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Structure of Porphyrins in Relation to Reversible Oxidation and Reduction Reactions[1]

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A variety of porphyrin derivatives were synthesized in order to determine the effect of certain structural features on the oxidation–reduction properties of these complexes. It was found that a fifth ring, such as the isocyclic ring of chlorins was without significant effect on the one-electron oxidation properties of a magnesium tetrahydroporphyrin (bacteriochlorophyll), Fe(II) porphyrin complexes, or Fe(II) chlorin complexes. On the other hand, large positive shifts of the midpoint potentials for oxidation of Fe(II) derivatives were found when the porphyrin ring was reduced to a chlorin structure.

INTRODUCTION

The question of the relationship between the structure of chlorophyll, its chemical reactivity, and its organization *in vivo* is most intriguing. Extensive studies of photosynthetic organisms have led to the general consensus that there are two different functions that these pigment molecules perform. On the one hand, it is believed that an aggregate of chlorophyll molecules serves as an antenna to gather light energy and to allow this light energy to arrive at a receiver molecule (*1*, *2*). On the other hand, a number of rapid photoinduced changes in absorbance (*3–11*) and EPR (*12–17*) signals have been carefully measured in plant and bacterial systems and these observations are in accord with the conclusion that the primary photochemical act involves the oxidation of chlorophyll (*18–23*).

It might be possible to arrange the structural features of the chlorophyll molecule in two general classes: (1) those features which affect the aggregation properties of the molecule and hence are important to its function as an antenna, and (2) those that affect the oxidation and reduction properties of the molecule. Katz and co-workers (*8–10*) have studied the aggregation of chlorophyll and porphyrins using the techniques of infrared and nuclear magnetic resonance spectroscopy. Their results indicate that

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in the absence of water, aggregation involves the interaction of the magnesium ion of one molecule with the C-9 carbonyl group of a second chlorophyll. Different spectral properties were related to different aggregate species, which can be generated by changing the solvent and the water content of the system.

A number of investigators have studied the oxidation of various metalloporphyrins and metallochlorins (*19*, *20*, *23*, *27–29*). Under proper conditions the reversible one-electron oxidation of the metalloporphyrins and metallochlorins could be observed to yield relatively stable cation radical species. Electrochemical considerations in the case of zinc and magnesium species lead to the conclusion that an electron is removed from the aromatic system rather than from the central metal ion. The oxidation–reduction potentials for any metal ion-substituted porphyrin or chlorin parallel the electronegativities of the central metal ion. Thus, it seems that the metal ion plays a role in determining both aggregation properties, and the oxidation and reduction properties, of chlorophyll and chlorophyll-like compounds.

Of the various structural features of the organic portion of these molecules the oxidation state of the macrocyclic ring system apparently has a significant effect on the oxidation–reduction properties. Fuhrhop (*28*) has carried out the only systematic investigation of this problem to date. It was found that the single-electron oxidation of a metallochlorin occurred at a potential approximately 300 mV less positive than the oxidation of the porphyrin complex of the same metal.

It has been the purpose of this present work to determine the influence of certain of the structural features of chlorophyll on its oxidation and reduction properties. A list of the derivatives and analogs studied, and a comparison of their features to that of chlorophyll, is presented in Table 1. A preliminary report of some of this data has been given (*30*).

TABLE 1

PORPHYRIN DERIVATIVES PREPARED

Compound	Isocyclic ring	Reduced ring IV	Electrophilic substituents at C-9 and C-10
Chlorophyll *a* (pheophorbide *a*)	+	+	+
Chlorin e_6	−	+	+
Pyropheophorbide *a*	+	+	−
Desoxyphylloerytherin	+	−	−
Hematoporphyrin IX	−	−	−
Protoporphyrin IX	−	−	−

For the purposes of these experiments, oxidations have been carried out on the zinc derivatives of the compounds listed in Table 1, and reductions have been carried out on the ferric derivatives of these compounds. Investigations have also been carried out on bacteriochlorophyll and bacteriopheophorbide: In addition to possessing the isocyclic ring, a reduced ring IV, and electrophilic substituents at C-9 and C-10, bacteriochlorophyll also has a reduced ring II.

MATERIALS AND METHODS

Solvents

All solvents used were reagent or spectroquality grade. Dry pyridine was prepared by distillation from calcium hydride and was stored over Linde A-5 molecular sieves which had been activated by heating at 120°C for 6 hr. The petroleum ether used was the 30–60°C boiling fraction. Diethyl ether was treated with ferrous sulfate and sulfuric acid to remove peroxides, then washed with water. Chloroform was washed with water, dried over calcium chloride, and filtered before use.

Redox Reagents

Potassium iridic chloride was purchased from City Chemical Corporation. Ferric perchlorate in the yellow hydrated form, $Fe(ClO_4)\cdot 6H_2O$, was obtained from the G. Frederick Smith Chemical Company, Columbus, OH and *N*-bromosuccinimide was manufactured by the J. T. Baker Company. All compounds were reagent grade, or better. They were used as received. The solutions were prepared immediately prior to use.

