The experiment with glutathione in 2.88 *M* HCl which indicates the formation of a thiol ester, coupled with the previous evidence for thiazoline formation, strongly suggests that glutathione may participate in a scheme similar to that of Fig. 4. The formation of an S-acyl derivative would at least partially explain the lack of reversibility when the once formed thiazoline derivative is taken to neutral solution and then returned to strong acid.[17] As in the case of N-acetyl-β-mercaptoethylamine the rate of thiazoline formation in glutathione is constant from 2 to 6 *M* acid. The rate constant is about half the value of k_4 observed for N-acetyl-β-mercaptoethylamine. However, both rates are subject to some uncertainty, due to amide hydrolysis.

Although the rate constants for thiazoline formation in the two compounds are of the same order of magnitude, this is not so for the equilibrium situation. For N-acetyl-β-mercaptoethylamine the equilibrium ratio $(TH^+)/(N\text{–}SH)(H^+)$ has a value of about 2.3 molar^{-1}. The comparable ratio for glutathione[6] is of the order of 3×10^{-3} molar^{-1}. The formation of thiazoline-4-carboxylic acid from N-formylcysteine[18] appears to proceed rapidly toward an equilibrium condition intermediate between the values characteristic for the formation of the thiazoline derivatives of glutathione and N-acetyl-β-mercaptoethylamine.

(17) Observed by several authors, cited in reference 6. See also the discussion of Préaux and Lontie, 5b.

(18) D. Cavallini, B. Mondovi and C. De Marco, *Experientia*, **13**, 436 (1957).

The occurrence of thiazoline rings in proteins has been advanced as one possible explanation for the "masking" of –SH groups, *e.g.*, in serum albumin.[19] Insofar as the properties of 2-methylthiazoline described here may provide information concerning proteins, little support is given to such a view except in solutions more acid than *p*H 1. Simpson and Saroff[19] obtained a maximum rate of decrease of sulfhydryl titer at *p*H 3; however, this is the same *p*H at which 2-methylthiazoline has a maximum rate of decomposition. For the physiological *p*H range the inferred equilibrium constant $K_{NT} = (N\text{–}SH)/(T) = 7 \times 10^4$ strongly favors the amide over the thiazoline form. The situation may be even more unfavorable for thiazoline formation if glutathione is typical of the proteins. However, the basic form of the thiazoline structure once formed may well be stable kinetically speaking. Although the thiazoline derivative may not hydrolyze, its rate of reaction with the ever-present amines would be quite significant in the *p*H 7–8 range. It is of course possible that particular proteins may contain regions in which a special type of configuration favors the formation of a thiazoline ring. The results of the study presented here, however, appear unfavorable to the thiazoline ring hypothesis as a general proposal to explain the "masked" sulfhydryl groups of proteins.

(19) R. B. Simpson and H. A. Saroff, THIS JOURNAL, **80**, 2129 (1958).

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, POLYTECHNIC INSTITUTE OF BROOKLYN]

Dye Sensitized Photoöxidation[1]

BY GERALD OSTER, JUDITH S. BELLIN,[2] ROBERT W. KIMBALL AND MALCOLM E. SCHRADER

RECEIVED APRIL 3, 1959

Only those dyes which are capable of being photoreduced can act as sensitizers for photoöxidation. Both reactions proceed through a metastable long-lived excited state of the dye. A kinetic study using proflavine as the sensitizer and *p*-toluenediamine as the substrate shows that the dye molecule in the long-lived state reacts with oxygen or forms a labile peroxide which in turn oxidizes the substrate. The quantum yield of the reaction decreases markedly with increasing dye concentration due to concentration quenching of the long-lived state and of the peroxide. The large limiting quantum yield achieved, namely, 3.0, is attributed to the formation of a polymer of the oxidized aromatic amine.

Introduction

Nearly sixty years ago, Raab[3] discovered that microörganisms, if stained with certain dyes, are inactivated by visible light. This phenomenon, sometimes referred to as "photodynamic action," requires the presence of oxygen and consists of a dye-sensitized photoöxidation of the substrate.[4] The dye itself is not consumed in the over-all process and may be used over and over again, as long as some autoxidizable substrate remains.

