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One-Electron Reduction of Ferrideuteroporphyrin IX and Reaction of the Oxidized and Reduced Forms with Chlorinated Methyl Radicals

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Abstract: α-Hydroxyisopropyl radicals prepared by fast electron irradiation of aqueous solutions containing 2-propanol and acetone were found to reduce monomeric ferrideuteroporphyrin to ferrodeuteroporphyrin with a rate constant of 3.7×10^8 $M^{-1}\,s^{-1}$. Relaxation of the coordination sphere is found to proceed with a rate constant $k \geq 5 \times 10^5\ s^{-1}$. Irradiation of such solutions in the added presence of various concentrations of CCl_4, $CHCl_3$, or CH_2Cl_2 enabled studies to be made of the reactions of ferri- and ferrodeuteroporphyrin with chlorinated methyl radicals. No reaction of ferrideuteroporphyrin with $\cdot CCl_3$ could be detected ($k \leq 10^6\ M^{-1}\,s^{-1}$). For ferrodeuteroporphyrin it appears that a rapid reaction ($k = (2 \pm 1) \times 10^9\ M^{-1}\,s^{-1}$) gives a complex ($DPFe^{II}\text{-}\cdot CH_{3-n}Cl_n$) which undergoes further reaction as shown by transient spectral modifications. These results suggest that ferrous cytochrome P450 is likely to react rapidly with chlorinated radicals. A model for the toxicity and detoxification of CCl_4 is proposed.

Introduction

Iron porphyrins are a matter of continuing interest owing to their involvement in fundamental biological processes.[2] Their most recently recognized role is in relation to the cytochrome P450 functions,[3] one of which involves the reduction of carbon tetrachloride, a hepatotoxic molecule, resulting in the formation of trichloromethyl radicals which are responsible for lethal cellular damages.[4,5] Also, under anaerobic reducing conditions, carbon tetrachloride and other polyhalogenated compounds react with cytochrome P450 leading to new derivatives[6,7] assigned to carbene complexes.[7] The formation of these derivatives involves a two-electron reduction of the halogenated molecule and could correspond to a further step of its metabolism. Recently, dichlorocarbene complexes of ferrous porphyrins considered as models for these cytochrome P450 derivatives have been characterized.[8,9] It has been shown that these complexes are obtained in solution when ferrous porphyrins are allowed to react with carbon tetrachloride according to the overall reaction[8]

$$\begin{aligned} 3\ \text{ferroporphyrins} + 1\ CCl_4 \rightarrow 2\ &\text{ferriporphyrins} \\ &+ 1\ \text{dichlorocarbeneferroporphyrin} \end{aligned}$$

The limiting step was attributed[8] to the reduction of carbon tetrachloride by the ferroporphyrin leading to trichloromethyl radicals ($k \simeq 10^2\ M^{-1}\,s^{-1}$). Moreover, the stoichiometry of the above reaction implies that no leaking of intermediate species occurs, which suggests a fast scavenging of the $\cdot CCl_3$ radicals by the porphyrin.

The present paper deals with a pulse radiolysis study of the fast reaction of iron porphyrins, especially the ferrous form, with $\cdot CCl_3$, $\cdot CHCl_2$, and $\cdot CH_2Cl$ radicals. This approach has required the use of an efficient system for reduction of the ferriporphyrin to the ferrous state with concomitant $\cdot CCl_3$, $\cdot CHCl_2$, or $\cdot CH_2Cl$ radical formation. Therefore, we also present a study of the reduction of the ferriporphyrin by α-hydroxyisopropyl radicals.