Hematoporphyrin

Hematoporphyrin dihydrochloride, lot No. 28 B-1000, was used as received from the Sigma Chemical Corporation.

Pheophorbide a

Pheophorbide *a* was generated in, and extracted from, fresh spinach leaves. The leaves were immersed in boiling water for 1 min, squeezed dry, and then placed in a Waring Blendor with a 6:20 mixture of petroleum ether and methanol which was 2% (v/v) in concentrated hydrochloric acid. The suspension was allowed to stand until its color had changed from green to brown, and it was then filtered through cheesecloth. The brown residue was extracted several times with a petroleum ether–methanol mixture which contained no hydrochloric acid. Approximately 100 ml of water were added to the pooled plant extracts. The aqueous phase which resulted was extracted several times with petroleum ether while the organic phase was washed an equal number of times with water. The pooled organic phases were brought to dryness in a rotary evaporator. The residue consisted of impure pheophorbide *a*. This residue produced a positive Molisch phase test. A solution of the residue in acetone had absorbance maxima at 666, 606, 558, 532, 508, and 409 nm. The reported absorbance maxima of methyl pheophorbide *a* in dioxane occur at 666, 610, 560, 535, and 506 nm (*31*).

Pheophorbide *a* methyl ester was prepared by treating an ether solution of the free acid with diazomethane, and recrystallizing the product from chloroform and petroleum ether at 194°K.

Elemental analysis of the dimethyl ester was carried out by the Microtech Corporation of Skokie, IL. The following results were obtained:

Anal. Calcd for Methyl Pheophorbide *a* $C_{36}H_{38}O_5N_4$: C, 71.26; H, 6.31; N, 9.24. Found: C, 69.87; H, 6.20; N, 9.11.

Pyropheophorbide a

Pyropheophorbide *a* was prepared from pheophorbide *a* by the method of Pennington et al. (*32*), and the product was recrystallized from chloroform–petroleum ether. A Molisch phase test of the product was negative. The pyropheophorbide *a* was dissolved in acetone and the spectrum recorded on a Cary-14 spectrophotometer.

The spectral properties in *acetone* are reported below: The first number is the absorbance maxima in nanometers, and the number in parentheses is the millimolar extinction coefficient at that wavelength; 668(40), 609(7), 560(4), 535(9), 508(10), 410(69). Wolf (*33*) reported the following spectral characteristics for methyl pyropheophorbide *a* in dioxane: 668(56), 610(8), 560(3), 536(10), 507(12), 474(4), 412(125).

The NMR spectrum of this compound was recorded in deuteropyridine solution using a Varian MA-100 Nuclear Magnetic Resonance Spectrophotometer.[4] The spectrum agreed well with that reported by Katz et al. (*24*) and displayed no significant peaks attributable to low molecular weight impurities.

Desoxyphylloerythrin

Desoxyphylloerythrin was prepared from pyropheophorbide *a* by the method of Wei et al. (*34*). The product was recrystallized from chloroform–petroleum ether. Some difficulty was experienced in the removal of insoluble matter formed during the severe conditions of the Wolff–Kishner reduction. The product was dissolved in chloroform and filtered through a Millipore 50-nm filter prior to recrystallization.

The spectral properties in acetone were: 619(2.8), 564(4.0), 532(3.2), 495(5.7), 397(93.0). The spectral properties of the methyl ester in dioxane, reported by Stern and Wenderlein (*35*) were: 615(6), 589(1.0), 564(6), 530(4), 496(17). The NMR spectrum of this compound in $CDCl_3$ was recorded on the Varian MA-100 instrument at Argonne National Laboratories and was consistent with that expected for desoxyphylloerythrin.

Chlorin e_6

Chlorin e_6 was prepared from pheophorbide by the method of Conant and Mayer (*36*). The product gave a negative Molisch phase test and had the following spectral properties in acetone solution: 664(30), 609(4), 559(3), 530(4.6), 500(7.8), 400(73). Wolf (*18*) has reported the following spectral characteristics for a solution of chlorin e_6 trimethyl ester in dioxane: 665(52), 609(5), 559(2), 530(5), 501(13), 402(154). The chlorin e_6 was treated with diazomethane to form a compound which was not readily crystalline. The elemental analysis is in good agreement with that which is expected for either the trimethyl or the dimethyl ester, but on paper chromatography the material behaved as though it had a free carboxyl group.

Anal. Calcd for trimethyl ester: C, 69.0; H, 6.85; N, 8.94; for dimethyl ester: C, 68.61; H, 6.58; N, 9.14. Found: C, 69.00; H, 6.35; N, 8.78.

