

On the Mechanism of Reduction of Porphyrins. A Pulse Radiolytic Study

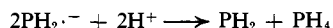
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Abstract: The mechanisms of reduction of hematoporphyrin, PH_2 , and its Zn complex, ZnP, by strong single-electron reducing agents in alkaline aqueous solutions were studied. It was found that for both compounds three intermediates are formed prior to the formation of the stable products. The mechanism of reduction of both compounds seems to be the following: (a) $\text{PH}_2/\text{ZnP} + \text{R}\cdot^- \rightarrow \text{PH}_3\cdot$ or $(\text{PH}_2\cdot^-)/(\text{ZnP}\cdot^-)$ ($\text{ZnP}\cdot^-$) + R (k_1), where k_1 depends on $\text{R}\cdot^-$; (b) $2\text{PH}_3\cdot/2\text{ZnP}\cdot \rightarrow (\text{PH}_3)_2/(\text{ZnP})_2$ ($k_2 = 2.1 \times 10^8 \text{ M}^{-1} \text{ sec}^{-1}$), where the dimer is plausibly a π -mer; (c) $(\text{PH}_3)_2/(\text{ZnP})_2 \rightarrow (\text{PH}_3)_2'/(\text{ZnP})_2'$ ($k_{\text{PH}_2} = 10 \text{ sec}^{-1}$; $k_{\text{ZnP}} = 17 \text{ sec}^{-1}$) (this reaction seems to be some kind of rearrangement of the dimer); (d) (here the mechanisms differ) $(\text{PH}_3)_2' \rightarrow \text{PH}_4$ or $(\text{PH}_3)_2' \rightarrow \text{PH}_4 + \text{PH}_2$ ($k = 0.11 \text{ sec}^{-1}$) and $(\text{ZnP})_2' \rightarrow \text{Zn}(\text{dihydroporphyrin}) + \text{ZnP}$, where PH_4 is a phlorin, whereas in the Zn(dihydroporphyrin) one of the hydrogen atoms is added on a peripheral position. This mechanism is nearly independent of pH in the $7 \leq \text{pH} \leq 13$ range and of the solvent water, methanol, or ethanol. The absorption spectra of all intermediates are reported. The reduction of hemin by e_{aq}^- or CH_3CHO^- on the other hand proceeds *via* direct reduction of the central Fe(III) to Fe(II) in one step.

Porphyrins, free base, their metallo complexes, and some of their reduced forms have an important role in many redox and energy-transfer processes. It is therefore not surprising that various aspects of the mechanism of reduction of porphyrins have been recently studied.¹⁻¹² Though, at least in nature, porphyrins react in protic media, most of the redox studies were carried out in aprotic solvents. The reason for this choice has been that the intermediates formed upon reduction are unstable in protic media and their nature cannot be studied by most conventional techniques.

The first product upon reduction of porphyrins, PH_2 , in aprotic solvents was shown to be the mono-anion $\text{PH}_2\cdot^-$.¹⁻¹² The radical anion was shown to undergo disproportionation in the presence of protons¹¹



the product being the parent porphyrin and the corresponding phlorin. The kinetics of disappearance of the radicals were shown to obey a second-order rate law, both by epr and optical absorption methods.⁶ However, the techniques used did not enable a detailed mechanistic study.

We have decided to study the mechanism of reduction of a porphyrin in aqueous solution by the pulse radiolytic technique.¹³ The reactions of several powerful

single-electron reducing agents thus formed with excess porphyrin were studied. The spectra of the intermediates formed and the mechanisms of their reactions were investigated. The formation of the corresponding phlorin as the final product was established.

Hematoporphyrin (IX), Figure 1, was chosen for the present study due to the following reasons. (a) It is soluble in a monomeric form in neutral and alkaline aqueous solutions.¹⁴ (b) All its side chains are saturated and thus are not expected to be reactive toward the aliphatic radicals used in this study.

The reduction of metalloporphyrins is known to differ from that of the free base in the following ways. (a) If the central metal atom is in a high oxidation state (e.g., Fe(III), Co(III), Cu(II)) it, and not the ligand, is reduced. However, reduction of the central atom might occur directly, or alternatively the ligand might be reduced first followed by an intramolecular electron-transfer reaction. (b) If the redox potential of the ligand is lower than that of the central cation the ligand is reduced;⁸ however, the final products of reduction were shown to differ from those obtained upon reduction of the free base.⁵ We have, therefore, decided to study the effect of the introduction of a metal cation into the porphyrin structure on the mechanism of reduction in two model cases: (a) hemin chloride, where the final product is expected to be the Fe(II) complex; (b) zinc hematoporphyrin (IX), where reduction is expected to occur on the ligand and the final product has been shown to be a dihydroporphyrin different from phlorin.⁵

Experimental Section

Materials. Hematoporphyrin (IX)·2HCl and hemin chloride were supplied by Nutritional Biochemical Corp. and used without further purification. Zinc hematoporphyrin (IX) was prepared in solution by adding a small excess of ZnSO_4 to a solution of hematoporphyrin at pH 13.0 and stirring for several hours. Completion of the formation was checked spectrophotometrically.

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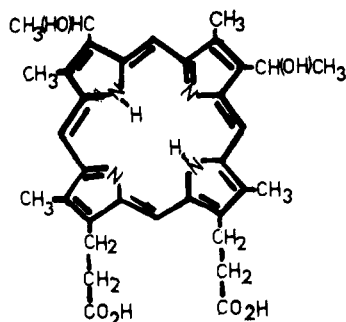


Figure 1. Hematoporphyrin.

The solutions of the three compounds were shown to obey the Beer-Lambert law in the concentration range used. *tert*-Butyl alcohol, Hopkins and Williams, was distilled over sodium and the 81.5–82.5° fraction was used. Nitrous oxide and argon were purified from oxygen traces by bubbling the gas through three washing bottles containing an acidic solution of V^{2+} over a zinc amalgam, followed by a washing bottle containing a sodium hydroxide solution and two washing bottles containing triply distilled water. All other chemicals used were of A.R. grade and were used without further treatment. All the water used for preparing the solutions was triply distilled.

Procedure. The procedure of preparation of samples for the pulse radiolytic experiments was identical with that described earlier in detail.¹⁵ The pulse radiolytic experiments were carried out using 0.1–1.5 μsec , 5 MeV, 200 mA pulses from the linear accelerator at the Hebrew University of Jerusalem. The dose per pulse was $2\text{--}20 \times 10^{19} \text{ eV l}^{-1}$. The irradiation cell was 4 cm long, the analyzing light traveling three times through the cell. The experimental setup in Jerusalem and the method used for evaluating the results have been described elsewhere in detail.¹⁶

In order to minimize photochemical decomposition of the solutes by the analyzing light, a Jena glass filter, GG-15 or GG-14, was introduced between the xenon lamp and the cell when the absorption was followed at $\lambda \geq 420 \text{ nm}$ or $\geq 500 \text{ nm}$, respectively. Furthermore, a mechanical shutter was kept closed until shortly before the pulse, and all solutions were kept in the dark.

