Photochemistry of a Water-Soluble Polymeric Derivative of Chlorophyll[1a]

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Chlorophyll a is chemically bound to a copolymer of maleic anhydride and methyl vinyl ether by aminolysis with 1,6-hexanediamine. Hydrolysis of the remaining anhydride groups by ammonia produces a water-soluble polyelectrolyte in which the pigment is monomolecularly dispersed. The pigment (an amide of Mg chlorin e_6) is fluorescent in aqueous media, and the fluorescence is partially polarized if excited with polarized light. Its photochemical properties are compared with those of a corresponding monomer, prepared similarly but with succinic anhydride instead of the copolymer. The reduction of phenosafranine by hydrazobenzene, photosensitized by the polymeric pigment, is compared in detail with that sensitized by the monomeric analog and with the previously studied reduction sensitized by ethyl chlorophyllide a. The rate of reduction is not retarded by product in the present systems as it was in the ethyl chlorophyllide system, but photostationary states are reached in which phenosafranine is more extensively reduced in the polymeric system than in the monomeric. The pigment is considerably harder to reduce photochemically in the polymeric system than in the monomeric.

Introduction

Numerous attempts have been made to link the photochemistry of chlorophyll in solution with that in plants by studying the physical and photochemical properties of chlorophyll and its derivatives in suspensions, emulsions,[2-6] and thin films,[7] and adsorbed to proteins[8-10] or other substrates.[11-13] One purpose of most of these efforts has been to study the behavior of chlorophyll in aqueous media, in which chlorophyll by itself is not soluble. The photochemistry of water-soluble derivatives of chlorophyll has been studied with the same purpose in mind.[14-16] We wish to report the photochemical properties of chlorophyll chemically bound to a water-soluble polymer, and to compare them in some detail with the properties of chlorophyll similarly bound to the corresponding monomer. Oster, *et al.*, have compared the photochemistry of chlorophyllin bound to polyvinylpyrrolidone with that of the unbound pigment.[15] Although the act of chemical binding converts chlorophyll to a derivative of Mg chlorin e_6, the photochemical behavior of this compound, like that of chlorophyllin, resembles that of chlorophyll in many respects.

The polymer chosen was initially a commercially available alternating copolymer of maleic anhydride and methyl vinyl ether. 1,6-Hexanediamine was bound at one end to chlorophyll by the aminolysis reaction of Fischer and Goebel,[17] and at the other end

(1) (a) This work was supported in part by National Science Foundation Grant No. GB-2089; (b) Lawrence Radiation Laboratory, University of California, Berkeley, Calif.; (c) Charles F. Kettering Research Laboratory.

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to the polymer by reaction with an anhydride group. The remaining anhydride groups were cleaved with ammonia or an amine to form the ammonium salt of a polycarboxylic acid.

The appropriate monomer for comparison was made by joining chlorophyll through hexanediamine to succinic anhydride. The nonionic compound, Mg chlorin e_6–6-butylamide phytyl methyl ester, was also prepared for comparison.

Several photochemical reactions were demonstrated for the chlorophyll–polymer, but the one selected for detailed study was the sensitized reduction of phenosafranine by hydrazobenzene. A mechanism has recently been proposed for this reduction, sensitized by ethyl chlorophyllide a in pyridine–ethanol mixtures.[18]

The polymer to which chlorophyll is bound is an anionic polyelectrolyte. In solutions of low ionic strength, it may therefore attract cations and repel anions with which the photoexcited pigment could react. An effect of this sort might operate *in vivo* to select the correct reagent for chlorophyll. A question to be kept in mind in comparing reactions of the polymeric and monomeric chlorins is whether any differences could be explained by this more general ionic effect, or whether they must be attributed to more local environmental causes.

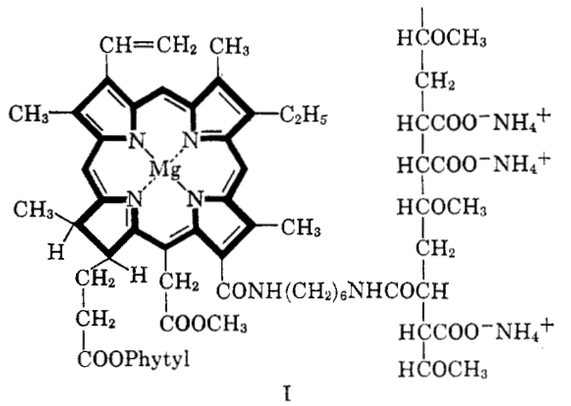
Experimental Section

Materials. Chlorophyll a was prepared from spinach by the method of Zscheile and Comar[19] and was purified chromatographically.

