Primary Processes in the Photobleaching of Eosin as Revealed by the Flash Technique

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(Received August 17, 1959)

A few years ago, Imamura and Koizumi[1] investigated kinetically the reductive photobleaching of xanthen dyes and proposed a reaction scheme which involved the formation of semiquinone radical with a lifetime of millisecond order. Now, techniques of flash photolysis and flash spectroscopy have been introduced in order to get direct evidence for their conclusion and to obtain more detailed information about the primary processes. The present paper is concerned chiefly with the spectra of the transient intermediates and their assignment along with some discussions about the reaction scheme. A rather remarkable result was that two types of intermediates with a lifetime of millisecond order were observed spectroscopically. One of these was assigned to semiquinone and the other to a certain kind of complex between dye and ethanol.

Experimental

Flash Lamps. — A photolysis flash lamp, six turn helical Terex tube, 10 mm. in diameter, the total length of which was about 1 m., was filled with argon at 4.5 cmHg pressure. It was surrounded by a cylindrical magnesia reflector. A spectroflash lamp was a Terex tube, 20 cm. in length and 15 mm. in diameter, having a flat quartz window, the argon pressure being 20 cmHg. Five 10 μF oil condensers were used for photolysis flash and one condenser of the same capacity for spectroflash, and both of them were charged up to 4 kV. Flashes were triggered by induction coils operated by strobo discharge tubes. The interval of firing was controlled from 0.3 to 700 millisec. by an electronic device. The half height widths of the flashes were measured by an oscilloscope and found to be approximately 60 and <20 μ sec., respectively, a crystal oscillator being used as a time measure.

Spectroscopic.—A Shimazdu QF-60 quartz spectrograph and Fuji Neopan plates were used.

Reaction Vessels. — The vessels were of 10 cm. length and 1 cm. diameter glass tubes with quartz plates cemented on both ends. The glass part of the vessel cuts off shorter wavelengths than 300 mμ.

Materials. — Merck eosin and Wako Pure Chemical Industries G. R. grade ethanol were used. The latter was transparent up to 260 mμ for a 10 cm. path. Throughout the investigation, 1×10^{-5} mol./l. ethanolic solution of eosin was degassed by repeated distillation in high vacuum, sealed in a reaction vessel and then was placed at the center of the helical flash lamp.

Calibration of the Flash Intensity. — Although the flashing condition was made as nearly the same as possible by monitoring the condenser voltage, some variation in intensity could not be avoided. To evaluate correctly the absorption spectra of the transient species from the trace of a microphotometer, the variation of the spectro-flash intensity was taken into account in the following way. From the readings of the trace for the solvent and solution, the logarithm of exposure was determined in the usual way. When the difference in the logarithm of exposure was plotted against the molar extinction coefficient (ε_λ) of eosin, a linear relationship was found to hold in the wavelength region above 480 mμ, where the transient species had no absorption. Since the relation

$$\Delta S_\lambda = \log I_\lambda - \log I'_\lambda + \varepsilon_\lambda cd$$

holds, where ΔS_λ is the difference in logarithm of exposure, I_λ and I'_λ are the intensity of the spectro-flash for solvent and solution, respectively; the intercept of the ordinate gives the value $\log I_\lambda - \log I'_\lambda$, which allows one to calibrate the intensity variation of the spectro-flash.

Results

The absorption spectra are shown in Fig. 1. The spectra of the initial solution were obtained by a Hitachi spectrophotometer EPU-2A. The time interval written is that between the start of the two flashes. As seen from the figure, after 0.6 millisec., the main absorption of eosin decreases to 60% of the initial solution, and at the same time a definite transient absorption comes into existence. Substracting from this the absorbance due to the remaining eosin, the quantity of which

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[1] M. Koizumi and M. Imamura, This Bulletin, **28**, 117 (1955); **29**, 899 (1956).

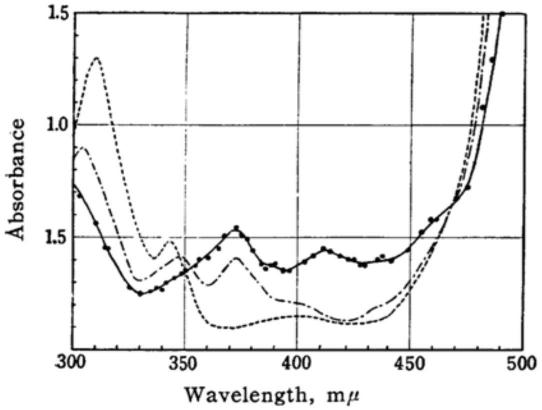

Fig. 1. Transient spectra of eosin.