It is the purpose of the present paper to describe certain experiments which help to elucidate the mechanism of photodynamic action. For most of the quantitative studies we have employed proflavin (3,6-diaminoacridine) as the sensitizer. The photoreductive properties[5] and the photodynamic action on biological substrates[6-8] of this dye and its analogs have been studied. As will be shown, there is a correlation between these two apparently diverse phenomena which also holds for dyes of other classes. As a model substrate, we have employed mainly *p*-toluenediamine. This substance is readily susceptible to photoöxidation,

(1) (a) Presented before the 135th National Meeting of the American Chemical Society, Boston, April 9, 1959. (b) This paper represents a part of the dissertation submitted in June, 1956, by Malcolm E. Schrader to the faculty of the Graduate School of the Polytechnic Institute of Brooklyn in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

(2) Public Health Service Research Fellow of the National Cancer Institute.

(3) O. Raab, *Z. Biol.*, **39**, 524 (1900).

(4) H. Blum, "Photodynamic Action and Diseases Caused by Light," Reinhold Publ. Corp., New York, N. Y., 1941.

(5) F. Millich and G. Oster, THIS JOURNAL, **81**, 1357 (1959).

(6) G. Oster and A. D. McLaren, *J. Gen. Physiol.*, **33**, 215 (1950).

(7) G. Oster, *Trans. Faraday Soc.*, **47**, 660 (1951).

(8) J. S. Bellin, R. W. Kimball and G. Oster, to be published.

by dye sensitization or by the direct action of ultraviolet light, to give a colored product.

Experimental

Materials.—The sources for the acridine dyes employed are listed elsewhere.[5] All other dyes were Histological Grade obtained from Fisher.

Polymethacrylic acid (PMA) was prepared and purified as described earlier.[9] Polyvinylpyrrolidone (PVP) (mol. wt. 5.6×10^6) was obtained from Schenley. Desoxyribonucleic acid (DNA) was obtained from British Drug House. The aromatic amines used as substrates were obtained from Eastman. All other chemicals were Reagent Grade obtained from Fisher. The mixtures of oxygen and nitrogen were prepared by Matheson.

Procedures.—Freshly prepared solutions of *p*-toluenediamine (hereafter referred to as PTD) in 0.1 *M* phosphate buffer at *p*H 7.0 are colorless and have a maximum in absorption at 235 mμ. On oxidation a yellow species, the imine, with a maximum at 466 mμ is initially formed. Further standing yields a pink species, with a maximum at 530 mμ. In the present studies the oxidation of PTD was followed by measuring the rate of formation of the pink product. Dilute solutions of PTD in the concentration range 10^{-4} to 10^{-3} *M* were oxidized completely to the pink form by slowly heating at 70° in the presence of oxygen and by photooxidizing the solutions at 25° using proflavine as the sensitizer. In both cases it was found that the molar extinction coefficient at 530 mμ calculated on the basis of the number of moles of PTD which had been oxidized was 1.03×10^3 l. mole^{-1}cm.$^{-1}$.

The dye-sensitized oxidations were all performed in a 0.1 *M* phosphate buffer at *p*H 7.0 and were carried out in an optical cell one centimeter in path length having provisions for bubbling mixtures of oxygen and nitrogen through the solutions prior to and during the course of the reaction. The solutions were irradiated using a 500-watt TDC slide projector at a distance of 10 cm. from the front surface of the cell. Ultraviolet light was eliminated by means of a Corning No. 3–74 pale yellow glass filter. The optical densities of the pink solutions were measured by means of a recording photometer with an interference filter having a maximum in transmission at 528 mμ placed in front of the phototube. A detailed description of this experimental arrangement has been published.[10] For quantum yield determinations a Bausch and Lomb interference filter transmitting maximally at 448 mμ was inserted into the slide projector and the intensity of the radiation was determined with a calibrated thermopile (Eppley Laboratories). The rate of oxidation of PTD then was determined by following the absorption of the pink product at 530 mμ in a Beckman DU spectrophotometer.