Samples of the alternating copolymer of maleic anhydride and methyl vinyl ether were gifts from General Aniline and Film Corp. A higher molecular weight sample, GANTREZ AN-169 (specific viscosity 2.6–3.5 at 1% concentration in butanone at 25°), was used for most purposes, but a lower molecular weight sample, GANTREZ AN-139 (specific viscosity 1.0–1.4), was more suitable for fluorescence polarization studies because it gave rise to less microgel.

1,6-Hexanediamine was sublimed *in vacuo* and stored under N_2. *n*-Butylamine was distilled and stored under N_2. Ascorbic acid and hydrazobenzene were recrystallized from ethanol–water. Phenosafranine was recrystallized from ethanol–cyclohexane and chromatographed on calcium carbonate with 2-propanol and ethanol as eluents. Hydroquinones were prepared by reduction of the ubiquinones UQ_2 and UQ_6 with sodium dithionite and extraction of the product into cyclohexane. Other reagents were used as received.

Preparation of Chlorophyll–Polymer (chl/p) (I). An excess of 1,6-hexanediamine was resublimed onto a film of chlorophyll a left by evaporation of an ethanol solution of the pigment. The reaction vessel was heated on a water bath to 45° to melt the diamine, into which the chlorophyll dissolved, and the aminolysis reaction was allowed to proceed for 90 min at that temperature, under N_2. Excess diamine was then removed by sublimation, leaving a film of Mg chlorin e_6–6-(6-aminohexylamide) phytyl methyl ester (chl/hexanediamine). As hexanediamine is an excellent cross-linking agent for the polymer, it was necessary



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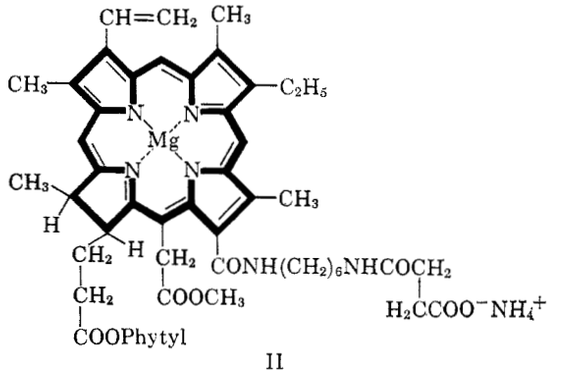

II

to remove every trace of it from the chlorin before reaction with the polymer. The chlorin was therefore dissolved in ethanol and precipitated by dilution with 0.1 *M* phosphate buffer of pH 10. The microcrystals were collected by filtration on a pad of Celite, washed with more buffer, air-dried, and dissolved off the pad with acetone. A solution of the polymer in acetone was added to the solution of the chlorin, and the reaction between them was allowed to proceed overnight in a refrigerator. Finally, the rest of the anhydride groups

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of the polymer were decomposed by treatment with 2 N NH_4OH, and excess ammonia and acetone were removed by flash evaporation. The chlorophyll–polymer is soluble in water and in mixtures with up to 80% ethanol.

The density of chlorin groups on the polymer chain could be controlled by varying the ratio of polymer anhydride groups to chl/hexanediamine. The samples used in the present work were prepared from an anhydride/chlorin ratio of 100:1. Polymers have been prepared with ratios from 1000:1 to 50:1, but polymers prepared with lower ratios contained too much gel to be useful.

The "chlorophyll–monomer" (chl/m) (II) was prepared by treating chl/hexanediamine with a 100-fold excess of succinic anhydride. The ammonium succinate formed by subsequent treatment of excess anhydride with ammonia was not separated from the chl/m, so that its effect could be compared with that of the succinate groups on the polymer. Chl/p and chl/m solutions differ, therefore, only in the connectivity of the groups to which the chlorin is attached.

Mg chlorin e_6–6-butylamide phytyl methyl ester (chl/butylamine) was prepared by reaction of chlorophyll with n-butylamine.[17]

The Mg-free derivatives of chl/p and chl/m, abbreviated pheo/p and pheo/m, were prepared by treatment of chl/p and chl/m with dilute HCl. Magnesium is slowly lost from the chlorins on storage in a refrigerator (about 10% per week), and many of the samples used for photochemistry contained a small amount of the Mg-free pigment, which appeared not to affect their reactivity unduly. Addition of 10% (v/v) pyridine to the stock solution helped to retain Mg.

Apparatus and Procedure. Absorption spectra were recorded on a Cary 14 spectrophotometer. Polarization of fluorescence was measured on an Aminco-Bowman spectrophotofluorometer. The fluorescence intensities were corrected for an instrumental asymmetry favoring perpendicularly polarized light over parallel polarized light.