Preparation of the Zinc Derivatives

A solution of about 10^{-5} moles of the porphyrin or chlorin in 10 ml of pyridine was heated to 60°C under nitrogen and a 10-fold molar excess of $ZnSO_4 \cdot 7H_2O$ in 10 ml

[4] We express our gratitude to Dr. J. J. Katz of Argonne National Laboratories for enabling us to make and interpret these spectra.

of acetic acid was added. The reaction was allowed to continue with stirring for about 1 hr. Twenty milliliters of chloroform and 200 ml of water were added to the cooled reaction mixture. The aqueous phase was separated and extracted with a further 20 ml of chloroform. The chloroform solution was washed with water, filtered through Whatman No. 1 filter paper to remove any solid impurities and allowed to evaporate to dryness. The solid was recrystallized from a petroleum ether–chloroform solution.

The zinc derivatives prepared in this manner displayed absorbance bands as listed in Table 2.

TABLE 2

ABSORBANCE BANDS OF ZINC PORPHYRIN DERIVATIVES

Compound	Solvent	λ_1 (nm)	λ_2 (nm)
Zinc desoxyphylloerthyrin	3% Methanol in $CHCl_3$	572	538
Zinc hematoporphyrin	Pyridine in Methanol	581	545
	Methanol	576	539
Zinc pyropheophorbide	Methanol	658	429
Zinc chlorin e_6	Methanol	629	410

Preparation of the Iron Derivatives

Ferrous acetate was generated *in situ* by the following method: Iron powder was collected on a Teflon-covered magnetic stirring bar when sodium borohydride was added to a ferrous acetate solution. This iron powder was dried by washing with diethyl ether. For the preparation of the iron derivatives of the chlorins and porphyrins, 10-fold molar excess of iron powder was added to 25 ml of glacial acetic acid in a three-necked flask. After the mixture had been made anaerobic with nitrogen, the temperature was raised to 100°C for 40 min, at the end of which time all the powder had dissolved. A solution of the pigment in 10 ml of glacial acetic acid was then added. A reaction time of 1 hr at 100°C was sufficient for the insertion of iron into the porphyrin. The solution was allowed to cool prior to being shaken in air. This last step insured the oxidation of the compound to the ferric state. Water and chloroform were then added to the acetic acid solution. The excess ferrous ion was removed from the chloroform solution of the pigment with several washings with acetate buffer at pH 5. The addition of several volumes of petroleum ether to the chloroform caused the precipitation of the ferric pigment. The precipitate was redissolved in chloroform and filtered through a 50-nm pore size cellulose acetate filter (Millipore VMWP, Millipore Corporation, black) to remove trace amounts of colloidal iron hydroxide which might be present. In some instances, recrystallization with petroleum ether was carried out at this point. Overall yields from this procedure were on the order of 30%.

Bacteriochlorophyll

Bacteriochlorophyll was isolated from *Rhodopseudomonas spheroides* and purified as previously described (*22*).

Redox Titrations

The glass apparatus for conducting simultaneous measurements of potential and spectra has been described previously (*7*, *37*).

The oxidative titrations of the zinc derivatives were run in three different solvent systems: dry methanol, aqueous methanol, and buffered aqueous detergent. For reactions in the first solvent, freshly opened anhydrous methanol was used. Wet methanol consisted of a 10% (v/v) solution of water in methanol. The aqueous buffered detergent was a 0.01 *M* potassium phosphate solution (pH 7.0) which contained 7.5×10^{-2}% of the neutral detergent Triton X-100. For the latter solution, the zinc porphyrin or chlorin was dissolved in a minimum volume of methanol and added to the aqueous detergent. The concentrations of the metallopigments are given in the results section for the experiments reported.

The reductive titrations of the iron derivatives were carried out in dry methanol, aqueous methanol, and aqueous buffered detergent. Dry methanol consisted of freshly opened anhydrous methanol which was made 0.5% in pyridine. Aqueous methanol was prepared by diluting 1 ml of 0.01 *M* potassium phosphate buffer (pH 7.0) to a final volume of 25 ml with methanol which was 0.5% in pyridine. The aqueous detergent was a solution of 0.2% Triton X-100 and 1% pyridine in 0.01 *M* potassium phosphate at pH 7.0. The concentrations of the iron pigments are reported in the results section for each titration.

In order to prevent the diffusion of the dense potassium chloride solution into the methanolic solutions, it was necessary to block the end of the salt bridge with an agar plug. The plug was formed by drawing a 1% solution of agar in saturated potassium chloride into the salt bridge. The agar was allowed to solidify with the salt bridge remaining in the beaker of agar. After the cooling was completed, the salt bridge was cut out of the agar block and the agar was carefully trimmed with a spatula. This procedure prevented the contraction of the agar up into the narrow-diameter glass tubing which comprised the lower tip of the salt bridge.

RESULTS

Oxidation of the Zinc Derivatives and Oxidation of Bacteriochlorophyll

A. Zn hematoporphyrin. The extent of reversibility of the oxidation and the spectral characteristics of the product were functions of the composition of the solvent and oxidant. When ferric perchlorate was used as the oxidant, the primary oxidized species had an absorbance maximum of 665 nm (Fig. 1). Good isobestic points were obtained during the titration if it was carried out rapidly. The midpoint potential was estimated from a plot of absorbance at 538 nm as a function of potential and was +0.610 V vs SCE which may be compared to the value of 0.525 V vs SCE reported by Fuhrhop and Mauzerall (*11*) for the oxidation of zinc octaethylporphyrin in methanol. The extent of reversibility was a function of the reducing agent used. When ascorbate served as reductant, reversibility measured by the absorbance at 538 nm was 78%, and when hydroquinone was used, the oxidation was apparently 95% reversible.