——: 0.6 millisec.
—·—: 5 millisec.
----: before flash

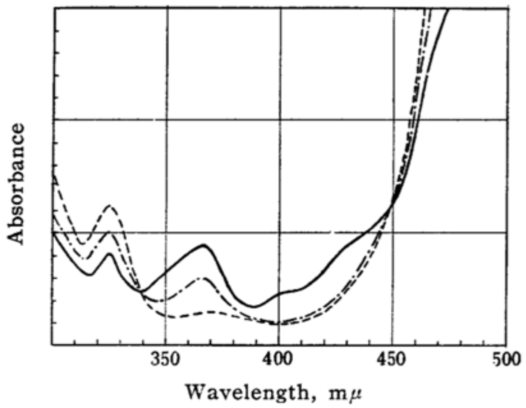

Fig. 3. Transient spectra of uranin.

——: 0.6 millisec.
—·—: 5 millisec.
----: before flash

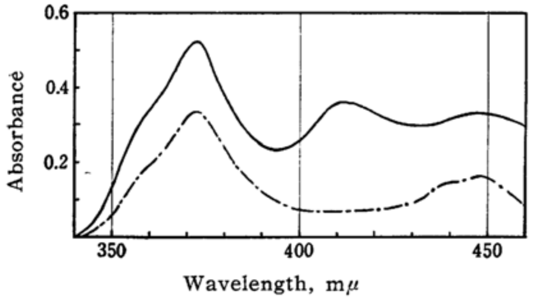

Fig. 2. Absorption spectra of transient species of eosin.

——: 0.6 millisec.
—·—: 5 millisec.

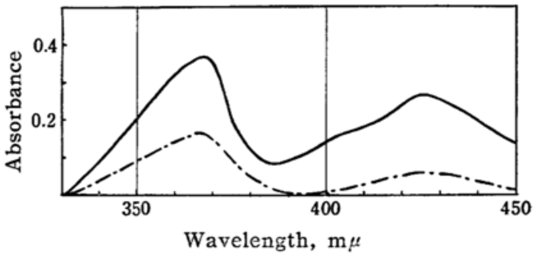

Fig. 4. Absorption spectra of transient species of ulanin.

——: 0.6 millisec.
—·—: 5 millisec.

can be evaluated from the depression of the absorbance in the region longer than 480 mμ, one can obtain the genuine spectra of transient species, which are shown in Fig. 2.

Three peaks are seen at 373, 410 and about 450 mμ. After 5 millisec., the 373 and 450 mμ peaks, which will be called A band from now on, still maintain considerable intensity, whereas the 410 mμ peak (B band) has disappeared. After several minutes, these transient absorption bands thoroughly vanish and the eosin spectra are recovered up to about 88% of the original solution. Twelve per cent loss is attributable, without doubt, to permanent bleaching.

In the presence of air, neither transient spectral change nor permanent bleaching was observed. The deaerated aqueous solution of eosin showed a slight transient depression of eosin absorption spectra, but no new spectra were observed in the region of the above mentioned A and B bands.

For uranin in ethanol also, transient spectra were observed by the flash illumination, but at 0.6 millisec. no clear peak corresponding to the 410 mμ peak of eosin was found (Figs. 3 and 4).

It is worth adding here that when only a photolysis flash is fired, a fluorescence spectrum can be taken on a plate, and it is to be noted that the fluorescence intensity varies markedly according to whether the sample is aerobic or not. On the contrary, the measurement by an ordinary fluorimeter (an accessory of the spectrophotometer), shows no such difference.

TABLE I. RELATIVE FLUORESCENCE INTENSITY AT THE PEAK WAVELENGTH

	Flash	Fluorimeter
Aerobic	100	100
In vacuo	5	100

Discussion

The appearance of two spectral bands A and B with different lifetimes indicates that at least two transient species are formed in the initial stage of the reductive photobleaching of eosin.

As regards the assignment of these two bands, there scarcely exists any doubt that the A band is due to the semiquinone radical of eosin on the basis of the kinetic studies of Imamura and Koizumi. The assignment of band B, however, is not so clear-cut. A question may arise whether this absorption is caused by the triplet state of dye, but this possibility can be denied from the following two reasons. The first reason is that the lifetime of the band, being some tenths of a millisecond, would be too long for a triplet. Considered from the results already published in literature, for instance, as reported by Porter et al.[2], triplet states have usually too short a lifetime at room temperature and in ordinary solvents of common fluidity, to be observed by the present apparatus. The second reason is that if it were due to triplet state, the similar band should be observed in aqueous solution because of lower reactivity of water to the triplet state than ethanol, a fact which has been established by kinetic studies of photobleaching[1].

The B band must, therefore, be attributed to some unknown molecular species which perhaps is formed prior to semiquinone. Now, as reported elsewhere[3], the influence of added substances on the bleaching rate of eosin strongly suggests the formation of a certain kind of molecular complex between the excited state of the dye and ethanol. If this scheme is accepted, it is quite natural to attribute the B band to the complex in question.

Furthermore, in order to interpret the other experimental results reported in this paper, it is quite desirable, indeed it seems rather indispensable, to introduce such a complex in the reaction scheme, as will be shown below.