Photoreactions were followed on a Perkin-Elmer Model 350 recording spectrophotometer, the sample compartment of which had been adapted to permit simultaneous irradiation and spectral measurement of the sample. With this instrument the transmittance scale can be expanded by a factor of 50, making possible detection of small light-induced changes in absorptivity. Light from a Sylvania Sun-Gun lamp was directed through a red sharp-cutoff filter (Corning 2-63) into one side of the 1-cm square cross-section reaction cell, in a direction perpendicular to the direction of the measuring beam. The light intensity incident on the sample cell was usually 8–9 $\times$ 10^4 ergs cm^{-2} sec^{-1}, as measured by a Yellow Springs Instrument Co.–Kettering radiometer. To eliminate scattered actinic light during kinetic runs with phenosafranine, in which spectral changes were followed at 530 nm, Spectrolab interference filters No. 1860 and 1860-1 were placed in the measuring and reference beams.

Although chl/p is fully reactive in aqueous solutions, *e.g.*, in 0.1 M tris(hydroxymethyl)methylamine (Tris)–HCl buffer of pH 7.5, most reactions reported here were conducted in a medium in which chl/m is also soluble, a mixture of equal parts of water, ethanol, and pyridine (1:1:1), pH 9.0; no foreign buffer was included, so that differences in the ionization properties of polymer and monomer might be accentuated. Unless otherwise stated, all reactions were carried out in this solvent, at chl/p and chl/m concentrations of 1.2 $\times$ 10^{-5} M. In sensitized reductions of phenosafranine (D^+) by hydrazobenzene (AH_2), their usual concentrations were 1.0 $\times$ 10^{-5} and 8 $\times$ 10^{-4} M, respectively.

For a sensitized photoreaction, stock solutions of the chlorophyll derivative and the oxidant were mixed with solvent in the reaction tube, which was then sealed with a serum cap and flushed 10 min with Matheson prepurified grade N_2, which had been passed through Oxsorbent and KOH solutions. An aliquot of stock solution of reductant, preserved under N_2, was added by syringe, and the contents flushed 5 min more with N_2.

Results

Spectral Properties. In a given solvent, the spectra of chl/p, chl/m, chl/butylamine, and chl/hexanediamine are almost identical. In the 1:1:1 mixture, the main red and Soret bands of both chl/p and chl/m are at 644 and 417 nm. In water the bands of chl/p are at 642 and 414 nm, and in ethanol the bands of chl/m are at 641 and 415 nm. The band widths and relative intensities in the spectrum of chl/p in water are normal for nonassociated chlorin, and the solution is fluorescent. The extinction coefficients of the red bands of chl/p and chl/m were taken to be the same as that of chl/butylamine, 6.3 $\times$ 10^4 M^{-1} cm^{-1} in acetone, determined by quantitative reaction of n-butylamine with chlorophyll a.

If the fluorescence of a molecule is excited with polarized light and if the molecule is unable to rotate freely during the lifetime of its excited state, the emitted fluorescence may be polarized to some extent. Attachment of the chlorin to the polymer chain somewhat restricts its movement, and it is not surprising that the fluorescence of chl/p retains polarization

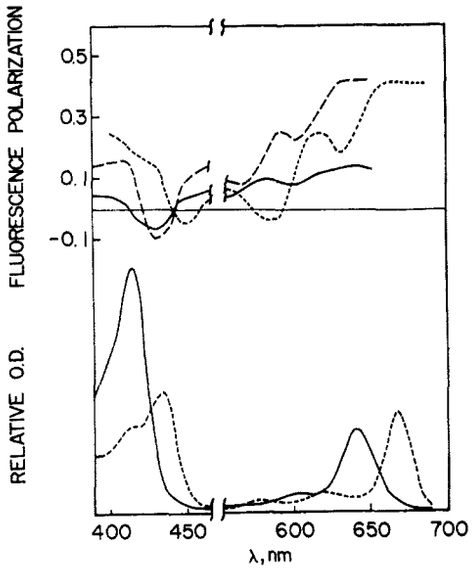

Figure 1. Absorption spectra of chlorophyll a (- - - -) and of chl/hexanediamine (———) in castor oil (lower). Polarization of fluorescence excitation spectra of chlorophyll a (- - - -) and of chl/hexanediamine (— — —) in castor oil, and of chl/p in Tris–HCl buffer, pH 7.5 (———) (upper).

even in aqueous solution at room temperature (Figure 1). The maximum degree of polarization, $p = +0.12$, is considerably less than that of chlorophyll a or chl/hexanediamine in castor oil (+0.42), but the wavelength dependence closely resembles that of the latter. (The degree of polarization p is defined as $(I_{\parallel} - I_{\perp})/(I_{\parallel} + I_{\perp})$, where $I_{\parallel}$ and $I_{\perp}$ are the intensities of fluorescence polarized parallel and perpendicular to the direction of polarization of the activating beam.) Our curve for the wavelength dependence of the degree of polarization of the fluorescence of chlorophyll a is in good agreement with previously published curves.[20,21]