The experiments have been performed with ferrideuteroporphyrin IX chloride (deuterohemin) in 2-propanol–phosphate buffer (pH 7.2) mixture, where this porphyrin was found to be a monomer. The only previous data on the reduction of simple monomeric porphyrins appear to have been on protohemin IX at alkaline pH,[10] on hemin *c*,[11] and on protohemin IX intercalated into sodium dodecyl sulfate micelles.[12]

Experimental Section

Materials. Ferrideuteroporphyrin IX chloride (deuterohemin) was prepared from ferriprotoporphyrin IX chloride via the resorcinol melt procedure[13] and recrystallized three times as follows. Crude deuterohemin (4 g) was dissolved in pyridine (40 mL). The solution was then filtered and added to a boiling NaCl saturated 90% acetic acid solution (550 mL) containing 35 mL of concentrated HCl. After cooling and standing for 24 h, the deuterohemin crystals were twice washed with 50% acetic acid and three times with water. Washing with ethanol and ethyl ether was performed after the last crystallization sequence. The crystals were then dried under vacuum. The purified deuterohemin moves as a single spot using polyamide 6 TLC plates (Macherey-Nagel) according to a procedure modified from Lamson et al.[14] Sodium dithionite was purchased from Merck. 2-Propanol was purified according to Baxendale and Mellows[15] to remove aldehydic and ketonic impurities. Carbon tetrachloride and methylene chloride (Baker BAR) were used without further purification. Chloroform was three times distilled before use.

Preparation of Deuterohemin Solutions. Deuterohemin being soluble only at alkaline pH, the following procedure was used to prepare neutral alcoholic solutions. Deuterohemin was dissolved in 1.5 mL of NaOH (0.1 M). The solution was diluted to 15 mL, and then 25 mL of KH_2PO_4 (10^{-2} M) was added followed by 50 mL of 2-propanol. The resulting solution was adjusted to 100 mL with phosphate buffer

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(6.2×10^{-3} M, pH 7.2). The pH of the aqueous solution was found to be 7.2 before addition of 2-propanol.

Ferrous deuteroporphyrin (deuteroheme) was prepared from deuterohemin by chemical reduction using a minimal amount of sodium dithionite and handled under inert atmosphere as described elsewhere.[16]

Physical Measurements. Optical absorption spectra were recorded using a Cary 219 spectrophotometer.

Pulse radiolysis experiments were performed with the apparatus located at the Paterson Laboratories of the Christie Hospital.[17] Irradiations were carried out with 10- or 20-ns pulses with doses ranging from 1 to 6 krad. The dose was estimated from the hydrated electron absorption at 700 nm[18] assuming that the molar extinction coefficients of hydrated electron in pure water and in water containing 2-propanol (6.5 M) are similar, i.e., 18 000 M^{-1} cm^{-1}. This assumption seems to be reasonable since addition of 2-propanol in water up to 10 M does not significantly change the absorption maximum energy and the half-bandwidth of the hydrated electron spectrum.[19] In water/2-propanol/acetone mixtures, it was assumed that the relative yields of primary species (e^-_{aq}, $OH^\cdot$, $H^\cdot$) were the same as in water and the initial concentration of α-hydroxyisopropyl radicals was computed as being equal to the e^-_{aq} concentration multiplied by $(G(e^-_{aq}) + G(OH^\cdot) + G(H^\cdot))/G(e^-_{aq})$, i.e., 6/2.7.[20]

Transients were monitored by standard spectrophotometric techniques. A Bausch and Lomb monochromator with slits giving a band of either 2.5 or 5 nm was used. In order to suppress photolysis a filter which removes light of wavelength lower than 370 nm was placed in front of the optical cell.

Solutions were deaerated by bubbling with high-purity argon (Air Products) and transferred into the radiolysis cell by overpressure. Great care had to be taken to avoid CCl_4, $CHCl_3$, or CH_2Cl_2 evaporation. Minimal amounts of solutions of these compounds were added in anaerobic conditions, just before experiments, to deuterohemin solution which had been previously deaerated. It is believed that the precautions taken eliminated any possibility of significant formation of $\dot{C}Cl_3O_2$ radicals or related compounds.[21]