Absorption spectra were measured by splitting the light beam into two monochromators. The wavelength of one of these monochromators was kept constant and was thus used as a monitor relative to which the absorptions at the different wavelengths were measured. The absolute molar absorption coefficients were determined by pulse radiolyzing, under exactly identical conditions, a standard solution ($1 \times 10^{-3} \text{ M K}_4\text{Fe}(\text{CN})_6$ saturated with N_2O), the solution to be analyzed, and the standard solution once again. The yield of $\text{Fe}(\text{CN})_6^{3-}$ was assumed to be 6.1,¹⁷ and $\epsilon_{420}(\text{Fe}(\text{CN})_6^{3-}) = 1000 \text{ M}^{-1} \text{ cm}^{-1}$ was used. The yields of the different intermediates were calculated from the known yields of the primary products,¹³ their measured specific rates of reaction with the solutes present, and the suggested mechanism of reaction (see below). The molar absorption coefficients were thus determined from the measured optical densities, the calculated yield of the intermediates, and the calculated amount of porphyrin destroyed by the pulse.

γ Irradiations. Low-dose-rate irradiations were carried out using a ^{60}Co γ -ray source (Gammacell 200, Atomic Energy Commission of Canada, Ltd.). The dose rate was of the order of $2 \times 10^{20} \text{ eV l}^{-1} \text{ min}^{-1}$ as determined by Fricke dosimeter.¹³ Solutions saturated with the desired gas were irradiated in a 4 cm long optical cell with suprasil windows.

Spectroscopic Measurements. The spectra of the stable products of irradiation were measured in a Cary 17 spectrophotometer. An identical cell containing an unirradiated solution was used as a blank. The spectra thus obtained are therefore difference spectra. The spectra were measured 3–10 min after the end of irradiation.

Results

Reduction of Hematoporphyrin (IX): Spectra of

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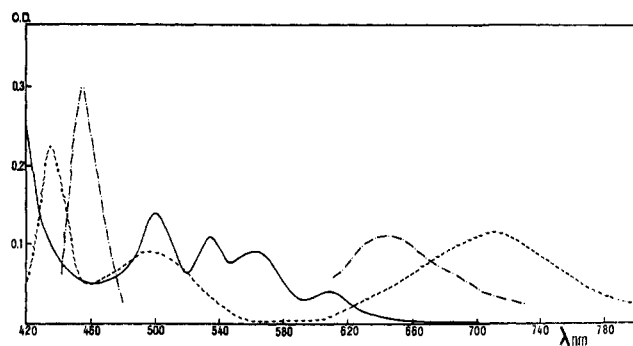


Figure 2. Difference spectra for the stable products of irradiation of PH_2 . The solution composition was $2 \times 10^{-5} \text{ M PH}_2$, $0.1 \text{ M (CH}_3)_2\text{CHOH}$, N_2O saturated. The dose was $1200 \pm 180 \text{ rads}$, in a 4-cm cell, measured 3 min after the end of irradiation. (—) Spectrum of unirradiated solution, reference cell contained water. (---) Irradiated solution at pH 13.0, reference cell contained an unirradiated solution. (- - -) Irradiated solution at pH 7.0, reference cell contained an unirradiated solution.

Stable Products. N_2O -saturated solutions containing 0.1 M 2-propanol and $2.0 \times 10^{-5} \text{ M}$ hematoporphyrin at different pH's were irradiated in the γ source by a dose of $1200 \pm 200 \text{ rads}$. The changes in the absorption spectra were measured. Typical results for pH 7.0 and 13.0 are given in Figure 2. At pH 9.0 the absorption spectrum was a 1:1 combination of the spectra obtained at pH 7.0 and 13.0. Irradiation of a solution at pH 7.0 which contained 0.1 M NaClO_4 in addition to the other solutes yielded a spectrum identical with that obtained at pH 13.0. Irradiation of solutions containing ethanol or methanol instead of 2-propanol or irradiation of the solutions by a short pulse from the linac yielded identical spectra. Shaking of the irradiated solutions with oxygen resulted nearly in the total disappearance of the difference spectra.

Pulse Radiolytic Experiments. When N_2O -saturated solutions containing $1\text{--}5 \times 10^{-5} \text{ M}$ hematoporphyrin and 0.1 M alcohol (methanol, ethanol, or 2-propanol) at pH 13.0 are irradiated by a short pulse four well separated in time processes are observed. Similar results are obtained in N_2O -saturated solutions containing 0.1 M 2-propanol in the pH range of 7.0–13.0 and in Ar-saturated solutions containing 0.1 M tert -butyl alcohol. We shall now describe the detailed results for each of these four reactions separately.

Kinetics of Reduction of Hematoporphyrin and the Absorption Spectrum of the First Intermediate. The specific rate of reaction of e_{aq}^- with hematoporphyrin was determined in Ar-saturated solutions containing 0.1 M tert -butyl alcohol at different pH's by following the disappearance of the absorbance of e_{aq}^- . As the intermediate formed also absorbs at the wavelength used, 600–650 nm, it was important to check that no intermediate absorbing at this wavelength is formed by the reactions of OH and H radicals. Identical solutions, but saturated with N_2O instead of Ar, were therefore irradiated. No intermediate absorbing in the range of 430–700 nm was formed in these solutions. The specific rates of reactions of e_{aq}^- thus determined are summarized in Table I. In the same table are summarized also the measured specific rates of reaction of $\cdot\text{CH}_2\text{O}^-$, $\text{CH}_3\dot{\text{C}}\text{HO}^-$, $(\text{CH}_3)_2\dot{\text{C}}\text{O}^-$, $\cdot\text{CO}_2^-$, and $(\text{CH}_3)_2\dot{\text{C}}\text{OH}$ radicals with hematoporphyrin under different conditions. The latter rates were measured by following the for-

Table I. Specific Rates of Reaction of Different Radicals with Hematoporphyrin^a

Radical	Solvent	pH	Other solutes, M	Gas	$k, M^{-1} \text{sec}^{-1}$
e_{aq}^-	H ₂ O	7.0	0.1 <i>tert</i> -Butyl alcohol	Ar	$1.5 \pm 0.2 \times 10^{10}$
	H ₂ O	7.0	0.1 <i>tert</i> -Butyl alcohol + 0.1 NaClO ₄	Ar	$2.7 \pm 0.3 \times 10^{10}$
(CH ₃) ₂ CHOH	H ₂ O	13.0	0.1 <i>tert</i> -Butyl alcohol	Ar	$2.5 \pm 0.3 \times 10^{10}$
	H ₂ O	7.0	0.1 2-Propanol	N ₂ O	$2.4 \pm 0.4 \times 10^8$
(CH ₃) ₂ CO ⁻	H ₂ O	13.0	0.1 2-Propanol	N ₂ O	$1.1 \pm 0.2 \times 10^9$
CH ₃ CHO ⁻	H ₂ O	13.0	0.1 Ethanol	N ₂ O	$7.0 \pm 1.0 \times 10^8$
	CH ₃ CH ₂ OH + 5% H ₂ O		0.1 NaOH	N ₂ O	$6 \pm 1 \times 10^8$
·CH ₂ O ⁻	H ₂ O	13.0	0.1 Methanol	N ₂ O	$3.3 \pm 0.5 \times 10^8$
	CH ₃ OH + 5% H ₂ O		0.1 NaOH	N ₂ O	$2.0 \pm 0.4 \times 10^8$
·CH ₂ OH	H ₂ O	7.0	0.1 Methanol	N ₂ O	$\leq 1 \times 10^7$
·CO ₂ ⁻	H ₂ O	13.0	0.1 HCO ₂ Na	N ₂ O	$4 \pm 1 \times 10^7$