For ultraviolet catalyzed autoxidations a Hanovia Sc-2537 low pressure mercury lamp was used in conjunction with a 43% acetic acid filter. Ninety per cent. of the lamp output transmitted through the filter is of wave length 254 mμ, none of it being of wave lengths shorter than 245 mμ.[6] The output of the lamp was determined with a uranyl oxalate actinometer.[11] Here again the oxidation was followed by determination of the absorption at 530 mμ.

Qualitative observations of fluorescence and phosphorescence were carried out visually in a dark room using a medium pressure mercury lamp with a Woods' glass filter passing mainly 365 mμ. Quantitative studies on the self-quenching of fluorescence of proflavine were carried out in the Aminco light scattering instrument using the frontal observation arrangement previously described.[12]

Three criteria were employed to evaluate the susceptibility of a dye to photoreduction in the presence of an electron donor: (1) the ability to induce photopolymerization of calcium acrylate or acrylamide[13]; (2) the ability to photosensitize the reduction of 2,3,5-triphenyl-tetrazolium chloride to the insoluble red formazan[9]; and (3) direct photobleaching to the reduced form of the dye which for yellow dyes, however, is rather difficult to observe visually.

(9) G. Oster and J. S. Bellin, THIS JOURNAL, **79**, 294 (1957).

(10) N. Wotherspoon and G. Oster, *ibid.*, **79**, 3992 (1957).

(11) E. J. Bowen, "Chemical Aspects of Light," 2nd Ed., Oxford University Press, Oxford, 1946, Appendix IV.

(12) G. Oster and Y. Nishijima, THIS JOURNAL, **78**, 913 (1956).

(13) G. Oster, *Nature*, **173**, 300 (1954).

Results

In addition to *p*-toluenediamine (PTD) these various aromatic amines are susceptible to dye-sensitized photooxidation at *p*H 7.0: 3,4-toluenediamine, *o*-phenylenediamine, *p*-phenylenediamine, N,N,N′,N′ tetramethyl - *p* - phenylenediamine, chloro-*p*-phenylenediamine, 4-chloro-*o*-phenylenediamine and *p*-aminophenol. On the other hand, *m*-phenylenediamine and 4-methoxy-6-methyl-*m*-phenylenediamine do not undergo photosensitized oxidation. Of all the photooxidizable compounds, PTD showed the greatest stability in the dark and was therefore chosen for the quantitative studies.

A number of dyes were tested for their ability to act as photosensitizers for the autoxidation of PTD. The dyes were employed in concentrations of about 10^{-4} *M* and the PTD at a concentration of 10^{-3} *M*, both in 0.1 *M* phosphate buffer at *p*H 7.0. In Table I are listed under Class A those dyes which sensitize a noticeable oxidation of PTD in 5 min. or less of illumination with white light. Most of the dyes listed actually show an effect in less than 1 min. of illumination. For many dyes, binding to high polymers enhances their photosensitizing ability. This is the case for eosin, erythrosin, rose bengal, thioflavin S and primulin yellow bound to polyvinylpyrrolidone and for thioflavin TG bound to desoxyribonucleic acid. On the other hand, proflavine when bound to desoxyribonucleic acid shows diminished activity.

Class B dyes are those which show no measurable photosensitizing action at least for illumination up to 20 min. Binding of these dyes to high polymers is without effect except in the case of ethyl violet and crystal violet. These latter dyes when bound to either polymethacrylic acid or to desoxyribonucleic acid are good sensitizers.

TABLE I

SENSITIZERS FOR PHOTOOXIDATION

Class A. Those Acting within 5 min. of Illumination

XANTHENES (fluorescein, 4,5-dichlorofluorescein, eosin, phloxine, erythrosin, rose bengal, pyronine Y); THIAZOLES (thiaflavin TG, thioflavin S, primulin yellow, Clayton yellow); THIAZINES (methylene blue, azure A, azure B, azure C); ACRIDINES (proflavin, acridine orange, enflavin, acridine yellow, 2,7-dimethoxy-3,6-diaminoacridine, 4,5-dimethyl-3,6-diaminoacridine, atebrin, acrizan R); PORPHYRINS (hematoporphyrin, potassium chlorophyllin); RIBOFLAVIN.

