OPTICAL DETECTION OF THE TRIPLET STATE OF URACIL

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Summary. A transient species has been observed in the ultraviolet flash photolysis of uracil in aqueous solution which is quenchable by O_2 and by uracil itself, but not by N_2O. This transient has a mean lifetime of 6.1 ± 0.5 μsec in the absence of quenching and an absorption spectrum which peaks at 380 nm. Analysis of the kinetics of quenching shows this transient to be the triplet state which is the direct precursor of the uracil dimer photoproduct.

The ultraviolet (UV) irradiation of uracil (and other pyrimidines) leads to the formation of permanent photoproducts of chemical and biological importance. Considerable evidence has been presented which suggests that the pyrimidine dimer photoproduct is formed in aqueous solution by the reaction of a molecule in an excited triplet state with another like molecule in its ground state(1,2,3,4). A scheme for such a reaction mechanism has been advanced by Sztumpf-Kulikowska, Shugar and Boag(1), and the kinetics predicted by such a scheme have been confirmed for uracil(3), orotic acid(4) and thymine(5). According to this model for uracil, represented as U, excitation occurs from the ground state singlet to an excited singlet state, followed by intersystem crossing to a lower-lying triplet state. Dimerization proceeds from this triplet state with rate constant $k_1[U]$(sec^{-1}) in competition with natural radiationless decay with rate constant k_3 (sec^{-1}), and with other quenching reactions. A convenient triplet quencher is molecular O_2(3), so that de-excitation of the triplet can also occur with rate constant $k_2[O_2]$(sec^{-1}).

When all de-excitation mechanisms are present the mean lifetime, τ, of the triplet state is given by

$$\tau = \frac{1}{k_1[U] + k_2[O_2] + k_3} \qquad (1)$$

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At infinite dilution of uracil and in the complete absence of quenchers, τ approaches τ_0, the natural lifetime of the triplet state in solution, given by

$$\tau_0 = \frac{1}{k_3} \tag{2}$$

Equations (1) and (2) may be combined to give

$$\frac{1}{\tau} = \frac{1}{\tau_0}\left[1 + \frac{k_1}{k_3}\left[U\right] + \frac{k_2}{k_3}\left[O_2\right]\right] \tag{3}$$

The direct detection of the triplet state of one such pyrimidine, orotic acid, by flash photolysis techniques has been reported by Herbert et al.(6). These authors describe a transient species in the UV excitation of orotic acid which obeys the kinetics predicted by the model already described, and which has a natural lifetime of 79 μsec. Moreover, the quenching rate constants agree well with the data obtained from steady-state methods(4). A similar species might be expected in the flash photolysis of uracil. However, using an estimate of the rate constant for oxygen quenching of triplet states, k_2(7), in combination with his own data, Brown(3) has pointed out that the lifetime of the uracil triplet state would be smaller than that for orotic acid by at least a factor of four. Such a lifetime would not be detected by the system used for orotic acid. Moreover, since the intersystem crossing efficiency for uracil is 10-fold smaller than that for orotic acid, the population of triplet states to be detected is reduced by a similar factor, and a more intense analyzing beam must be employed.

We now report the detection of a transient species in the flash photolysis of uracil in aqueous solution which exhibits the kinetics predicted for the triplet state with rate constants which are in good agreement with the data of Brown and Johns(3). This transient species has a natural lifetime of 6.1 ± 0.5 μsec. and is the excited precursor state of the dimer photoproduct.

Methods

The methods used in these experiments are improvements over those already described(5). Uracil was irradiated in a cylindrical cell 3 cm in diameter and 15 cm in length enclosed in a double-elliptical reflecting cavity which also enclosed the two flash lamps. These lamps were 20 cm long and 10 mm in inside diameter, and were partially filled with a centred quartz rod 9 mm in diameter. The plasma was formed in the annular space in air at reduced pressure. The flash resulted from the discharge of a 0.75 μF condenser charged to 23 kv through a near-critically damped circuit which included the flash lamps in series. An analyzing beam of high output was produced by pulsing an Osram XBO-150 w/1 Xenon lamp above its continuous level from a current source for a period of 20 msec which included the analyzing period. A 20-fold gain in light level is achieved in this way without the sacrifice of lamp stability. The absorption signal was detected through a Spex monochromator by an RCA 1P28 photomultiplier tube and displayed and photographed on a Tektronix 556 oscilloscope.