^a All solutions contained $1-5 \times 10^{-6} M$ hematoporphyrin and were at $25 \pm 2^\circ$. The dose was $2-4 \times 10^{19} \text{ eV l.}^{-1} \text{ pulse}^{-1}$.

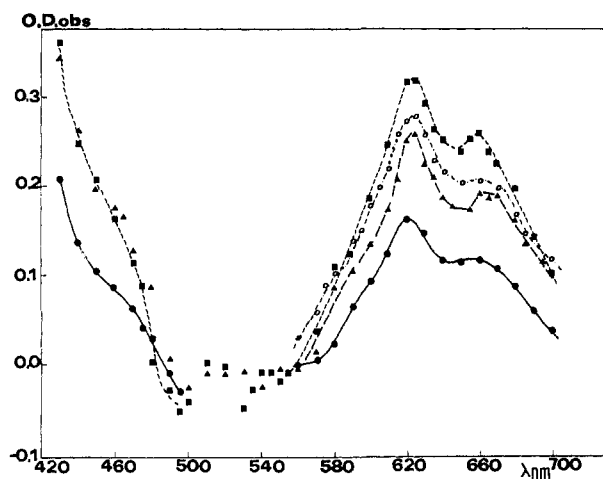


Figure 3. Uncorrected absorption of $\text{PH}_3\cdot$ (or $\text{PH}_2\cdot^-$) in alkaline solutions formed by different reducing radicals. (●) Reducing radical e_{aq}^- . The solution composition was $1 \times 10^{-5} M \text{PH}_2$, 0.1 M *tert*-butyl alcohol, 0.1 M NaOH, Ar saturated, and the dose per pulse was $3.3 \times 10^{19} \text{ eV l.}^{-1}$. (■) Reducing radical $(\text{CH}_3)_2\text{CO}^-$. The solution composition was $1 \times 10^{-5} M \text{PH}_2$, 0.1 M $(\text{CH}_3)_2\text{CHOH}$, 0.1 M NaOH, N₂O saturated, and the dose per pulse was $3.3 \times 10^{19} \text{ eV l.}^{-1}$. (▲) Reducing radical $\cdot\text{CH}_2\text{O}^-$. The solution composition was $1 \times 10^{-5} M \text{PH}_2$, 0.1 M CH_3OH , N₂O saturated, and the dose per pulse was $2.2 \times 10^{19} \text{ eV l.}^{-1}$. (○) Reducing radical $\cdot\text{CH}_2\text{O}^-$. The solvent is $\text{CH}_3\text{OH} + 5\% \text{H}_2\text{O}$, for the solution containing 0.1 M NaOH, N₂O saturated, and the dose per pulse was $4.9 \times 10^{19} \text{ eV l.}^{-1}$.

mation of a transient absorption, at 600–650 nm, which obeyed always a pseudo-first-order rate law.

The observed optical densities due to the intermediates formed in the latter reactions were measured as a function of wavelength, immediately after the formation reaction ended (20–1000 μsec after the pulse). Some typical results are plotted in Figure 3 (a negative optical density means that the molar absorption coefficient of the intermediate is smaller than that of hematoporphyrin). The molar absorption coefficients of the intermediate ϵ_i^a , at the different wavelengths were calculated using the formulas $\epsilon_i^a = (\text{OD}/l) + \epsilon_p$, where OD is the measured optical density, l is the optical path length, ϵ_p is the molar extinction coefficient of PH_2 , and c is the concentration of the intermediate. The latter concentration was calculated from the dose per pulse, the yield of the primary radicals, and the specific rates of reaction of disproportionation or dimerization of the reducing radicals and of their reaction with PH_2 . A typical spectrum of the intermediates thus calculated is

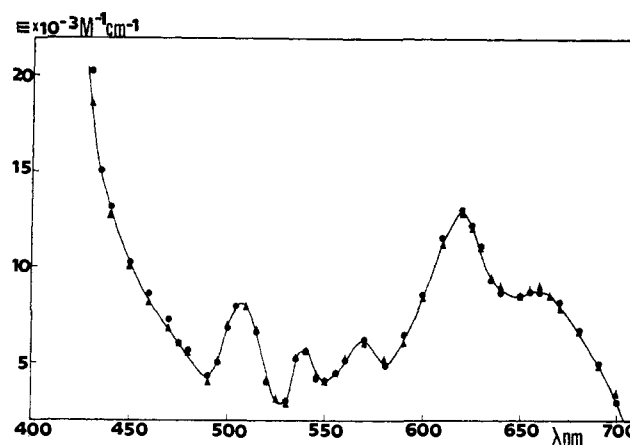
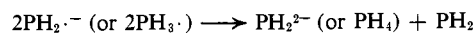


Figure 4. Absorption spectrum of the $\text{PH}_3\cdot$ (or $\text{PH}_2\cdot^-$) radicals in alkaline solutions. This spectrum is obtained from the data in Figure 3 by correcting for the destruction of PH_2 ; see text. (▲) Reducing radical e_{aq}^- . (●) Reducing radical $(\text{CH}_3)_2\text{CO}^-$.

given in Figure 4. Similar spectra for the intermediates were obtained independent of the reducing radical used. In neutral solutions the absorption bands in the 600–700-nm range are shifted by about 20 nm to the red and are somewhat broader.