Class B. Those Not Acting within 20 min. of Illumination

XANTHENES (rhodamine B); INDAMINES (Bindschedler's green, toluylene blue); Azo (Janus green B, methyl orange, azoflavin S, acid tartrazine, trypan blue, acid orange XX, Bordeaux S, quinalizarin, Azorubin, azobenzene *p*-sulfonic acid, chlorophenine); AZINES (neutral red, phenosafranine, pinkakryptal green, safranine O, azocarmine); OXAZINES (brilliant cresyl green, Nile blue, cresyl violet); PHENYL METHANES (auramine O, acid fuchsin, aniline blue, brilliant green, wool fast blue, crystal violet, ethyl violet); ANTHRAQUINONES (alizarine red, 1-nitroanthroquinone sulfonic acid); CYANINES (3,3′-diethyl oxacarbocyanine, pinacyanol); ACRIDINE (9-β-hydroxyethylaminoacridine, N,N′-bis-9-acridyl-ethylenediamine, 10-methylacridine, 3-aminoacridine, 2,7-diaminoacridine, 3,7,9-triaminocridine); NITRO (Martius yellow, picric acid); INDOPHENOL.

Certain properties of these dyes are correlated with their ability to act as photosensitizers. All the dyes of Class A at a concentration of 10^{-5} *M* are: (1) fluorescent, (2) serve as photosensitizers

acting within 5 min. for the polymerization of 20% calcium acrylate in the presence of 0.01% allyl thiourea, (3) serve as photosensitizers acting within 5 min. for the reduction of 0.1% 2,3,5-triphenyltetrazolium chloride. Most of the dyes in class A exhibit a noticeable fading (photoreduction) on illumination in the presence of 0.01% allyl thiourea. Many of the dyes of Class B are also fluorescent but do not serve as photosensitizers for polymerization or for reduction at least for irradiation up to 20 min. None of the dyes of Class B exhibits photobleaching in the presence of 0.01% allyl thiourea. Crystal violet and ethyl violet exhibit these properties only when bound to the polymeric acids. For the xanthene dyes of Class A binding to polyvinylpyrrolidone enhances these same properties. On the other hand, binding of proflavine to desoxyribonucleic acid suppresses its fluorescence and diminishes its photosensitizing ability for polymerization, yet the dye is photobleached readily in the bound state.

The rate of oxidation of PTD in 0.1 M phosphate buffer at pH 7.0 sensitized by proflavin was studied in detail. The initial rate of autoxidation of PTD was found to be directly proportional to the intensity of the actinic radiation. As shown in Fig. 1, the rate is practically independent of oxygen concentration for oxygen–nitrogen gas

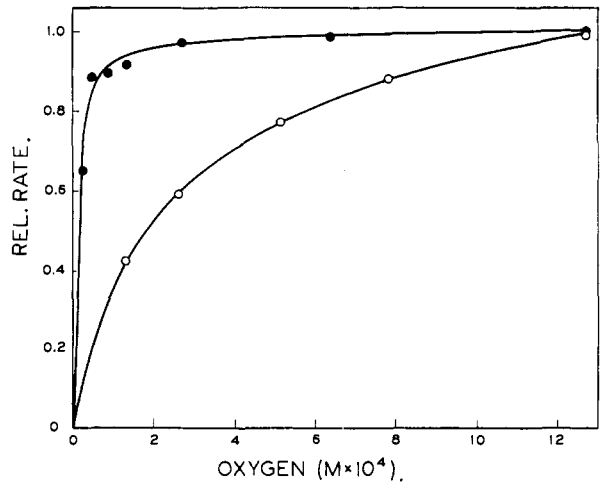

Fig. 1.—Variation of rate of photoöxidation of PTD with concentration of oxygen; PTD concn. 2×10^{-3} M. Open circles, proflavine concentration 2×10^{-5} M, 0.1 M phosphate buffer pH 7.0, illumination with white light; closed circles, 0.1 M acetate buffer, pH 3.0, illumination with ultraviolet light of wave length 254 mμ.