When the oxidation of zinc hematoporphyrin was attempted in methanol which contained 10% (v/v) phosphate buffer, the course of the reaction was quite different.

The primary oxidized species in this case was green. This decayed very rapidly to give a brown solution: It was not possible to measure the absorbance of the primary species but we feel that it may have been the species with an absorbance maximum at 665 nm observed in dry methanol. The secondary brown species, or mixture of species, had an absorbance maximum at 605 nm. Reductant caused no significant subsequent changes in the spectrum. Oxidation of zinc hematoporphyrin in 0.01 *M* potassium phosphate buffer (pH 7.0) containing 7.5×10^{-2} % Triton X-100 proceeded to give a product with a spectrum identical to that of the product of the oxidation in wet methanol. The

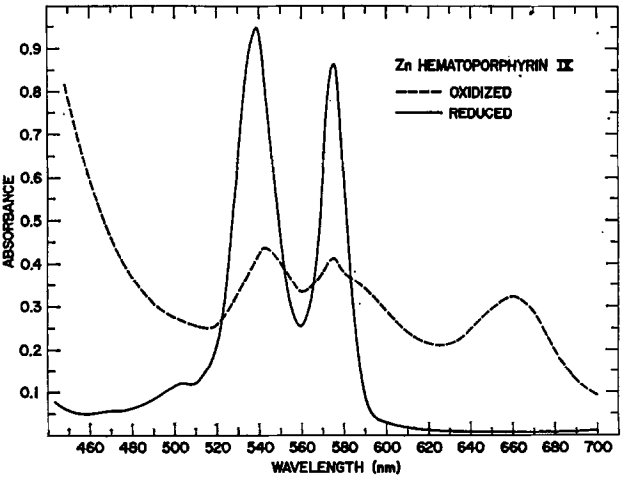

FIG. 1. Oxidation of Zn(II) hematoporphyrin IX with ferric perchlorate in methanol. Concentration of Zn hematoporphyrin IX = 5.1×10^{-5} *M*, 1-cm cuvet, room temperature, anaerobic.

oxidation in the aqueous detergent was carried out with potassium iridic chloride and the reduction was attempted with buffered potassium ferrocyanide.

B. Zinc desoxyphylloerythrin. When zinc desoxyphylloerythrin was oxidized with ferric perchlorate in dry methanol and reduced very rapidly with sodium dithionite the reaction was reversible as judged by comparison of the spectra of the starting material and the product. However, if sufficient time was allowed for the potential to become stable after the addition of each increment of oxidant, the product had a very different spectrum with an additional band appearing at approximately 610 nm. It was not possible to estimate the midpoint potential for the oxidation.

C. Zinc pyropheophorbide a and zinc chlorin e_6. Both zinc pyropheophorbide *a* and zinc chlorin e_6 displayed a rapid and irreversible loss of absorbance when oxidized in anhydrous methanol. No matter how rapidly the oxidation and subsequent reduction were carried out, it was never possible to regenerate anything resembling the original spectrum.

D. Bacteriochlorophyll. Some of our results for the oxidative titration of bacteriochlorophyll have been reported previously (*22*). Since the time of that report we have amplified these studies. We investigated the condition of our bacteriochlorophyll preparations by means of NMR spectroscopy. It was especially important to determine

the amount of C-10 hydrogen present in the starting material, since this would indicate whether ring V were intact. Table 3 shows data from these measurements. Although there was peak broadening due to aggregation, the band which has been assigned to the C-10 hydrogen was present in an amount equal to that of each of the methine bridge hydrogens. Ring V is, thus, intact in the fresh bacteriochlorophyll.

It is desirable to determine the reversibility of the oxidative reaction by means of NMR, since significant changes in the structure of the molecule may not be reflected in spectral changes in the visible region. A bacteriochlorophyll sample was oxidatively titrated to 90% completion with iodine, according to the spectral changes, and then reduced with sodium hydrosulfite. Pertinent NMR data of the product are also shown in Table 3. There are no indications of significant differences between the starting material and the product.

TABLE 3

NMR DATA FOR BACTERIOCHLOROPHYLL

	Position (ppm)[a]		
Hydrogens	Fresh BCHl	BCHl after Ox–Red	Bacteriopheophytin (24)
α	8.96	8.97	8.96
β	8.52	8.52	8.47
δ	8.38	8.38	8.40
C-10	6.01	5.99	6.08
	Peak height ratios[b]		
α	1.00	1.00	1.00
β	0.91	0.82	0.95
δ	0.86	0.91	1.00
C-10	1.14	1.21	1.24

[a] $CDCl_3$ was employed as the solvent. The peak due to the small amount of $CHCl_3$ present was used as the reference point and assigned a value of 7.20 ppm.
[b] These ratios are based on an assignment of 1.0 to the α peak.