1) From the result that at 0.6 millisec. after the firing of the main flash the quantity of the existent dye drops to about 60% of that of the initial solution, it is very natural to consider that at a certain early period after flashing, nearly all the dye molecules exist as intermediates whose lives are in the order of 10^{-3} sec. or so.

Supposing that a semiquinone is the only one species as such an intermediate, then nearly 50% of dye would be bleached permanently, if one assumes, as is plausible, that semiquinone is stabilized by a dismutation process, $2DH \rightarrow D + DH_2$. Contrary to this expectation, only 12% permanent bleaching is produced by a single flash. In other words, about 24% of the dye is converted into semiquinone by one flash, hence this result necessitates that some other transient species like the complex considered above be existent, and that most of this transient species be deactivated without going to the reaction product.

2) The absence of photobleaching as well as of transient spectrum in aerobic condition is intimately connected with the fact formerly reported, that the reductive photobleaching is completely inhibited by the existence of a very small quantity of oxygen[1]. Imamura and Koizumi who at that time did not conceive of the formation of the complex, estimating the lifetime of the species attacked by oxygen to be 10^{-3} sec. or so from the quantity of oxygen necessary to suppress completely the bleaching reaction, concluded that semiquinone be mainly destroyed by oxygen, rejecting the possibility of the triplet state (with much shorter lifetime) being deactivated. But if so, B band should still be observed in flashing the aerated alcoholic solution. Thus, their scheme is not consistent with the present results and it is necessary to consider that the molecular complex now introduced be deactivated effectively by oxygen.

3) The results of fluorescence measurements are also consistent with the formation of such a complex. Thus by flash illumination in vacuo, a major part of the dye is converted into the complex at an early stage and survives at the end of the flash. Consequently, the illumination has poor efficiency for the fluorescence, since only a little part of dye remains in the ground state throughout the later period of flash. Whereas the complex is rapidly destroyed into its components by the attack of oxygen, yielding the dye molecule in the ground state. This process enables each molecule of dye to emit fluorescence repeatedly many times in the duration of the flash, resulting in a large increase of the fluorescence intensity. Such a situation is not expected if one assumes that

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[2] Cf. N. K. Bridge and G. Porter, *Proc. Roy. Soc.*, **A244**, 259 (1958).

[3] K. Uchida, S. Kato and M. Koizumi, This Bulletin, **33**, 169 (1960).

oxygen mainly attacks the semiquinone radical. In measurements by an ordinary fluorimeter on the other hand, the low intensity of exciting light causes almost no change in the population of the ground state irrespective of whether a sample is free from air or not, hence the same fluorescence intensity results in the two cases.

The above qualitative discussion, though it seems quite plausible, is in fact not conclusive so long as only the fluorescence experiments for ethanol solutions are concerned, because the results are equally well interpreted if one assumes that the triplet state instead of the complex is efficiently deactivated by oxygen. The problem will be clarified if a quantitative comparison is made for the fluorescence intensity of the evacuated and aerated aqueous solution, where neither semiquinone nor complex is formed. At the present stage of investigation, the complex-decomposition-scheme may be said, in view of the discussion in 2), to be more plausible than the triplet state-deactivation-scheme, although the latter process may perhaps take place to some extent.

Summarizing the above discussion one can conclude the following reaction scheme for the reductive photo-bleaching of eosin.

$$D + h\nu \rightarrow D^* \quad (1)$$

$$D^* \rightarrow D + h\nu' \quad (2)$$

$$D^* \rightarrow D^t \quad (3)$$

$$D^t + RH \rightarrow X \quad (4)$$

$$X \rightarrow D + RH \quad (5)$$

$$X \rightarrow DH\cdot + R\cdot \quad (6)$$

$$2DH\cdot \rightarrow D + DH_2 \quad (7)$$

In the presence of air

$$X + O_2 \rightarrow D + RH + O_2 \quad (8)$$

and in addition to reaction 8, perhaps, D^t may also be deactivated to a lesser extent. The process of reaction 8 is faster than reaction 5 or 6 even when the oxygen content is very small.

For uranin also, a similar scheme may hold, but the lifetime of X seems to be much shorter.

Summary

Reductive photobleaching of eosin and uranin in ethanol solutions was studied by the flash technique. Transient spectra of semiquinone with a lifetime of millisecond order was established. In addition, another transient spectra with a life of the same order was observed in the case of eosin. This was interpreted to be due to a certain complex intermediate between ethanol and eosin, formed prior to semiquinone. The aerated alcoholic solution and the evacuated aqueous solution, when illuminated by a flash, showed no transient spectra in the wavelength region of the above two intermediates. The fluorescence of the aerated ethanol solution produced by the flash had about twenty times as large intensity as that of the evacuated solution induced under otherwise similar condition. A scheme for the primary processes of the reductive photobleaching involving the above complex can interpret all these results satisfactorily.

The authors express their gratitude to the Mainichi Press for a grant of subsidy.

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