Uracil solutions were prepared in triply quartz-distilled water and the concentrations were obtained from optical density readings from a Zeiss spectrophotometer using an extinction coefficient of 8.2×10^3 l M^{-1} cm^{-1} at 259 nm(8). Oxygen concentration in solution was controlled by bubbling a known mixture of oxygen and nitrogen through the solution for at least 30 minutes prior to photolysis. Oxygen concentrations were calculated from the solubility of oxygen, 1.3×10^{-3}M, in water at the experimental temperature (25°C), and the mole percent of oxygen in the gas mixture.

The flash lamp system as described produced an exciting light pulse with a half-width of 0.9 μsec. The pulse was more than 99% complete in 3 μsec. The lamp produced approximately 6×10^{17} photons per flash in the UV region up to 320 nm, as measured by the dye, malachite green leucocyanide(9).

Results

Figure 1 shows a typical trace obtained in the flash photolysis of uracil in solution. In this figure X is the observed transient, S is the tail of the scattered light from the flash observed without the analyzing

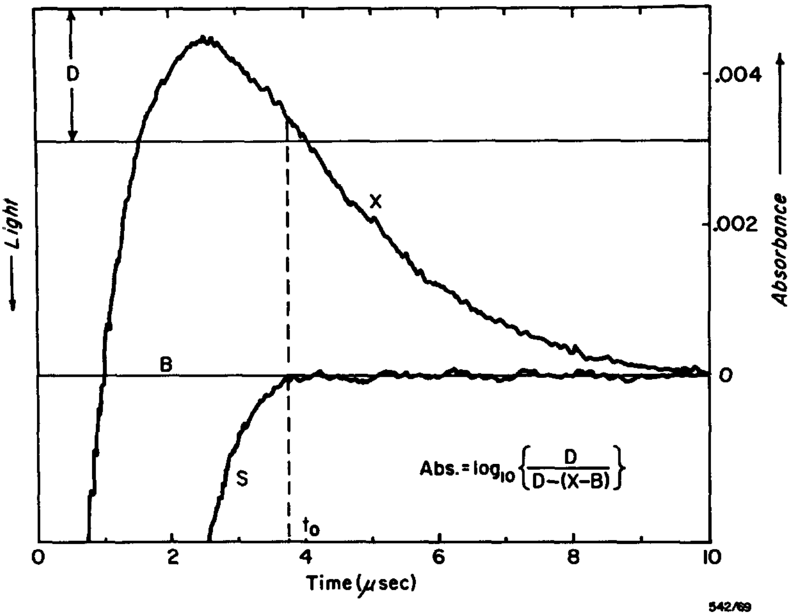

Fig. 1. A typical transient observed in the flash photolysis of uracil in aqueous solution at 350 nm. (6.7×10^{-5} molar uracil, pH5.9; 7.8×10^{-5} molar O_2) Notation is as described in the text.

light, and B is the baseline. D is the pulsed output of the analyzing lamp through the cell measured directly before the flash at a sensitivity 0.004 that of the signal traces. The relationship between signal levels and absorbance is shown and an auxiliary absorbance scale for the transient is included at the right. Traces such as this were analyzed for times greater than t_0 using a computer programme the output of which demonstrated that each trace represented an exponential decrease with time. The response of the analyzing system to a 20 nanosecond light pulse showed a time constant of 0.4 μsec which is four times faster than the shortest transient lifetime which was analyzed.

The transient such as shown in Fig. 1 has a peak absorption at 380 nm and absorbs from beyond 500 nm to slightly below 300 nm. Because of this spectrum and the output of our analyzing light further quenching studies were performed at 350 nm. Since we were unable to quench this transient