Photochemical Properties. Photoreduction. Perhaps the best known photochemical reaction of chlorophyll is its photoreduction by ascorbic acid in pyridine, discovered by Krasnovskii.[22] Rackow and König reported that the photoreduction of chl/butylamine was very slow and irreversible.[23] Photoreduction of chl/p is also very slow. In Tris–HCl buffer containing 10% pyridine and 10^{-3} M ascorbic acid, the chlorin was only about 30% reduced to a product with an absorption band around 530 nm, even after 15 min of light of intensity of 1.5×10^5 ergs cm^{-2} sec^{-1}. The product reached a maximum concentration, and on continued illumination both it and the chlorin were bleached. After exposure to air for several hours the original chlorin was almost completely regenerated.

Neither chl/p nor chl/m was reduced at an appreciable rate by hydrazobenzene. Certain photoreductions were noted in connection with the sensitized reduction of phenosafranine and will be described later.

Sensitized Reactions. Chlorophyllin sensitizes the photoreduction of the azo dye, fast red S, by ascorbic acid in water at pH 7.[15] Similarly, chl/p sensitized the reduction of the dye (10^{-5} M) by hydrazobenzene in the 1:1:1 solvent to the extent of 90% in 2 min. However, no reduction was noted with ascorbic acid, for which we can offer no explanation.

N-Methylphenazinium methyl sulfate (PMS) and the ubihydroquinones, $(UQ_2)H_2$ or $(UQ_6)H_2$, equilibrate in the dark according to eq 1. Chlorophyll and

$$PMS + UQH_2 \rightleftharpoons PMSH_2 + UQ \qquad (1)$$

chloroplasts photochemically displace this equilibrium toward the left in the presence of the detergent Triton X-100.[3] The original equilibrium is restored in the dark. Chl/p has the same effect in 55% aqueous ethanol, pH 7.5 Tris–HCl buffer, with or without Triton X-100. With UQ_6, the displacements of optical density in the light and the dark amounted to about 0.05 at 388 nm (PMS band) and were attained in about 20 sec.

Phenosafranine. No photosensitized reduction of this dye was noted with chl/p as the sensitizer and ascorbic acid as the reductant, probably because of a very rapid back reaction.[18,24] With cysteine as reductant, a quasi-stationary state was attained after a few seconds of light, involving a 3% increase in the transmittance at the phenosafranine peak, which reverted rapidly in the dark. On this was superimposed a slow photoreduction of the dye, which was not reversed in the dark without admission of air. A similar situation existed with $FeCl_2$ as reductant, in which slow precipitation of $Fe(OH)_3$ was probably responsible for the shift of the quasi-stationary state toward further reduction of dye.

With hydrazobenzene as reductant, and chl/p or chl/m as sensitizer, photostationary states were also attained within 1–2 min after onset of illumination, but these corresponded to much greater reduction of dye than with the other reductants. More dye was reduced at the stationary state with chl/p as sensitizer than with chl/m, under otherwise identical conditions; Figure 2 compares the spectra at the photostationary

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(21) W. Arnold and E. S. Meek, *Arch. Biochem. Biophys.*, **60**, 82 (1956).
(22) A. A. Krasnovskii, *Dokl. Akad. Nauk SSSR*, **60**, 421 (1948).
(23) B. Rackow and H. König, *Z. Elektrochem.*, **62**, 482 (1958).
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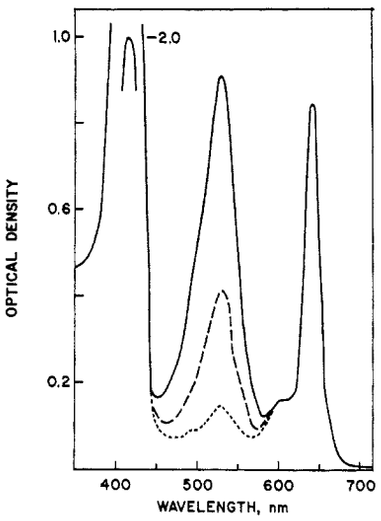

Figure 2. Sensitized photoreduction of phenosafranine by hydrazobenzene: initial concentrations, 1.2×10^{-5} M chl/p or chl/m, 1.25×10^{-5} M phenosafranine, 8×10^{-4} M hydrazobenzene in 1:1:1 water–ethanol–pyridine; initial spectrum (———); photostationary state with chl/m (— — —); photostationary state with chl/p (- - - -). Spectra normalized to Soret band optical density of 2.