mixtures containing more than 5% oxygen and decreases below this concentration. The solubility of pure oxygen in water at 760 mm. pressure is taken to be 1.28×10^{-3} mole/l. and the data of Fig. 1 assume also that Henry's law is applicable. The dependence of the initial rate on oxygen concentration (O_2) is given by the empirical expression $(O_2)/(\alpha+(O_2))$ where α is approximately 1.25×10^{-5} mole/l. In the case of the ultraviolet-catalyzed autoxidation of PTD a similar rate expression is applicable but here $\alpha = 1.80 \times 10^{-4}$ mole/l.

As seen from the data of Fig. 2, the dependence of the rate of dye sensitized photoöxidation on

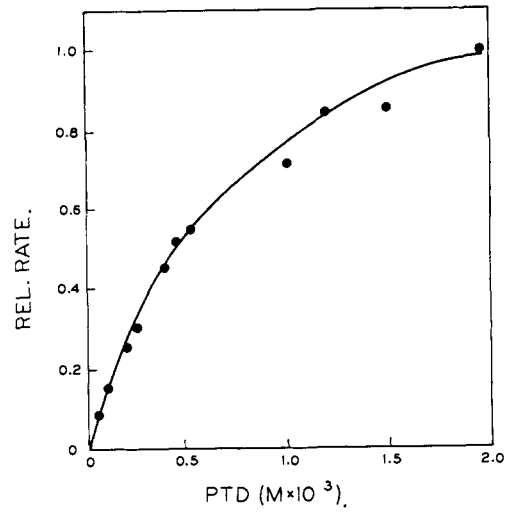

Fig. 2.—Variation of rate of photoöxidation with concentration of PTD. Dye concn. 2×10^{-5} M, oxygen concn. 2.78×10^{-4} M in 0.1 M phosphate buffer pH 7.0.

PTD concentration (S) is given by the empirical expression $(S)/(\beta + (S))$ where $\beta = 4.6 \times 10^{-4}$ mole/l. For very low concentrations of proflavine (less than 10^{-6} M) and high PTD concentration (2×10^{-3} M) in solutions saturated with oxygen, the quantum yield of autoxidation is 3 moles of PTD consumed per einstein of blue light absorbed. At very high concentrations of PTD exceeding 10^{-2} M there is actually a decrease in rate with increasing PTD concentration.

The quantum yield decreases markedly when the dye concentration is increased (Fig. 3). The quantum yield Φ decreases with increasing dye concen-

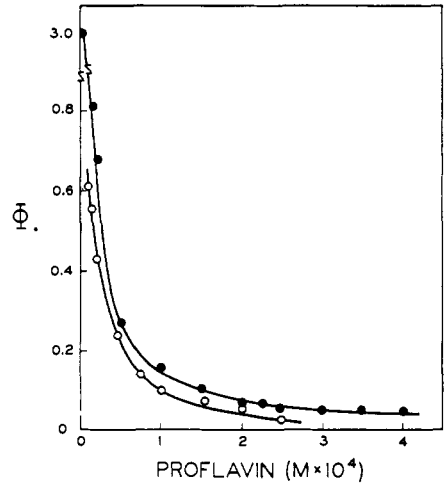

Fig. 3.—Variation of quantum yield of photoöxidation with concentration of dye; PTD concentration 2×10^{-3} M; 0.1 M phosphate buffer pH 7.0. Closed circles 2.78×10^{-4} M oxygen; open circles 2.78×10^{-5} M oxygen.

tration (D) according to the Stern–Volmer relation $\Phi = \gamma/(1 + \delta(D))$. For oxygen concentrations above 6.4×10^{-4} mole/l. (corresponding to a gas mixture of 5% oxygen) δ and γ are independent of oxygen and have the values 2.07×10^{5} l./mole and 3.0, respectively. For low oxygen concentration,

the value of δ increases with decreasing oxygen concentration. For example, with a gas mixture containing 2% oxygen $\delta = 2.40 \times 10^5$ l./mole but γ is essentially unchanged.