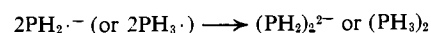
Spectrum of the Second Intermediate and Its Mechanism of Formation. The kinetics of formation of the second intermediate obeyed always a second-order rate law, in respect to the first intermediate. In order to calculate the specific rate of this reaction k_2 , the yields and the molar absorption coefficients of the products, ϵ_i^b , have to be known. The calculation of the latter depends on the mechanism of reaction. Two plausible mechanisms for the observed second-order reaction are considered (see Discussion). (a) A disproportionation reaction



and then

$$\epsilon_i^b = \frac{2(\text{OD})}{cl} + \epsilon_p$$

(where c is the initial concentration of the first intermediate). (b) A dimerization reaction



and then

$$\epsilon_i^b = \frac{2(\text{OD})}{cl} + 2\epsilon_p$$

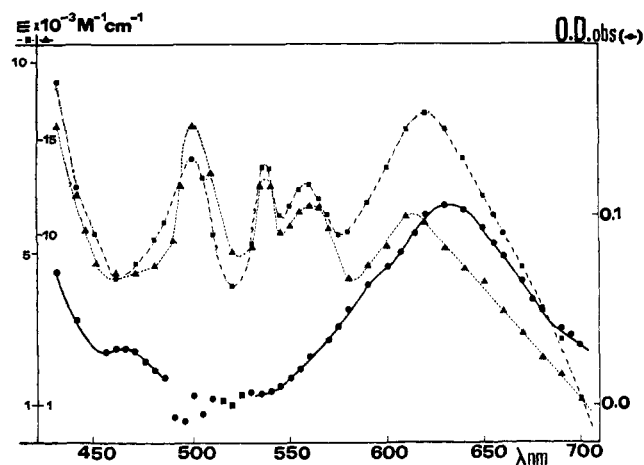


Figure 5. Absorption spectrum of the second intermediate. The solution composition was $1 \times 10^{-5} M$ PH_2 , $0.1 M$ $(\text{CH}_3)_2\text{CHOH}$, $0.1 M$ NaOH , N_2O saturated. The dose per pulse was 3.3×10^{-19} eV l^{-1} measured 100 msec after the pulse: (O) experimentally observed optical densities; (▲) corrected absorption spectrum, assuming a dimerization mechanism; (■) corrected absorption spectrum assuming a disproportionation mechanism.

In Figure 5 are plotted the observed optical densities, as well as the calculated absorption spectra, according to both mechanisms.

The specific rates of the second-order reaction in alkaline solutions were calculated from the slopes of the kinetic plots, k_{obsd} , and the absorption coefficients obtained for both suggested mechanisms. If the reaction is a disproportionation reaction, one obtains $k_2 = \frac{1}{2}k_{\text{obsd}}l(\epsilon_i^b - 2\epsilon_i^a + \epsilon_p)$, whereas if the reaction is a dimerization reaction, one obtains $k_2 = \frac{1}{2}k_{\text{obsd}}l(\epsilon_i^b - 2\epsilon_i^a)$. Thus, $k_2 = 2.0 \pm 0.4 \times 10^8 M^{-1} \text{sec}^{-1}$ and $k_2 = 2.1 \pm 0.4 \times 10^8 M^{-1} \text{sec}^{-1}$ for the two mechanisms, respectively, obtained. These rates are independent of the original reducing agent, e_{aq}^- , $\cdot\text{CH}_2\text{O}^-$, $\text{CH}_3\dot{\text{C}}\text{HO}^-$, $(\text{CH}_3)_2\dot{\text{C}}\text{O}^-$, and $\cdot\text{CO}_2^-$. In Table II are summarized

Table II. Observed Rates for the Second-Order Reaction of $\text{PH}_3\cdot$ (or $\text{PH}_2\cdot^-$) Radicals^a

$[\text{PH}_2]$, M	Pulse length, μsec	Solvent	$[\text{NaOH}]$, M	λ , nm	k_{obsd} , $\times 10^{-3}$, sec^{-1}
4×10^{-5}	0.1	H_2O	1×10^{-7}	700	4.0
4×10^{-5}	0.2	H_2O	1×10^{-7}	700	4.0
4×10^{-5}	0.2	H_2O	1×10^{-7}	440	1.5
5×10^{-5}	0.2	H_2O	0.1	440	1.5
2×10^{-5}	0.1	H_2O	0.1	600	3.8
2×10^{-5}	0.1	H_2O	0.1	700	4.0
1×10^{-5}	0.1	$\text{C}_2\text{H}_5\text{OH} + 5\% \text{H}_2\text{O}$	0.1	600	3.8
4×10^{-5}	0.1	$\text{C}_2\text{H}_5\text{OH} + 5\% \text{H}_2\text{O}$	0.1	600	4.0
2×10^{-5}	0.1	$\text{CH}_3\text{OH} + 5\% \text{H}_2\text{O}$	0.1	700	4.0
2×10^{-5}	0.1	$\text{CH}_3\text{OH} + 5\% \text{H}_2\text{O}$	0.1	600	4.0
2×10^{-5}	0.1	$\text{CH}_3\text{OH} + 5\% \text{H}_2\text{O}$	0.1	440	1.5

^a Error limit $\pm 10\%$.

the observed rates of the second-order reaction in neutral aqueous solutions and in alcohols as solvents. As the yields of the primary products in the latter are not known accurately, we could not calculate k_2 under these conditions. However, k_{obsd} at the different wavelengths are very similar to those in alkaline solutions. It is reasonable to assume that the molar absorption

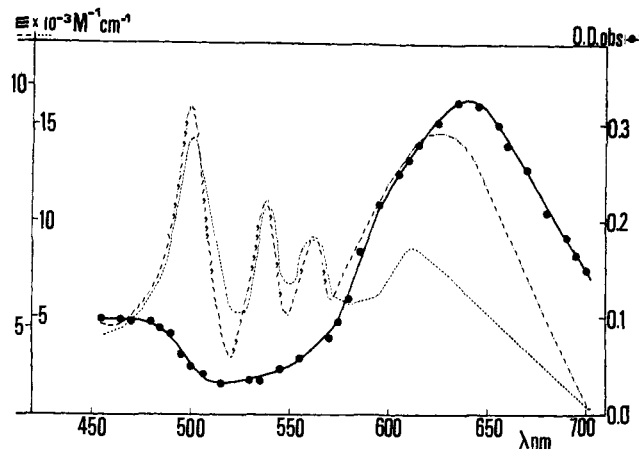


Figure 6. Absorption spectrum of the third intermediate. The solution composition was $2 \times 10^{-5} M$ PH_2 , $0.1 M$ $(\text{CH}_3)_2\text{CHOH}$, $0.1 M$ NaOH , N_2O saturated. The dose per pulse was 7.2×10^{19} eV l^{-1} measured 1 sec after the pulse: (●) experimentally observed optical densities; (---) corrected absorption spectrum assuming a dimerization mechanism; (-.-) corrected absorption spectrum assuming a disproportionation mechanism.

coefficients do not differ by more than a factor of 2 from those in aqueous alkaline solutions. Therefore, it seems that k_2 is nearly independent on pH or on a change of water to an alcoholic solvent.

Spectrum of the Third Intermediate and Its Mechanism of Formation. After the formation of the second intermediate was over, a third reaction leading to the formation of a third intermediate was observed. This reaction caused only slight changes in the optical densities at most wavelengths. The spectrum of the third intermediate is plotted in Figure 6. Again two calculated spectra are shown, depending on the assumed mechanism (see in the preceding paragraph and the Discussion).

The kinetics of the third reaction obeyed always a first-order rate law. The specific rate of this reaction, $k_{\text{obsd}} = 10 \pm 2 \text{sec}^{-1}$ is independent of the concentration of PH_2 , of the dose per pulse, and of the solvent in alkaline media. In neutral aqueous solutions $k_{\text{obsd}} = 4 \pm 1 \text{sec}^{-1}$ was obtained.