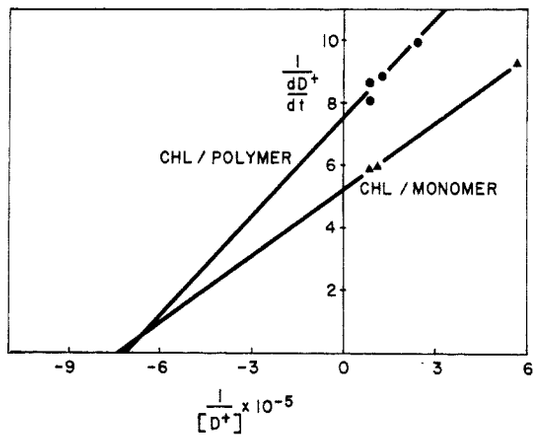

Figure 3. Dependence of initial rate of sensitized reduction on phenosafranine concentration (see eq 2 of text).

states. The dye was not regenerated to any great extent in the dark, although there was often some slow recovery, apparently due to oxidizing impurities or oxygen leakage. Reexposure to light rapidly restored the photostationary state.

Although initial rates were somewhat faster in the monomeric system than in the polymeric, the extent of reduction at the photostationary states represents the most significant difference between the two systems. We have investigated the effects of various alterations in the conditions of the reaction on the initial rate and the photostationary state composition, in an attempt to locate the cause of this difference.

In the absence of phenosafranine, there was no detectable reaction between chl/p or chl/m and hydrazobenzene. In the absence of hydrazobenzene, however, there was a rapid, reversible, small (<1%) decrease in the phenosafranine peak on illumination with red light in the presence of chl/p. The sensitized reduction of phenosafranine is therefore probably initiated by oxidation of the photoexcited chlorin by phenosafranine, as was proposed for the ethyl chlorophyllide sensitized reduction.[18]

Although the initial rate of dye reduction was proportional to light intensity down to one-third of the usual level, the composition of the photostationary state was unchanged. This observation and the absence of a fast dark reaction show that the photostationary state arises from competition between forward and reverse photochemical reactions and not between a forward photochemical reaction and a reverse dark reaction.

With either chl/p or chl/m as sensitizer, the initial rate of dye reduction varied with its concentration according to

$$d[D^+]/dt = (d[D^+]/dt)_\infty k_2[D^+]/(k_1 + k_2[D^+]) \quad (2)$$

in which $(d[D^+]/dt)_\infty$ is the limiting rate at large dye concentration. Values of k_2/k_1, obtained from the $1/[D^+]$ intercept of Figure 3, were about 7.2×10^5 M^{-1} for both chl/p and chl/m. The limiting rates $(d[D^+]/dt)_\infty$ varied somewhat from sample to sample but were always comparable for chl/p and chl/m. The per cent of dye reduced at the photostationary state did not vary with initial dye concentration.

Both the initial rate of reduction and the per cent dye reduced at the photostationary state depend on the concentration of hydrazobenzene (Figure 4). The dependence does not follow an equation like (2) but is better described by (3), with $x = 0.21$, 0.29, and 0.36 for chl/p, chl/m, and chl/butylamine.

$$d[D^+]/dt = -k[AH_2]^x \quad (3)$$

The presence of the polymer only affects the photostationary state when the chlorin is bound to it. Addition of the polymer, decomposed with ammonia but without pigment, to the chl/m-sensitized system did not increase the amount of dye reduced at the photostationary state.

Lowering of the pH by addition of acetic acid considerably decreased the initial rate and the per cent dye reduced at the photostationary state in the chl/p-sensitized system, but left the chl/m-sensitized system comparatively unaltered. With chl/p at pH 6.6 and