The intensity of fluorescence of proflavine in 0.1 M buffer both at pH 7.0 and at pH 4.0 is proportional to the dye concentration up to about 10^{-4} M. In solutions of 0.01 M buffer, however, deviations from linearity are appreciable at dye concentrations as low as $5 \times 10^{-6} M$.[5] Proflavine in glycerol cooled to about $-120°$ exhibits in the absence of oxygen a strong orange phosphorescence when excited with blue light or with near ultraviolet light. The phosphorescence is strongly quenched with oxygen. PTD is a much weaker quencher; in order to achieve the same degree of phosphorescence quenching the concentration of PTD must be about 25 times that of oxygen. In water the fluorescence of proflavine is quenched by PTD according to the Stern–Volmer relation and the quenching constant (concentration of PTD necessary to reduce the fluorescence by one half) is 26.3 l./mole. The fluorescence of an aqueous solution of proflavine is not appreciably quenched when saturated with oxygen at atmospheric pressures.

The addition of small amounts of certain reducing agents to a mixture of proflavine and PTD causes inhibition of the photoöxidation of the aromatic amine. The PTD oxidation is inhibited completely for a period of time, after which the reaction rate becomes the same as that for the uninhibited system. The induction period is proportional to the concentration of inhibitor. If oxygen is removed from the illuminated system during a period of time equivalent to the inhibition period and then oxygen is admitted, a further inhibition period of same duration results. Thus, light, dye and oxygen are necessary to overcome the inhibition of the photoöxidation. In aerated solutions at pH 7.0 containing 2×10^{-3} M PTD and 2×10^{-5} M proflavine, the addition of 5×10^{-4} M inhibitor caused inhibition periods as follows: glutathione, 4.5 min.; ascorbic acid, 3.2 min.; and cysteine, 2.5 min. Glucose, allylthiourea, thiourea, potassium oxalate, ethylenediaminetetraacetic acid, citric acid and potassium iodide are ineffective as inhibitors at least up to a concentration of 2×10^{-3} M.

Discussion

Only those dyes which are capable of being photoreduced act as photosensitizers for the oxidation of PTD. It is clear, therefore, that the same excited state of the dye is involved in both types of reaction. It has been demonstrated that the photoreduction of dyes involves a long-lived electronically excited state (probably triplet).[14] Molecules in this state can abstract electrons from mild reducing agents or certain chelating agents. PTD, on the other hand, is not an electron donor for light excited dye molecules even when oxygen is rigorously excluded. In the presence of oxygen, however, the long-lived species reacts preferentially with oxygen and thereby inhibits photoreduction. Oxygen is a powerful quencher of the phosphorescent triplet species (more powerful a quencher than PTD, KI, etc.). We therefore postulate that the long lived state reacts with oxygen to form some dye peroxide which in turn oxidizes the substrate (here PTD). This is in general agreement with the schemes proposed by Schenck[15] and by Livingston[16] for other sensitized photoöxidations.

Our data are compatible with the following scheme: (1) $D + h\nu \rightarrow D^*$ (absorption of visible light to give dye in first singlet electronically excited; (2) $D^* \rightarrow D + h\nu_f$ and/or heat (fluorescence and/or internal conversion to ground state); (3) $D^* \rightarrow D'$(transition to long-lived state); (4) $D' \rightarrow D + h\nu_p$/and heat (phosphorescence and/or internal conversion from the long-lived state); (5) $D' + D \rightarrow 2D$ (concentration quenching of long-lived state); (6) $D' + O_2 \rightarrow DO_2$ (formation of photoperoxide); (7) $DO_2 \rightarrow D + O_2$ (spontaneous decomposition of photoperoxide); (8) $DO_2 + D \rightarrow 2D + O_2$ (concentration quenching of photoperoxide); (9) $DO_2 + S \rightarrow D + SO_2$ (oxidation of substrate). Assuming steady-state concentrations for the transient species D*, D', DO_2, we obtain for the quantum yield of photoöxidation of the substrate

$$\Phi = \frac{k_3}{k_2 + k_3} \frac{k_6(O_2)}{k_4 + k_5(D) + k_6(O_2)} \times \frac{k_9(S)}{k_7 + K_8(D) + k_9(S)}$$