Kinetics of Formation of the Stable Products. After the formation of the third intermediate was over a fourth reaction was observed. The kinetics of this reaction obeyed always a first-order rate law. The specific rate of this reaction in alkaline solutions, $0.1 M$ NaOH , is $k_4 = 0.10 \pm 0.01 \text{sec}^{-1}$ independent of the solvent (water, methanol, or ethanol), the reducing radical used, the concentration of PH_2 , or the dose per pulse. In neutral aqueous solutions the specific rate of this reaction is $k_4 = (4 \pm 2) \times 10^{-2} \text{sec}^{-1}$. (The high error limit is due to the fact that our scope was limited to 50 sec, so that the transmittance at the end of the reaction was determined using a digital voltmeter.)

No further changes in the transmittance of the solutions were detected for several minutes, as judged by reading the output of the photomultiplier by a digital voltmeter. The absorption spectrum after 3 min was measured in a Cary 14 spectrophotometer and was found to be identical with that given in Figure 2.

The possibility that the third or fourth reactions are due to an impurity or a side product of radiation was checked by adding $1 \times 10^{-4} M$ of H_2O_2 , CH_3CHO , or

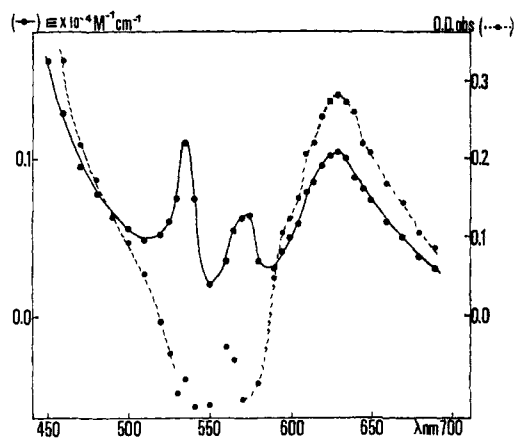


Figure 7. Absorption spectrum of $\text{ZnPH}\cdot$ (or $\text{ZnP}\cdot^-$) radicals. The solution composition was $1 \times 10^{-5} M \text{ZnP}$, $1 \times 10^{-4} M \text{ZnSO}_4$, $1.0 M (\text{CH}_3)_2\text{CHOH}$, $0.1 M \text{NaOH}$, N_2O saturated. The dose per pulse was $4.0 \times 10^{19} \text{ eV l}^{-1}$: (---) experimentally observed optical densities, (—) corrected absorption spectrum.

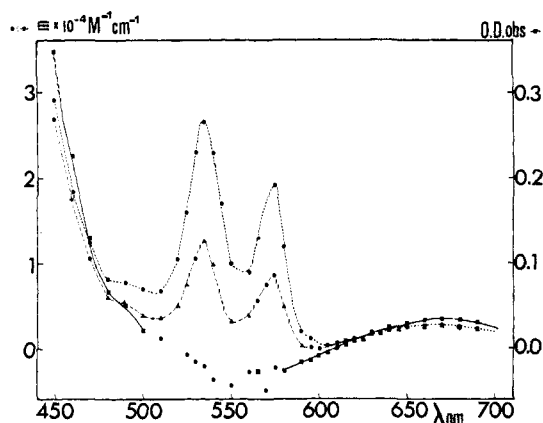


Figure 8. Absorption spectrum of the second intermediate in the reduction of ZnP . Experimental conditions as in Figure 7: (■) experimentally observed optical densities, (●) corrected absorption spectrum assuming a dimerization mechanism, (▲) corrected absorption spectrum assuming a disproportionation mechanism.

$(\text{CH}_3)_2\text{CO}$ to the solutions. No effect on the kinetics by any of these additives was observed. The possibility that the analyzing light affects the results was checked by chopping the light; this too was found to have no effect on the results.

Reduction of Zinc Hematoporphyrin. The mechanism of reduction of ZnP was studied in order to compare it with the corresponding mechanism for PH_2 . However, we have decided to use in this case only one reductant, $(\text{CH}_3)_2\dot{\text{C}}\text{O}^-$. Aqueous N_2O -saturated solutions at pH 13.0 containing $1.0 M$ 2-propanol were used throughout this study. The results have shown that the reduction of ZnP by $(\text{CH}_3)_2\dot{\text{C}}\text{O}^-$ involves four consecutive reactions until stable products are formed.

(a) The reaction of $(\text{CH}_3)_2\dot{\text{C}}\text{O}^-$ with ZnP obeyed a pseudo-first-order rate law, with $k_{(\text{CH}_3)_2\dot{\text{C}}\text{O}^- \rightarrow \text{ZnP}} = 1.0 \pm 0.1 \times 10^9 M^{-1} \text{ sec}^{-1}$. The absorption spectrum of the intermediate formed in this reaction is shown in Figure 7.

(b) The first reaction is followed by a reaction which obeys a second-order rate law in respect of the first intermediate. The specific rate of this reaction is 2.1

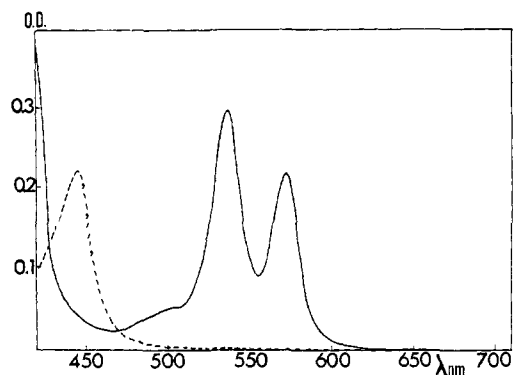


Figure 9. (—) Absorption spectrum of ZnP . The solution composition $2 \times 10^{-5} M \text{ZnP}$, $1 \times 10^{-4} M \text{ZnSO}_4$, $0.1 M \text{NaOH}$, $1.0 M (\text{CH}_3)_2\text{CHOH}$. The cell length was 1.0 cm . (---) Same solution irradiated by $1200 \pm 180 \text{ rads}$. The reference cell contained an unirradiated solution. The cell length was 4 cm .

$\pm 0.4 \times 10^8 M^{-1} \text{ sec}^{-1}$, independent of the assumed mechanism (dimerization or disproportionation). The absorption spectrum of the second intermediate, according to the two plausible mechanisms (see above in the corresponding section on PH_2), is plotted in Figure 8.

(c) The second reaction is followed by a first-order reaction with $k_{\text{obsd}} = 17 \pm 2 \text{ sec}^{-1}$. The changes in the OD due to this reaction are very small and the spectrum of the third intermediate is therefore very similar to that of the second intermediate. The kinetics of the reaction could be followed only in the $430\text{--}500\text{-nm}$ range and even there the changes in OD are smaller than 20%.