Finally, we were able to use the more stable bacteriochlorophyll system to determine the effect of ring V on the oxidation potential of the metalloorganic system. A methanol solution containing 1.0% water and sufficient Tris buffer (pH 7.0) so that its final concentration was 1×10^{-3} *M* was used for these experiments. At a temperature of 0°C, bacteriochlorophyll could be reversibly oxidized in the solvent. Figure 2 shows the absorbance changes which accompanied this oxidation. The midpoint potential of 0.00 V vs SCE is significantly different from that of bacteriochlorophyll in anhydrous methanol [$E^{0\prime} = 0.27$ V vs SCE (*20*, *22*)].

An aliquot of the bacteriochlorophyll solution in methanol was brought to pH 11.9 for 1 hr in the absence of air at 25°C. Under these conditions, ring opening should be quantitative. The alkaline treatment caused only minor changes in the absorbance spectrum of the bacteriochlorophyll. The solution was then adjusted to pH 7 with

Tris–HCl and subjected to oxidative titration at low temperature. Absorption spectra of the oxidized and reduced forms are shown in Fig. 2 and compared with the spectra of the untreated bacteriochlorophyll. The titration appeared to be identical to that of bacteriochlorophyll under the same conditions and we consider the midpoint potential of –0.02 V vs SCE to be indistinguishable from that of the intact bacteriochlorophyll under these conditions.

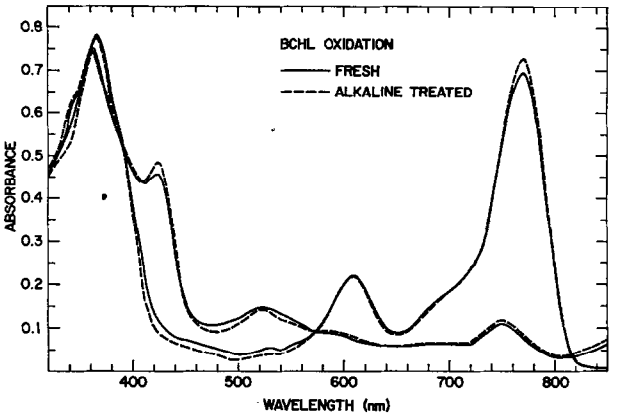

FIG. 2. Absorbance spectra of bacteriochlorophyll (solid curves) and alkaline-treated bacteriochlorophyll (dashed curves) and also their one-electron oxidized forms (lower curves at 770, 605 and 365 nm and higher curves at 520 and 425 nm). Concentration of bacteriochlorophyll = 1.3×10^{-5} *M*; 1-cm cuvet; 3°C, anaerobic.

Reductive Titration of Fe(III) Derivatives

The preparation of the iron derivatives proceeded easily, except in the case of bacteriopheophorbide. Apparently, destruction of the organic ligand occurs even at low temperatures. To our knowledge, no one has yet reported the preparation of the Fe(II) or Fe(III) derivatives of bacteriopheophorbide. Absorbance changes accompanying iron insertion into pheophorbide *a* are shown in Fig. 3.

The results of the reductive titrations of the iron derivatives are shown in Table 4. It was not possible to carry out titrations of all the derivatives in all the solvent systems, since some of the material was present in only minute quantities. In all the reductions, isosbestic points were good and the reversibility as measured spectrophotometrically was excellent.

The spectra of the ferric and ferrous forms of the derivatives of hematoporphyrin, desoxyphylloerythrin, pyropheophorbide *a*, pheophorbide *a*, and chlorin e_6 are shown in Figs. 4–8. The spectra of the ferric and ferrous derivatives of hematoporphyrin (Fig. 4) and protoporphyrin IX agree well with those reported in the literature [see Falk (*31*) for a comprehensive survey]. The midpoint potentials derived from the titration data for these latter two compounds are in good agreement with those presented by other investigations for iron hematoporphyrin and protoporphyrin IX in similar solvents (*38*, *39*). The reductive titration of Fe(III) desoxyphylloerythrin revealed that its midpoint potential was in the range expected for iron porphyrins. The spectral changes observed (Fig. 5) were also those expected for an iron porphyrin.

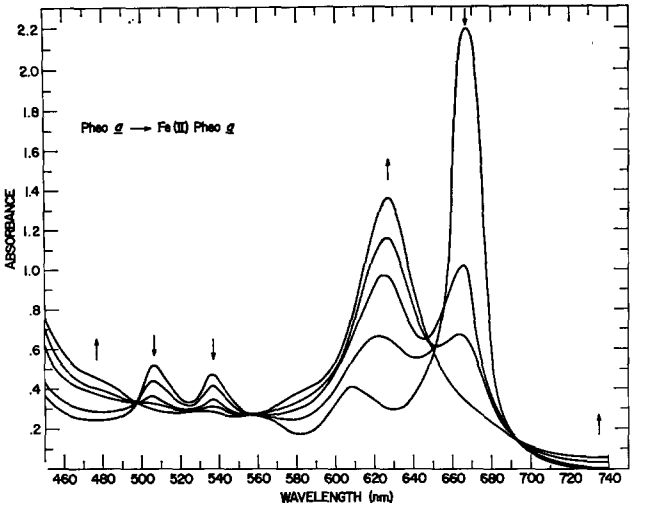

FIG. 3. Change in absorbance spectrum accompanying iron insertion into pheophorbide *a* in acetic acid. Initial concentration of pheophorbide $a = 2.5 \times 10^{-5}$ *M*; 1-cm cuvet; room temperature; anaerobic. The iron concentration was about 20 times greater than that of the pheophorbide *a*.