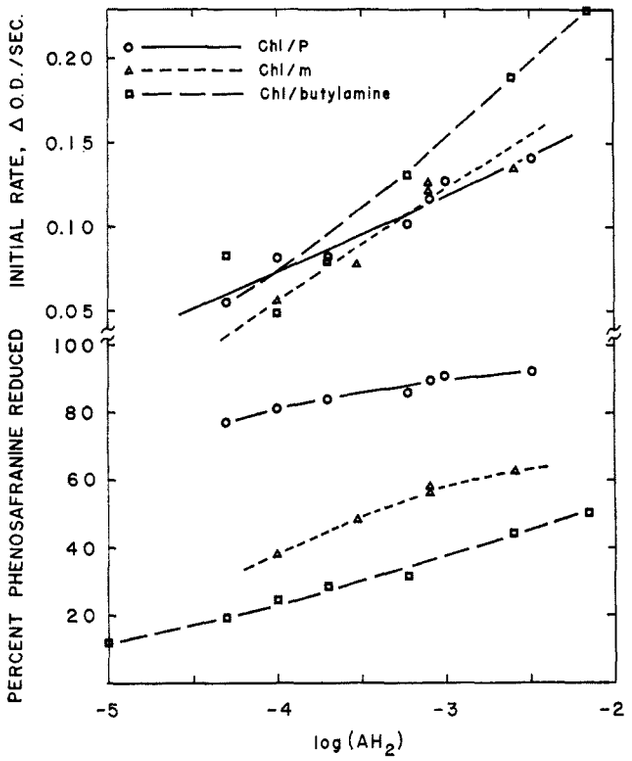

Figure 4. Dependence of initial rate (upper) and photostationary state composition (lower) on initial hydrazobenzene concentration: initial concentrations, 1.1×10^{-5} M chl/p or chl/m and 1.0×10^{-5} M phenosafranine in 1:1:1 water–ethanol–pyridine, 1.5×10^{-5} M chl/butylamine, and 1.2×10^{-5} M phenosafranine in 70% ethanol–30% 0.2 M Tris–HCl buffer, pH 7.5. Initial rate expressed as rate of optical density change at 530 nm (phenosafranine band).

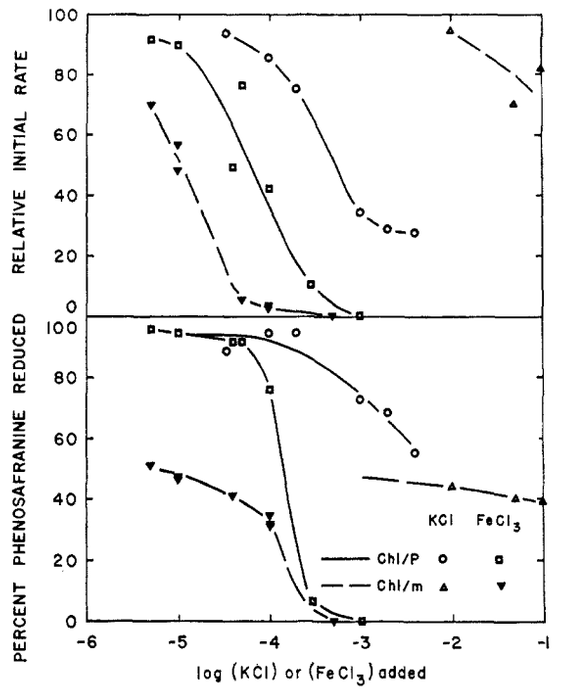

Figure 5. Effects of addition of KCl and $FeCl_3$ on rate and photostationary state of sensitized reduction of phenosafranine by hydrazobenzene: *ca.* 1.0×10^{-5} M phenosafranine, 1.2×10^{-5} M chl/p or chl/m, and 8×10^{-4} M hydrazobenzene. Initial rates expressed as per cent of rate with no added salt ($FeCl_3$ is reduced to $FeCl_2$ by hydrazobenzene before the photoreaction begins).

5.95, the initial rates were 41 and 22% of their usual value at pH 9.0, and the dye was only 56 and 39% reduced at the photostationary state.

Increase of the ionic strength by addition of KCl also decreased the initial rate and the per cent of dye reduced at the photostationary state in the chl/p-sensitized system and had almost no effect in the chl/m-sensitized system (Figure 5). Above 10^{-3} M KCl, the polymer began to precipitate, and the initial rates in Figure 5 have been adjusted to correct for loss of pigment from this cause, assuming that the rate is proportional to pigment concentration.

The effect of pH is not clearly separated from that of ionic strength, because the amounts of acetic acid added to lower the pH (1 and 10% of the pyridine) were such as to significantly increase the ionic strength.

A number of runs were made with $FeCl_3$ added to the reaction mixture before illumination. Addition of $FeCl_3$ has two effects: oxidation of an equivalent amount of hydrazobenzene to azobenzene and, thereby, introduction of $FeCl_2$ as a reductant. Points for these runs are also included in Figure 5. Unlike KCl, addition of $FeCl_3$ depresses the initial rate in the chl/m system at lower concentrations than in the chl/p system; the fall of the rate to ~0 may be ascribed to displacement of hydrazobenzene as reductant by $FeCl_2$. However, the composition of the photostationary state is not much altered until the amount of $FeCl_3$ exceeds 10% of that of hydrazobenzene.

A few runs were made with hydrazobenzene stock solutions that had become yellow owing to partial oxidation to azobenzene by air. Although the amount of azobenzene in these runs obviously exceeded the amount that would be formed during a sensitized reduction, the initial rates and photostationary states were hardly different from those in the absence of azobenzene.