(d) The third reaction is followed by a fourth step with $t_{1/2} \geq 5 \text{ sec}$. However, by chopping the analyzing light it was found that the decomposition of the third intermediate is accelerated photochemically. Therefore, we were unable to determine the specific rate of this reaction.

The difference spectrum between the stable products formed by irradiation in the γ source (in the dark) and an unirradiated blank is plotted in Figure 9.

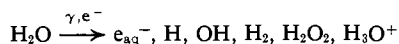
Reduction of Hemin. In order to obtain monomeric solutions of hemin, we have dissolved chlorohemin in aqueous solutions at pH 13.0 containing 30% w/v of *tert*-butyl alcohol or ethanol.¹⁸ The specific rate of reaction of e_{aq}^- with hemin, in Ar-saturated solutions containing *tert*-butyl alcohol, was found to be $k_{e_{\text{aq}}^- \rightarrow \text{hemin}} = 6.0 \pm 1.0 \times 10^9 M^{-1} \text{ sec}^{-1}$. The specific rate of reduction of hemin by $\text{CH}_3\dot{\text{C}}\text{HO}^-$ radicals, in N_2O -saturated solutions, was found to be $k_{\text{CH}_3\dot{\text{C}}\text{HO}^- \rightarrow \text{hemin}} = 9.0 \pm 1.0 \times 10^8 M^{-1} \text{ sec}^{-1}$. No further reaction was observed after the reduction by either radical was over.

Spectral studies have shown that the reduced product has a much lower absorption than hemin between 600 and 650 nm. On the other hand, the reduced product has a strong absorption band with a maximum around 560 nm. We could not determine the absolute absorption coefficients of the product as the primary yields of radicals in the solvent mixtures used are unknown.

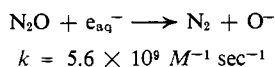
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Discussion

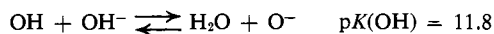
The radiolysis of water may be described by¹³



The yields of the products in neutral dilute solutions being $G_{e_{\text{aq}}^-} = 2.6$, $G_{\text{OH}} = 2.65$, $G_{\text{H}} = 0.6$, $G_{\text{H}_2} = 0.45$, $G_{\text{H}_2\text{O}_2} = 0.75$ (G is defined as the number of molecules of product formed by absorption of 100 eV by the sample). The hydrated electron reacts with N_2O according to¹⁹

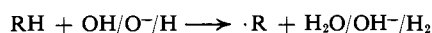


which is followed by the equilibrium reaction¹⁹

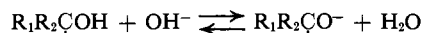


In neutral solutions saturated with N_2O , $2 \times 10^{-2} \text{ M}$, the yield of OH , or O^- depending on pH, is therefore increased to $G(\text{OH}) = 5.25$. (A somewhat higher yield is plausible owing to the effect of N_2O on G_{H_2} .¹⁷)

The radicals OH , O^- , and H react with saturated aliphatic compounds according to¹³



There is a high preference for hydrogen abstraction by these reactions from a site α to a functional group, e.g., OH , CO_2H . Thus, in N_2O -saturated solutions containing methanol, ethanol, 2-propanol, formate, or *tert*-butyl alcohol all the primary radicals are transformed into $\cdot\text{CH}_2\text{OH}$, $\text{CH}_3\dot{\text{C}}\text{HOH}$, $(\text{CH}_3)_2\dot{\text{C}}\text{OH}$, $\cdot\text{CO}_2^-$ and $\cdot\text{CH}_2\text{C}(\text{OH})(\text{CH}_3)_2$, respectively. The specific rates of formation of these radicals from the primary products,²⁰ as well as their uv^{21,22} and epr²³ spectra, mechanism of disappearance (by dimerization or disproportionation), are well known.^{21,22} The radicals $\cdot\text{CH}_2\text{OH}$, $\text{CH}_3\dot{\text{C}}\text{HOH}$, and $(\text{CH}_3)_2\dot{\text{C}}\text{OH}$ behave as weak acids



with the pK 's 10.7, 11.6, and 12.2, respectively.²⁴ The radicals $(\text{CH}_3)_2\dot{\text{C}}\text{O}^-$, $\text{CH}_3\dot{\text{C}}\text{HO}^-$, $\dot{\text{C}}\text{H}_2\text{O}^-$, and $\dot{\text{C}}\text{O}_2^-$ are strong reducing agents. The following reversible half-wave potentials for their oxidation -2.2 , -1.9 , -1.73 , and $-1.34 \text{ V vs. calomel}$, respectively, have been reported.²⁵ For the acidic form of these radicals the polarographic half-wave potentials are considerably lower, indicating large overpotentials. Thus, in irradiated N_2O -saturated solutions containing a high concentration of $(\text{CH}_3)_2\text{CHOH}$, $\text{CH}_3\text{CH}_2\text{OH}$, CH_3OH , or HCO_2^- , all the primary radicals formed are transformed into strong reducing radicals. On the other hand, the radical $\cdot\text{CH}_2\text{C}(\text{OH})(\text{CH}_3)_2$ is known to be a very weak redox agent and Ar-saturated solutions containing *tert*-butyl alcohol are therefore used to

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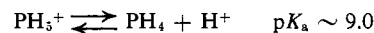
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study the reactions of e_{aq}^- without the interference of the effects due to OH radicals.

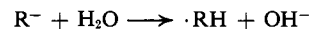
Identification of the Stable Product Obtained by the Radiolytic Reduction of PH_2 . The spectrum of the product (Figure 2) is in good agreement with the known spectrum of phlorin in neutral aqueous solutions. Furthermore, the pH effect on the spectrum which indicates a pK_a of about 9.0 with a blue shift at $\text{pH} > 9$ is also in agreement with the pK_a of phlorin



and the absorption spectrum of PH_4 .² The salt effect on the spectrum and the reactivity toward O_2 also indicate that the product obtained is a phlorin.² We conclude, therefore, that the radiolytically obtained reduction product is phlorin which is known to be the product obtained also by photochemical, electrochemical, or chemical methods.²

Mechanism of Reduction of PH_2 and Identification of the Intermediates. The observation that the absorption spectrum of the first intermediate is independent of the reducing agent used (e_{aq}^- , $(\text{CH}_3)_2\dot{\text{C}}\text{O}^-$, $\text{CH}_3\dot{\text{C}}\text{HO}^-$, $\cdot\text{CH}_2\text{O}^-$, and $\cdot\text{CO}_2^-$, Figures 3 and 4) indicates that the same intermediate is formed by all these radicals. The product of the reaction of e_{aq}^- with PH_2 has to be a reduced form, most probably $\cdot\text{PH}_2^-$ or $\cdot\text{PH}_3$, and it is therefore suggested that this is also the product of reaction of the other radicals. This conclusion is supported also by the fact that the specific rates of reaction of $(\text{CH}_3)_2\dot{\text{C}}\text{O}^-$, $\text{CH}_3\dot{\text{C}}\text{HO}^-$, $\cdot\text{CH}_2\text{O}^-$, and $\cdot\text{CO}_2^-$ with PH_2 decrease along this series which is also the order of decreasing redox potential of these radicals.²⁵ The observation that the $(\text{CH}_3)_2\dot{\text{C}}\text{HOH}$ and $\cdot\text{CH}_2\text{OH}$ radicals are less reactive than their alkaline analogues and that the $\cdot\text{CH}_2\text{C}(\text{OH})(\text{CH}_3)_2$ radical is unreactive is also in accord with the suggestion that the observed reaction is a redox process.