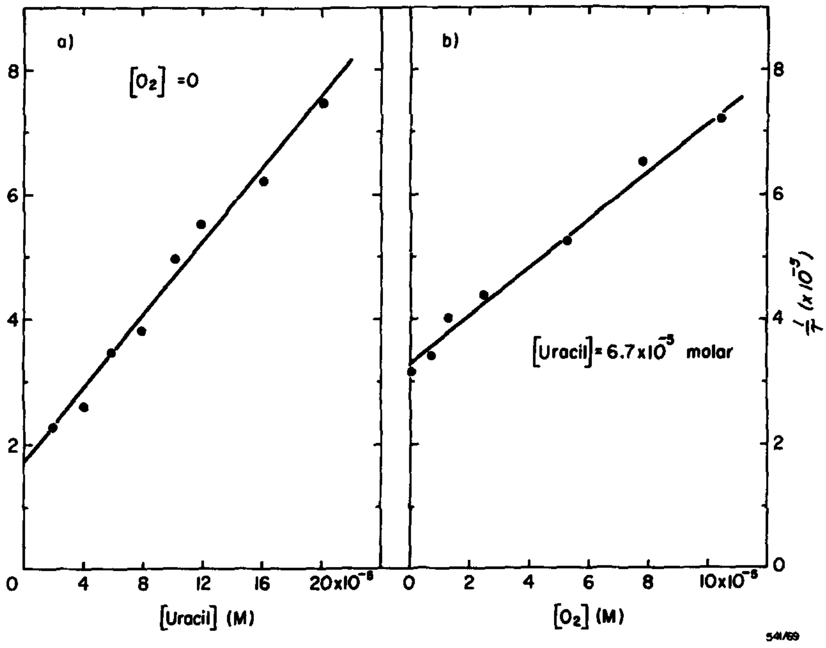

Fig. 2. Studies in quenching of the uracil triplet by
a) other molecules of uracil
b) molecular O_2 dissolved in solution.

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by saturating our solution with N_2O, it is not a transient produced by e^-_{aq} attack, similar to that suggested by Theard(10).

The transient which has been described is quenched both by oxygen and by other molecules of uracil, as has been suggested for the triplet state of uracil. According to the scheme previously described, as shown in equation (3), a linear relationship should exist between the reciprocal of the measured lifetime, $1/\tau$, and the concentration of uracil and of oxygen independently.

Figure 2(a) shows that a plot of $1/\tau$ versus uracil concentration for a number of experiments performed in the absence of oxygen is indeed a straight line. The intercept of this line at zero uracil concentration leads directly to a value of the natural lifetime, τ_o = 5.8 μsec. Moreover, from equation (3), the slope of this line is simply k_1, since $k_3\tau_o = 1$. From this slope one obtains a value for k_1 of 2.9×10^9 M^{-1} sec^{-1}. The ratio $k_3/k_1 = 6.0 \times 10^{-5}M$, derived from this data, is in excellent agreement with the value of $5.9 \times 10^{-5}M$ obtained by Brown and Johns by product analysis of the dimers produced in uracil(3).

Similarly, Figure 2 (b) shows that a plot of $1/\tau$ versus oxygen concentration for a particular uracil concentration of $6.7 \times 10^{-5}M$ is also linear. Using the values obtained in the previous result and equation (3), one derives from the intercept of this line a value for τ_o of 6.4 μsec. As before, the slope of this graph is k_2, since $k_3\tau_o = 1$. From this data a value for k_2 of 3.9×10^9 M^{-1} sec^{-1} results. The ratio, k_2/k_1, from these values is 1.5; this is one extreme of the range of values obtained by Brown and Johns(3) of 0.8 to 1.5, for different exciting wavelengths. We, of course, have no fine control over exciting wavelength.

A summary of these results is presented in Table 1. Further investigations of this triplet state, together with those of a similar transient observed in thymine, are underway.

Table 1. Kinetic Data for Reactions of the Uracil Triplet

$\tau_o = 6.1 \pm 0.5$ μsec

$k_3 = 1.6 \times 10^5$ sec^{-1}

$k_1 = 2.9 \times 10^9$ M^{-1} sec^{-1}

$k_2 = 3.9 \times 10^9$ M^{-1} sec^{-1}

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References

1. Sztumpf-Kulikowska, E., Shugar, D. and Boag, J.W. Photochem. Photobiol. 6, 41 (1967).

2. Greenstock, C.L., Brown, I.H., Hunt, J.W. and Johns, H.E. Biochem. Biophys. Res. Comm. 27, 431 (1967).
3. Brown, I.H. and Johns, H.E. Photochem. Photobiol. 8, 273 (1968).
4. Whillans, D.W. and Johns, H.E. Photochem. Photobiol. 9, 329 (1969).
5. Fisher, G.J. and Johns, H.E. Photochem. and Photobiol. (1969) in press.
6. Herbert, M.A., Hunt, J.W. and Johns, H.E. Biochem. Biophys. Res. Comm. 33, 643 (1968).
7. Kasche, V. and Lindqvist, J. J. Phys. Chem. 68, 817 (1964).
8. Shugar, D. and Fox, J.J. Biochim. et Biophys Acta 9, 199 (1952).
9. Fisher, G.J., LeBlanc, J.C. and Johns, H.E. Photochem. Photobiol. 6, 757 (1967).
10. Theard, L.M., Peterson, F.C. and Myers Jr., L.S. International Conference on Radiation Chemistry. Published in Radiation Chemistry Vol. I, American Chemical Society 1968.