In order to clarify the nature of the back-reaction leading to the photostationary state, the interaction of chl/m and chl/p with leucophenosafranine (DH) was examined. The photoreduction of a chlorin by leucophenosafranine alone is hard to study, because of extreme sensitivity of the reduced dye to O_2 in

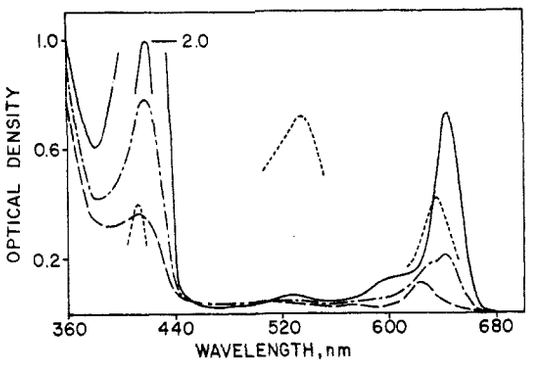

Figure 6. Photoreduction of chl/m by leucophenosafranine (1.25×10^{-5} *M*) preserved by slight excess of sodium dithionite, in 1:1:1 water–ethanol–pyridine: spectrum before illumination (———); after 5-min illumination (– · – ·), after 10-min illumination (— — —), location of bands after exposure to air overnight (- - - - -).

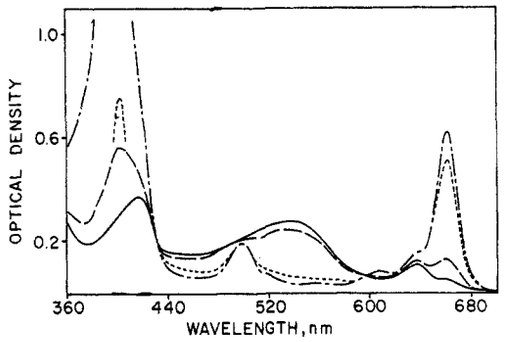

Figure 7. Photoreduction of pheo/m by hydrazobenzene (8×10^{-4} *M*) in 1:1:1 water–ethanol–pyridine: spectrum before photoreduction (— – —), after 2-min illumination (– – – –), after 10-min illumination (———), after exposure to air for 1 hr (- - - - -).

basic solution and suppression of the reaction by phenosafranine. The reaction can be followed if a small quantity of sodium dithionite is added to keep the dye in its reduced form. Neither chl/p nor chl/m react photochemically with dithionite alone rapidly enough to interfere.

The photoreduction of chl/m by leucophenosafranine/dithionite is shown in Figure 6. The only product which absorbs appreciably in the visible (623 and 412 nm) probably belongs to the class of hypochlorins.[25] The product of its oxidation by air absorbed at 635 nm and was presumably the *meso* derivative of the original Mg chlorin.

The photoreduction of chl/p was very much slower. After 10 min illumination, only 25% of the chl/p had been reduced, even with 2×10^{-4} *M* leucophenosafranine, and the bands of no reduced product were yet apparent.

If pheo/p is present with chl/p during sensitized reduction of phenosafranine, it is slowly reduced if illumination is continued past the time required to establish the photostationary state. Pheo/m is rapidly reduced by hydrazobenzene alone (Figure 7) to a product similar to those reported from other derivatives of chlorin e_6.[14,15]

Pheo/p sensitized the photoreduction of phenosafranine by hydrazobenzene as rapidly as did chl/p, and the reaction was quantitative; the ensuing reduction of the chlorin was less than 1% as fast. With pheo/m, an initial sensitized reduction of phenosafranine to about half its original concentration was followed by gradual restoration of the phenosafranine and reduction of the chlorin. The net result was reduction of pheo/m by hydrazobenzene, mediated by leucophenosafranine. The reduction of pheo/m continued after the light was turned off, but was probably accelerated by light.

Discussion

Chlorophyll, chemically bound to polymer in the way described, has in aqueous solutions the spectral, fluorescence, and photochemical properties expected of a monomolecularly dispersed pigment. It might therefore be used to study photochemical reactions in systems in which unbound chlorophyll derivatives would aggregate. However, the system has the drawback that the polymer is very susceptible to cross-linking reactions, so that solutions of it generally contain a certain amount of microgel.

The photochemistry of chl/p, chl/m, pheo/p, and pheo/m is very much like that of other chlorin e_6 derivatives and differs from that of chlorophyll most sharply in the ease and manner of photoreduction. The greater resistance of chl/p than of chl/m to photoreduction may have a simple explanation. The reduction of ethyl chlorophyllide a apparently requires the termination step (eq 4).[26] The rate of this step

$$\mathrm{ChlH}\cdot + \mathrm{ChlH}\cdot \longrightarrow \mathrm{ChlH_2} + \mathrm{Chl} \qquad (4)$$

for chl/p would be limited by the rate of diffusion of the polymer and therefore would be very slow compared with the rate for chl/m. However, the slow rate of permanent, two-electron reduction of chl/p and pheo/p would not prevent their acting efficiently as one-electron transfer agents in photosensitized reactions.