TABLE 4

OXIDATION–REDUCTION MIDPOINT POTENTIALS OF IRON PORPHYRIN AND IRON CHLORIN COMPLEXES

Compound	Concn. (*M*)	Triton X-100 (%)	Pyridine (%)	$E^{0'}$[b] (V)
A. In aqueous detergent[a]				
Fe hematoporphyrin IX	1.43×10^{-5}	0.04	0.25	0.049
Fe desoxyphylloerythrin	8.3×10^{-5}	0.21	1.00	0.110
Fe chlorin e_6	9.0×10^{-5}	0.21	0.31	0.188
Fe pyropheophorbide *a*	3.7×10^{-5}	0.21	0.40	0.320
B. Wet methanol[c, d]				
Fe desoxyphylloerythrin	7.6×10^{-5}		0.50	−0.088
Fe chlorin e_6	5.0×10^{-5}		0.50	+0.085
Fe pyropheophorbide *a*	8.1×10^{-5}		0.24	+0.118
C. Dry methanol[d]				
Fe protoporphyrin IX	8.0×10^{-5}		0.50	+0.042
	5.1×10^{-5}		0.50	+0.055
Fe desoxyphylloerythrin	9.5×10^{-5}		0.50	−0.005
Fe chlorin e_6	7.0×10^{-5}		0.50	+0.205
Fe pheophorbide *a*	5.2×10^{-5}		0.50	+0.255
Fe pyropheophorbide *a*	8.1×10^{-5}		0.24	+0.274
	7.7×10^{-5}		0.50	+0.229

[a] Solutions were 0.01 *M* in potassium phosphate buffer (pH 7.0).

[b] A value of +0.245 V was used for the potential of the saturated calomel electrode. Values reported are relative to the standard hydrogen electrode.

[c] Solutions contained 4% H_2O and were 0.01 *M* in potassium phosphate buffer (pH 7.0).

[d] A value of +0.040 was subtracted from the measured values to correct for junction effects arising at the methanol-saturated KCl interface.

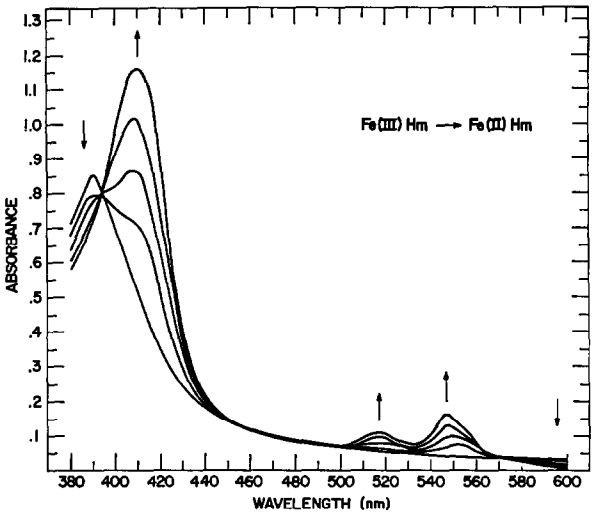

FIG. 4. Change in absorbance spectrum accompanying reduction of Fe(III) hematoporphyrin IX in 0.01 *M* phosphate buffer (pH 7.0) containing 0.25% pyridine and 0.04% Triton X-100. Fe hematoporphyrin concentration = 1.4×10^{-5} *M*; room temperature; 1-cm cuvet; reductant, $Na_2S_2O_4$ in methanol; anaerobic.

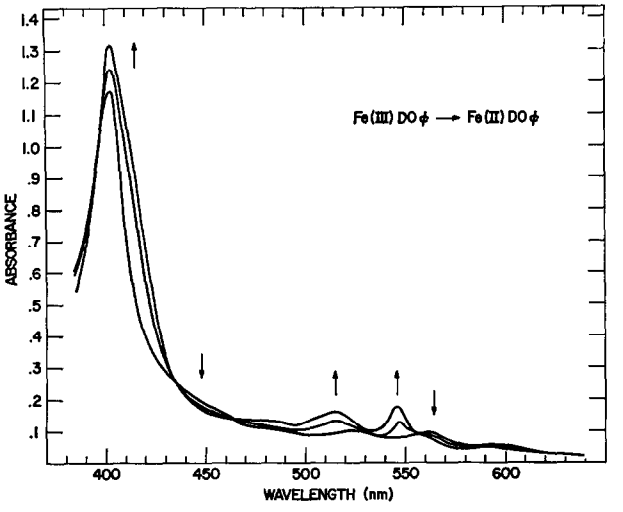

FIG. 5. Change in absorbance spectrum accompanying reduction of Fe(III) desoxyphylloerythrin in methanol containing 0.5% pyridine. Fe desoxyphylloerythrin = 2.5×10^{-5} *M*; 1-cm cuvet; room temperature; reductant, $Na_2S_2O_4$; anaerobic.