Furthermore, the most characteristic absorption band of the first intermediate, with $\lambda_{\text{max}} = 620 \pm 5 \text{ nm}$ and $\epsilon_{\text{max}} = 13,000 \text{ M}^{-1} \text{ cm}^{-1}$, and a shoulder at longer wavelength, is very similar to the known absorption spectrum of the $\cdot\text{PH}_2^-$ radical in aprotic solvents.^{3,4,11} It would thus seem reasonable to conclude that the first intermediate observed is the $\cdot\text{PH}_2^-$ radical. However, it is known that the rate of reaction of most aromatic anion radicals, including different azabenzenes, with water is very high²⁶⁻²⁸



For the azabenzene anion radicals it was shown that the added proton adds to a nitrogen atom.^{28,29} It is therefore plausible that the observed intermediate is the $\cdot\text{PH}_3$ radical and not $\cdot\text{PH}_2^-$ (this would mean that the reaction $\cdot\text{PH}_2^- + \text{H}_2\text{O} \rightarrow \cdot\text{PH}_3 + \text{OH}^-$ has a half-life shorter than $1 \mu\text{sec}$). It is reasonable to assume that the absorption spectra of $\cdot\text{PH}_3$ and $\cdot\text{PH}_2^-$ are similar.

It was hoped that the use of methanol or ethanol as solvents might help in indicating whether the observed intermediate is $\cdot\text{PH}_3$ or $\cdot\text{PH}_2^-$. However, as both the spectroscopic and kinetic data are nearly identical in

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the three solvents used, we are unable to resolve this question.

The second reaction observed is second order with respect to the first intermediate with a specific rate independent of the pH or the solvent used (Table II). These observations indicate that protonation is not the rate determining process for this reaction. The second-order rate law suggests a disproportionation or dimerization reaction. A disproportionation of $\cdot\text{PH}_3$ or $\cdot\text{PH}_2^-$ would yield PH_2 and PH_2^{2-} , PH_3^- , PH_4 , or PH_5^+ . As the formation of the second intermediate is followed by two further reactions yielding PH_4 or PH_5^+ , the final product at different pH's (see above), these products cannot be the second intermediate. Identification of this intermediate as PH_3^- or PH_2^{2-} seems also unlikely as their transformation into the stable products should involve only protonation processes, whereas the specific rates of the third and fourth reactions are nearly pH and solvent independent. Furthermore, the absorption spectrum of PH_2^{2-} which can be formed electrochemically in aprotic solvents¹¹ does not resemble the spectrum of the second intermediate. PH_2^{2-} is known to be unstable even in these solvents¹¹ and is therefore not expected to exist in water for periods of the order of 100 msec.

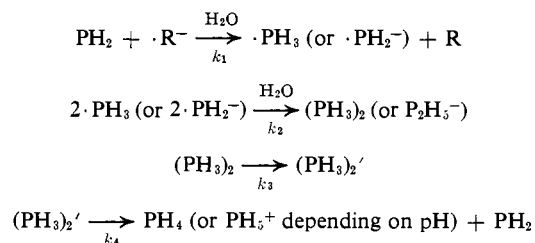
We thus conclude that the second reaction is a dimerization process and not a disproportionation reaction. The data available seem to be too scarce to prove the nature of the dimer formed. The spectrum of the dimer is similar to that of the monomer but for the band in the 600–700-nm range. The latter band has a different shape and intensity. It is tempting to speculate that the dimer is a π -mer and that the latter band is a charge-transfer band. π -mers are known to be very stable in aqueous media for different pyridinyl radicals.^{29,30} Their spectra are known to be similar to those of the monomers with the addition of an intense charge-transfer band at the long-wavelength end of the spectra.²⁹ It is plausible that $\cdot\text{PH}_3$ radicals might form similar dimers.

The specific rate of the second-order disappearance reaction of several $\cdot\text{PH}_3$ radicals in aqueous solution was measured by the epr technique. The results indicate that the rate increases with the decrease in the number, n , of CO_2H groups as substituents on the ring.⁶ Thus, for $n = 6$, $k \sim 10^4 M^{-1} \text{sec}^{-1}$, for $n = 4$, $k = 10^6 M^{-1} \text{sec}^{-1}$, and for $n = 2$, $k \gg 10^6 M^{-1} \text{sec}^{-1}$.⁶ The specific rate observed for the hematoporphyrin radical, $n = 2$, $k \sim 2 \times 10^6 M^{-1} \text{sec}^{-1}$, is in accord with these observations.

The third reaction observed is a first-order reaction with a specific rate which is independent of the PH_2 concentration and the solvent used in alkaline solutions and only slightly dependent on pH. Two plausible mechanisms might be suggested for this reaction: (a) decomposition of the dimer into $\text{PH}_4 + \text{PH}_2$ where the phlorin, PH_4 , is in a different configuration than the stable phlorin; (b) a rearrangement of the dimer. As the absorption spectrum of the third product resembles that of the second intermediate and differs considerably from that of phlorin, we prefer the latter mechanism.

The fourth reaction observed is a first-order process yielding the stable product, phlorin. It is therefore

suggested that it involves the decomposition of the dimer into $\text{PH}_4 + \text{PH}_2$. (Alternatively, rearrangement of an unstable form of phlorin into the stable form has to be suggested if alternative a is accepted for the third reaction.) It is suggested, therefore, that the mechanism of reduction of hematoporphyrin by one electron-reducing radicals in protic media is the following



where k_1 depends on the nature of the reducing radical $\cdot\text{R}^-$, $k_2 = (2.1 \pm 0.4) \times 10^8 M^{-1} \text{sec}^{-1}$, $k_3 = 10 \pm 2 \text{sec}^{-1}$, and $k_4 = 0.11 \pm 0.02 \text{sec}^{-1}$; $(\text{PH}_3)_2$ and $(\text{PH}_3)_2'$ are suggested to be two different forms of the dimer of the radical $\cdot\text{PH}_3$, most probably a π -mer.