The reduction of phenosafranine sensitized by chl/p or chl/m differs from that sensitized by ethyl chloro-

(25) G. R. Seely, *J. Am. Chem. Soc.*, **88**, 3417 (1966).
(26) G. R. Seely and A. Folkmanis, *ibid.*, **86**, 2763 (1964).

phyllide[18] in that a photostationary state is attained in the former. The ethyl chlorophyllide sensitized reduction is greatly retarded by a reaction product, probably leucophenosafranine, but there is no evidence for approach to a truly stationary state. On the other hand, there is no indication that the kind of retardation encountered in the chlorophyllide system is operating here, because with chl/p, and $[AH_2] > 8 \times 10^{-4}$ M, 90% of the dye is reduced within 1 min, and the rate depends on the ratio $[D^+]/[DH]$ rather than on [DH] alone. Therefore, the photostationary state cannot be just an exaggerated form of the retardation encountered in the chlorophyllide system. To explain the retardation, a metastable complex between hydrazobenzene and oxidized chlorophyllide was postulated; in the present systems, such a complex, if formed at all, must not be long-lived.

The mechanism of the forward reaction (reduction of dye) is probably the same, basically, as that proposed for the ethyl chlorophyllide sensitized reaction, *viz.*

$$\text{Chl} \xrightarrow{\text{light}} \text{Chl}^* \longrightarrow \text{Chl}' \qquad (5)$$

$$\text{Chl}' \xrightarrow{k_1} \text{Chl} \qquad (6)$$

$$\text{Chl}' + \text{D}^+ \xrightarrow{k_2} \text{Chl}^+ + \text{D}\cdot \qquad (7)$$

$$\text{Chl}^+ + \text{AH}_2 \longrightarrow \text{Chl} + \text{AH}\cdot + \text{H}^+ \qquad (8)$$

Chl, Chl*, and Chl′ represent chl/p or chl/m in its ground, singlet-excited, and triplet-excited states. Follow-up reactions convert D· and AH· in part to the final products, DH and A.

The photochemical back-reaction probably begins by reaction of Chl′ with one of the products of the forward reaction, azobenzene or leucophenosafranine. As the presence of azobenzene has little effect on the initial rate of photostationary state, primary reaction with this substance is most unlikely. It is more likely that the initial reaction is with leucophenosafranine, and that the mechanism is analogous to that proposed for the reduction of azobenzene sensitized by ethyl chlorophyllide (eq 9 and 10).[27] As the azobenzene concentration is not rate controlling, reaction 10 must be fast enough to compete favorably with reoxidation of ChlH· by D^+. The follow-up reactions

$$\text{Chl}' + \text{DH} \longrightarrow \text{ChlH}\cdot + \text{D}\cdot \qquad (9)$$

$$\text{ChlH}\cdot + \text{A} \longrightarrow \text{Chl} + \text{AH}\cdot \qquad (10)$$

that dispose of D· and AH· would be the same as in the forward reaction.

One of the questions originally asked was whether the anionic polymer might accelerate the reduction of phenosafranine by concentrating the cationic dye in the vicinity of the photosensitizer. The dependence of initial rate on [KCl] and pH would support an affirmative answer, but the more gradual change in the photostationary state with [KCl] and the identity of the ratios k_2/k_1 (see Figure 3, eq 2, 6, and 7) for chl/p and chl/m do not. It cannot therefore be concluded that concentration of dye about the polymer has any definite effect on the kinetics of the reaction.

The correlation between the dependences of initial rate and photostationary state composition on hydrazobenzene concentration (Figure 4) suggests that the relative rates of step 8 and of reactions competing with it (*e.g.*, eq 11) are an important factor in determining the amount of dye reduced at the photostationary state. The apparent fractional-order dependence on $[AH_2]$ suggests that the mechanism of the forward reaction is rather complex, but the data presently available cannot support a more detailed kinetic treatment. The difference between the chl/p and chl/m sensitized reactions appears not to arise from long-range effects of the polymer, but from a difference in the ease of reduction of the pigment, or from ionic or other environmental effects in the immediate vicinity of the pigment.

$$\text{Chl}^+ + \text{D}\cdot \longrightarrow \text{Chl} + \text{D}^+ \qquad (11)$$

(27) G. R. Seely, *J. Phys. Chem.*, **69**, 2779 (1965).