In the cases of ferric pheophorbide *a*, ferric pyropheophorbide *a*, and ferric chlorin e_6, the midpoint potentials observed for the reductions were significantly higher than those for the reduction of the ferric porphyrins. In addition, the intensity and position of the absorbance bands at wavelengths greater than 500 nm were red-shifted relative

to those of the iron porphyrins (Figs. 6–8). The spectral pattern in each case was that of a chlorin, with an absorption band above 600 nm.

Although good spectroscopic reversibility was observed in all the reductive titrations, a plot of log[Ox]/[Red] vs E_h did not give the expected value of $nF/RT = 0.059$ in all

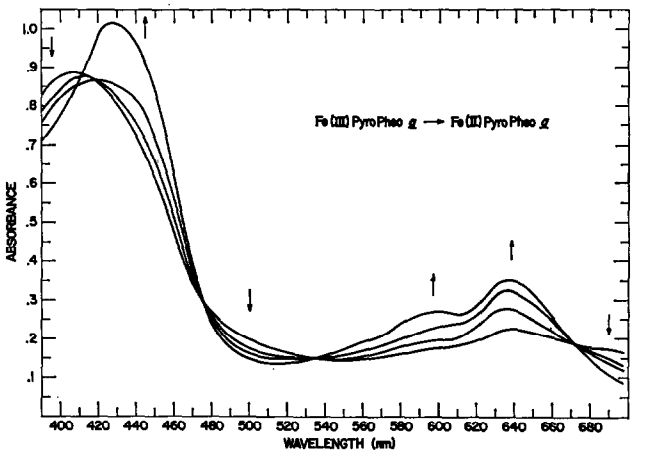

FIG. 6. Change in absorbance spectrum accompanying reduction of Fe(III) pyropheophorbide *a* in methanol containing 0.5% pyridine. Fe pyropheophorbide $a = 3.4 \times 10^{-5}$ *M*; 1-cm cuvet; room temperature; reductant $Na_2S_2O_4$; anaerobic.

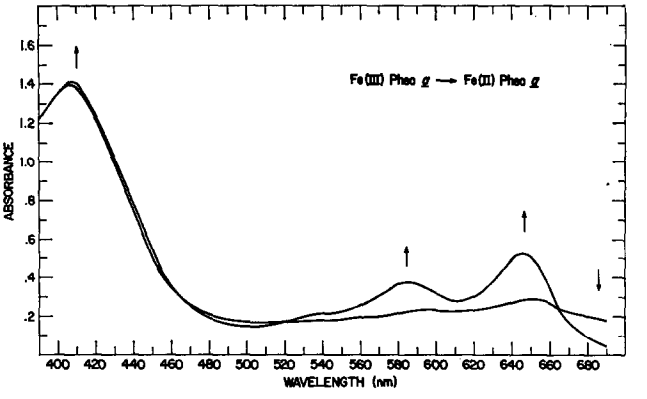

FIG. 7. Change in absorbance spectrum accompanying reduction of Fe(III) pheophorbide *a* in methanol containing 0.5% pyridine. Fe pheophorbide $a = 5.2 \times 10^{-5}$ *M*; 1-cm cuvet; room temperature; reductant, $Na_2S_2O_4$; anaerobic.

the experiments. In many experiments, the value was near 0.090. We found no consistent variation in this quantity. At the present time we attribute the deviation from the theoretical value for a single electron reduction to aggregation effects. These effects might be minimized in some cases by the utilization of other ligands; in particular, azide or cyanide might be useful.

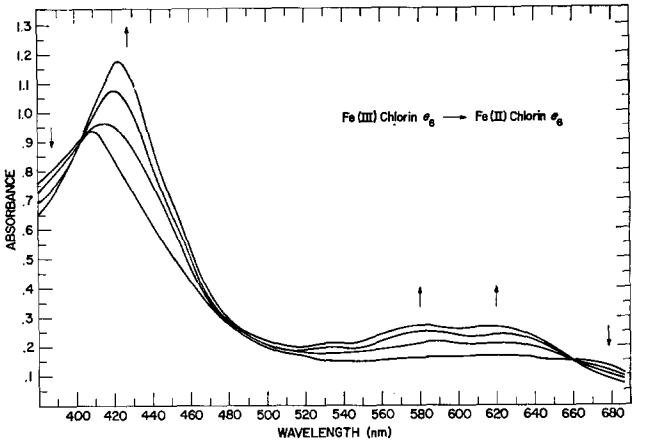

FIG. 8. Change in absorbance spectrum accompanying reduction of Fe(III) chlorin e_6 in methanol containing 0.5% pyridine and 4% of 0.01 *M* potassium phosphate buffer, (pH 7.0). Fe chlorin e_6 = 5.0×10^{-5} *M*; 1-cm cuvet; room temperature; reductant, $Na_2S_2O_4$; anaerobic.