Comparing this expression with the empirical results, we obtain $k_4/k_6 = 5.0 \times 10^{-7}$ mole/l., and $k_7/k_9 = \beta = 4.6 \times 10^{-4}$ mole/l. Knowing (O_2) and (S) for the dependence of Φ upon (D) for low (O_2) and for high (O_2) we calculate from the resulting simultaneous linear equations, after neglecting terms quadratic in (D), that $k_5/k_6 = 0.60$ and $k_8/k_9 = 414$.

The large value of the limiting quantum yield, namely, 3 moles of PTD oxidized per einstein absorbed, probably arises from the fact that more than one PTD molecule is involved in the formation of molecules of the oxidized product; that is, one PTD molecule is photoöxidized and this reacts with two or more other PTD molecules to give the highly conjugated species (a Bandrowski base). Proflavine is a highly fluorescent dye with a quantum yield of fluorescence of 0.35.[7] Thus the maximum yield of photoöxidation, namely, $k_3/(k_2+k_3)$, cannot exceed 0.65. We therefore conclude that the pink product consists of polymeric molecules made up of at least 4–5 oxidized PTD molecules.[17]

Since k_5 is nearly equal to k_6, a long-lived excited dye molecule has practically equal probability of being deactivated by an unexcited dye molecule or reacting with an oxygen molecule. This result suggests that every encounter of a molecule with the long-lived excited dye leads to a reaction. If this is the case, then the lifetime of the long-lived state, namely, $1/k_4$, is k_6/k_4 divided by the number of diffusional encounters in water at room temperature, for a molar solution 6.6×10^9, or the lifetime is 3.0×10^{-4} sec. The lifetime of the first excited state of the dye, on the other hand, is about 5.0

(14) See G. Oster and A. H. Adelman, THIS JOURNAL, **78**, 913 (1956), and subsequent articles.

(15) G. O. Schenck, *Naturwiss.*, **35**, 28 (1948).

(16) R. Livingston and K. E. Owens, THIS JOURNAL, **78**, 3301 (1956).

(17) *Cf.* C. J. Sunde and W. M. Lauer, *J. Org. Chem.*, **17**, 609 (1952).

$\times 10^{-3}$sec.[7] The ultraviolet catalyzed reaction proceeds *via* an excited state of PTD with a lifetime of 8.4×10^{-7} sec. as computed from the observed value of α.

The probability of the dye peroxide DO_2 reacting with unexcited dye is 414 times greater than with PTD. If we regard the former reaction as one in which every encounter is effective, then the lifetime of the peroxide, namely, $1/k_7$, is given by $(k_8/k_7)(k_9/k_8)$ divided by 6.6×10^9 or 1.4×10^{-4} sec. It should be pointed out, however, that nothing in our data requires the *de facto* existence of the dye peroxide DO_2. The formation of an excited oxygen molecule would be equally compatible with our results. This possibility has been denied, however, on spectroscopic grounds (for summary of arguments, see ref. 4, p. 68).

Our scheme differs from that of Livingston[16] in certain respects. In that study, the chlorophyll-photosensitized autoxidation of allylthiourea, the spontaneous decay of the long-lived state (our step 4) was not considered. Furthermore, the fluorescence of chlorophyll unlike that of proflavine is quenched by small amounts of oxygen. Hence we have not included the step involving the direct attack of oxygen on D*, as did Livingston. Since the concentrations of PTD employed do not appreciably quench the fluorescence of proflavin, we can neglect this contribution. The decline in rate at very high PTD concentrations (10^{-2} *M*) would be due to this step. Our scheme also differs in the early steps for the formation of the long-lived state from that proposed for proflavine photoreduction.[5] There it was found that with increasing dye concentration the quantum yield of photoreduction increased and the fluorescence yield decreased even at concentrations of dye below 10^{-4} *M*. In the present study the quantum yield of photooxidation decreased with increasing dye concentration. Furthermore, the fluorescence yield is independent of dye concentration up to 10^{-4} *M* in dye. We attribute these differences to salt effects which become important for low buffer concentrations but which are suppressed in the present work by operating in a medium of 0.1 *M* buffer.