Mechanism of Reduction of ZnP. The absorption spectrum of the first intermediate formed by the pseudo-first-order reaction of $(\text{CH}_3)_2\text{CHO}^-$, a one-electron reducing agent, with ZnP resembles the known spectrum of $\text{ZnP}\cdot^-$ in aprotic solvents.^{3,12} The specific rate of this reaction, as well as that of the following second-order step, is identical with those found for the corresponding reaction of the free base PH_2 . It is therefore reasonable to identify the first intermediate as $\text{ZnP}\cdot^-$, or as $\text{ZnPH}\cdot$, and the second intermediate as $(\text{ZnPH})_2$, or as $\text{Zn}_2\text{P}_2\text{H}^-$. (Similar reasons to those discussed above for the corresponding reaction of $\cdot\text{PH}_3$ exclude the possibility that the second reaction is a disproportionation reaction or that the product is $(\text{ZnP})_2^{2-}$.)

The third reaction observed causes only slight changes in the absorption spectrum and has a specific rate of reaction which is about twice that observed for the corresponding reaction for the free base. It is suggested that this reaction involves a rearrangement of the dimer $(\text{ZnPH})_2$ in parallel to the mechanism suggested above for PH_2 . On the other hand, we could not measure the kinetics of the fourth reaction due to photochemical decomposition of the third intermediate or of the final products by the analyzing light. No photochemical reactions were observed during the corresponding reactions of the free base. Furthermore, the absorption spectrum of the stable product as obtained by irradiation in the dark, see Figure 9, differs appreciably from that of phlorin. This is evident from the absence of the absorption band in the 600–750-nm region. The observed spectrum is similar to that of the product obtained by photochemical reduction of ZnP in ethanol.⁵ This product has been suggested to be dihydroporphyrin with one hydrogen atom on a peripheral position instead of the pyrrolic nitrogen which is occupied by the metal atom.⁵ It is suggested that the major difference in the mechanism of reduction of PH_2 and ZnP is that different dihydroporphyrins are formed upon the decomposition of the dimer of the first intermediate. The product formed upon the reduction of ZnP is less stable, at least photochemically, than phlorin.

Reduction of Hemin. The results clearly indicate that the reduction of hemin by e_{aq}^- and CH_3CHO^-

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radicals yields no intermediates before the stable product is formed. (Alternatively, if an intermediate is formed it is transferred into the stable products with a specific rate $k \geq 5 \times 10^5 \text{ sec}^{-1}$.) The spectral properties of the product clearly indicate that it is the Fe(II)-hemin complex.

Acknowledgment. The authors wish to thank Professor J. Manassen for many helpful discussions. We wish also to express our thanks to Miss E. Wajzman for technical assistance and to the linac group at the Hebrew University of Jerusalem for their care in operating the linac and maintaining the electronic equipment.

A Spectral and Thermodynamic Study of the 9-Cyclohexyladenine-Iodine Complex in Organic Solvents

Lung-Nan Lin, Sherril D. Christian,* and Jerry D. Childs

Contribution from the Department of Chemistry, University of Oklahoma, Norman, Oklahoma 73069. Received October 12, 1973

Abstract: The conventional spectral method and the polyiodide spectral-solubility method have been employed to obtain thermodynamic data and spectral parameters for the complex of 9-cyclohexyladenine with iodine in CCl_4 and heptane solution. Results of the two methods are in reasonable agreement, and 9-cyclohexyladenine- I_2 is shown to exist primarily as a charge-transfer complex stabilized by donation of n electrons from a ring nitrogen. Evidence is also presented for the existence of a 1:2 cyclohexyladenine- I_2 complex in CCl_4 at iodine concentrations on the order of 0.001 M .

In spite of the effort that has been spent on studies of weak molecular complexes of biologically important compounds, there is little direct evidence that charge-transfer forces play an important role in biochemistry. It is commonly assumed that charge transfer is involved in the relatively long-range interactions between the bases of nucleic acids and that numerous biological control mechanisms probably rely on transfer of charge from donor to acceptor molecules. A large part of the work on model biological charge-transfer systems has involved the purines and pyrimidines. There have been reports that purines and pyrimidines form π complexes with aromatic donors and acceptors such as chloranil, steroids, and the flavins.^{1,2} However, weak complexes of these types are undoubtedly stabilized in large part by interactions (such as dispersion forces and dipolar interactions) other than charge-transfer (CT) forces.^{3,4} A recent report from this laboratory included initial structural, thermodynamic, and spectral information about the 1:1 charge-transfer complex of 9-cyclohexyladenine (CHA) with I_2 , which is stabilized primarily by partial donation of an n electron from a ring nitrogen.⁵ The present article presents detailed results of the solution studies, obtained with a conventional spectral method of the Benesi-Hildebrand type⁶ and a polyiodide spectral-solubility method^{7,8} developed in this laboratory.

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(7) J. D. Childs, S. D. Christian, and J. Grundnes, *J. Amer. Chem. Soc.*, **94**, 5657 (1972); J. D. Childs, Ph.D. Dissertation, University of Oklahoma, Norman, Okla., 1971.

Difficulties in applying the Benesi-Hildebrand technique are encountered because of the limited solubility of CHA in organic solvents; the solubility method is a convenient alternative technique for obtaining spectral and thermodynamic data for such a system.

Experimental Section

Chemicals. Heptane (Baker Chemical Co. Defined purity) was purified by standard techniques⁹ and stored over drying agent (Linde 4A molecular sieve) in a 3-ft glass column. CCl_4 (Matheson Co. ACS analyzed reagent) was distilled from P_2O_5 and stored in the same way. Iodine (Malinckrodt, resublimed reagent grade) and tetramethylammonium iodide (Matheson, 99+%) were used without further purification. 9-Cyclohexyladenine (Cat. No. 2013), obtained from Cyclo Chemical Co., was used directly without further treatment.

Instrument. A single-beam Beckman DU-2 spectrophotometer equipped with a temperature-regulated cell holder was used in the spectral measurements. The temperature of the cell holder was controlled to within $\pm 0.2^\circ$ by circulating water from an auxiliary thermostated circulator (Haake). A separate refrigerator unit provided cooling for the Haake circulator when needed for experimental temperatures near or below ambient.

Polyiodide Solubility Method. The method for preparing tetramethylammonium pentaiodide (TMAI_5)¹⁰ and the experimental method⁷ for obtaining K_{DA} and ϵ_{DA} have been reported previously. Application of this method requires the accurate determination of the wavelength of the isosbestic point prior to spectral measurements of equilibrated solutions. This point was located by an isothermal method in which the total concentration of iodine ($[\text{I}_2]$) is held constant while the total concentration of CHA ($[\text{CHA}]$) is varied and also by varying the temperature while holding both $[\text{I}_2]$ and $[\text{CHA}]$ constant. Typical spectra are shown in Figure 1; the isosbestic point is located at 473 nm. In the solubility experiments, the increase in absorbance at the isosbestic point which occurs in the presence of added donor is attributed solely to the formation of the complex.¹¹ Since the extinction coefficients of

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(10) F. D. Chattaway and G. J. Hoyle, *J. Chem. Soc.*, **123**, 654 (1923).

(11) In the case of moderately strong complexes, such as $\text{CHA} \cdot \text{I}_2$, corrections for variation of activity coefficients with donor concentra-