DISCUSSION

It appears that the presence of the isocyclic ring does not have a significant effect on the redox properties of metalloporphyrins and metallochlorins. The oxidation state of the organic macrocycle is indicated to be the prime factor in the determination of the midpoint potentials. The basicity of the pyrrole nitrogens in chlorins is less than their basicity in porphyrins (*31*). The difference in midpoint potentials for the iron III/II equilibria in chlorin and porphyrin complexes is in agreement with this relationship (see Table 4). The present data for iron complexes are also in agreement with the earlier data of Loach and Calvin (*37, 40*) which showed that Mn Methyl Pheophorbide *a* has a higher midpoint potential than Mn Hematoporphyrin IX dimethyl ester for the Mn III/II equilibria.

The lack of influence of the isocyclic ring on the oxidation properties of the macrocyclic ligand was especially clear in the case of bacteriochlorophyll. The oxidation potential for bacteriochlorophyll which had been subjected to alkaline hydrolysis and opening of ring V was almost identical to that of the intact material. The results of these studies of the redox properties correlate well with the spectral properties. Chlorins can be distinguished from porphyrins by the presence of a prominent absorbance band above 600 nm with an extinction nearly equal to that of the Soret band. The presence of the fifth ring in desoxyphylloerythrin does not cause the spectrum to be appreciably different from the spectra of porphyrins without this ring. Furthermore, it has long been known that the opening of the isocyclic ring in chlorophylls *a* and *b* and in bacteriochlorophyll (see Fig. 2) does not effect a large perturbation in the spectra. It would seem likely then, that reduction of ring IV in chlorophyll *a* and *b* and rings II and IV in the bacteriochlorophyll serves to modify both the absorbance properties of the chromophore and the redox properties as an electron donor, and perhaps as an electron acceptor. The function of the isocyclic ring appears to be limited to its role in the formation of chlorophyll aggregates.

The oxidations of the zinc derivatives of hematoporphyrin, desoxyphylloerythrin, pyropheophorbide *a*, and chlorin e_6 display a distinctive qualitative pattern. The stability of the oxidized form has the order porphyrin > porphyrin with isocyclic ring > chlorin. The irreversibility of the oxidation may be a result of nucleophilic attack on the radical cation, as suggested by Fuhrhop and Mauzerall (*19*, *20*), or the result of the dismutation of the radical cation to yield the dication and the reduced species, followed by nucleophilic attack on the dication, as suggested by Dolphin and co-workers (*23*, *27*). However, the significant stability of the oxidized tetrahydroporphyrin, bacteriochlorophyll, is not in keeping with this pattern, if it is assumed that the Mg^{2+} derivatives would show a pattern similar to that of the zinc derivatives. The fact that the oxidation of bacteriochlorophyll is easily reversible in systems which contain large amounts of water indicates a slow rate of nucleophilic attack on the radical cation, or an unfavorable rate for the dismutation process, or both. The surprising stability just referred to, compared with the destruction of the bacteriopheophorbide during iron insertion, is indicative of the unique, and not well-understood, chemical nature of the tetrahydroporphyrin nucleus.

Finally, we would like to point out an anomaly in the data relating to the *in vivo* photosynthetic systems. The studies of Goedheer (*41*), Fuhrhop and Mauzerall (*19*, *20*) and Dolphin et al. (*23*, *27*), have shown that bacteriochlorophyll is more readily oxidized than chlorophyll *a*. That is, the midpoint potential for the oxidation of chlorophyll *a* in methanolic solvent is 0.55 V vs SCE (*19*, *20*, *41*) while the midpoint potential for what is presumably the same reaction in bacteriochlorophyll is 0.27 V vs SCE (*19*, *20*). However, the midpoint potentials for the oxidation of the primary electron donors have been found to be 0.43 V vs SHE in spinach chloroplasts (*11*, *16*, *10*) or 0.44 V vs SHE in bacterial chromatophores (*7*, *14*, *17*): these values are essentially identical. Moreover, nearly all photosynthetic systems have been found to display strikingly similar redox properties for at least one of their primary electron donor molecules (if they have several). It is not possible to compare in a quantitative fashion potential measurements made in aqueous solutions against a standard hydrogen electrode with those made in alcoholic or aprotic solvent against the saturated calomel electrode. Yet it seems that there is a large discrepancy between the *in vivo* and *in vitro* oxidation–reduction potentials of bacteriochlorophyll. This is, presumably, a result of the interaction of the trap bacteriochlorophyll with its environment in the photosynthetic membrane system, especially with the molecule which is the electron acceptor. We are also faced with the troublesome possibility that the similar redox properties of plant and bacterial photosystems may merely be coincidental.

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