The inhibitors for the reaction are all characterized by the fact that they are easily autoxidized. Thus very weak reducing agents such as glucose are ineffectual, but ascorbic acid which is more readily autoxidized than is PTD is a good inhibitor. Some substances such as PTD are good quenchers for the long-lived state as shown by their retarding effect in photoreduction of dyes.[14] In the presence of oxygen, however, reaction of the long-lived state with oxygen is much more highly favored than reaction with PTD. In fact, the form of the rate data on PTD concentration seems to rule out this latter step as being of any consequence in photooxidations.

We are at a loss to understand why the proflavine sensitized photooxidation is diminished on binding the dye to polymer since the rate of photoreduction is enhanced under these conditions. This anomaly in behavior is also reflected in the results of our studies on the photodynamic action using various biological substrates.[8] Proflavine is exceptional in that despite its powerful photosensitizing effect when it is free in solution, it is ineffectual in mediating photodynamic action on, for example, desoxyribonucleic acid (transforming principle), suggesting that it is in the bound state in the presence of this substrate.

BROOKLYN, N. Y.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, POLYTECHNIC INSTITUTE OF BROOKLYN]

Photoreduction of Dyes in Rigid Media. II. Photoredox Properties of Thiazine Dyes[1a,1b,1c]

BY BARRET BROYDE AND GERALD OSTER

RECEIVED MARCH 19, 1959

Thiazines undergo photoreduction to their leuco forms when incorporated into high viscosity glasses of polyhydroxy compounds. The rate of photoreduction is proportional to the square root of the diffusion coefficient suggesting a diffusion-controlled process in which stationary state conditions are not achieved. Illumination of highly concentrated dye glasses containing an added mild reducing agent yields an intermediate color which reverts to the original dye on softening the glass. This species is an entrapped dimer of the normal dye. Near ultraviolet light irradiation of the leuco glass yields the normal dye and other colored species. In acid glasses a red form is produced and in basic glasses a yellow form is the result, both forms reverting to the leuco species on softening the glass. The ratio of red to blue forms increases when the viscosity of the medium is increased. These intermediate species are believed to be a semiquinone and a diradical, or a molecule in the triplet state entrapped in the rigid medium.

Introduction

Thiazine dyes in solution are readily photoreduced in the presence of mild reducing agents[2] and certain chelating agents.[3] The rate of photoreduction was found to decrease when the viscosity of the medium was increased, suggesting that a

(1) (a) Presented before the 135th National Meeting of the American Chemical Society, Boston, April 9, 1959; (b) this paper represents a part of the dissertation to be submitted by Barret Broyde to the faculty of the Graduate School of the Polytechnic Institute of Brooklyn in partial fulfillment of the requirements for the degee of Doctor of Philosophy; (c) this work was supported by the United States Air Force through the Air Force Office of Scientific Research of the Air Force Research and Development Command under Contract No. AF 49(638)-293 and by the U. S. Atomic Energy Commission, Contract No. AT(30-1)-2206.

(2) M. Mudrovcic, *Z. wiss. Photo.*, **26**, 171 (1928); K. Weber, *Z. physik. Chem.*, **B15**, 18 (1931); G. Holst, *ibid.*, **B169**, 9 (1934); J. Weiss, *Trans. Faraday Soc.*, **32**, 1331 (1936); E. Rabinowitch, *J. Chem. Phys.*, **8**, 551 (1940); M. Pestemer, *Z. Elektrochem.*, **58**, 121 (1954); H. Hardwick, THIS JOURNAL, **80**, 5667 (1958).

(3) G. Oster and N. Wotherspoon, *ibid.*, **79**, 4836 (1957).