. B. *Photochem. Photobiol. 9* (1969) 479.
11. Moan, J. and Kaalhus, O. *J. Chem. Phys. 61* (1974) 3556.

Received October 7, 1976.