Results and Discussion

Water radiolysis essentially produces hydrated electrons (e^-_{aq}), hydroxyl radicals ($OH^\cdot$), and hydrogen atoms ($H^\cdot$) at yields, $G(e^-_{aq}) = 2.7$, $G(OH^\cdot) = 2.7$, $G(H^\cdot) = 0.6$.[20] Hydroxyl radicals and hydrogen atoms have been shown to quickly react with primary or secondary alcohols leading mainly to α-hydrogen abstraction. In the case of 2-propanol, the following principal reactions occur:[22]

$$(CH_3)_2CHOH + OH^\cdot \rightarrow (CH_3)_2\dot{C}OH + H_2O \qquad (1)$$

$$k_1 \simeq 10^9\ M^{-1}\ s^{-1}$$

$$(CH_3)_2CHOH + H^\cdot \rightarrow (CH_3)_2\dot{C}OH + H_2 \qquad (2)$$

$$k_2 \simeq 10^8\ M^{-1}\ s^{-1}$$

Hydrated electrons react with acetone[22] according to

$$(CH_3)_2CO + e^-_{aq} \rightarrow (CH_3)_2CO^- \qquad (3)$$

$$k_3 = 6 \times 10^9\ M^{-1}\ s^{-1}$$

The radical produced is the alkaline form of $(CH_3)_2\dot{C}OH$:[23]

$$(CH_3)_2\dot{C}OH \rightleftarrows (CH_3)_2CO^- + H^+\ (pK_a = 12.2) \qquad (4)$$

Thus, in neutral aqueous solutions containing 2-propanol (6.5 M) and acetone (3.4×10^{-2} M) all primary species are scavenged in less than 100 ns, the main product being the α-hydroxyisopropyl radical $(CH_3)_2\dot{C}OH$. This radical can dimerize or disproportionate according to

$$2(CH_3)_2\dot{C}OH \rightarrow \text{products} \qquad (5)$$

with $2k_5 = 1.4 \times 10^9\ M^{-1}\ s^{-1}$ [24] or react with a solute.

The α-hydroxyisopropyl radical (as other alcohol radicals) is known to be a reducing agent, whose reduction potential is known.[23] Thus, the reaction of α-hydroxyisopropyl radicals with a solute can be written as

$$(CH_3)_2\dot{C}OH + S \rightarrow (CH_3)_2CO + S^- + H^+ \qquad (6)$$

S being in large excess, pseudo-first-order conditions prevail, and the concentration of S which is reduced to S^- can be calculated as[25]

$$[S^-] = \frac{k_6'}{2k_5} \ln \left\{ 1 + \frac{2k_5}{k_6'} [(CH_3)_2\dot{C}OH]_0 \right\} \qquad (7)$$

where $[(CH_3)_2\dot{C}OH]_0$ is the initial concentration of α-hydroxyisopropyl radicals and $k_6' = k_6[S]$. In all experiments presented below, the amount of α-hydroxyisopropyl radicals which react together does not exceed 12% of their initial concentration.

Reduction of Ferrideuteroporphyrin (Deuterohemin) by α-Hydroxyisopropyl Radicals. According to Maehly and Akeson,[26] ethanol and methanol stabilize and disperse aqueous solutions of proto- and deuterohemin, the monomerization being almost complete in 50% alcohol solution. 2-Propanol was found to give similar results, this alcohol being even more efficient (C. Bizet and D. Brault, unpublished results). At neutral or alkaline pH, monomeric ferriporphyrins have a counterion coordinated to the ferric ion balancing the charges.[2] This counterion is protonated below pH $\simeq$ 5 in detergent[27] or alcohol/water[26] solutions. The same considerations apply to deuterohemin in water/2-propanol mixtures. Thus, in the experimental conditions prevailing below (phosphate buffer pH 7.2/2-propanol mixtures) the ferric ion is coordinated by a counterion which can be OH^- or the alcoholate ion $(CH_3)_2CHO^-$.

2-Propanol was also found to disperse deuteroheme solutions. Referring to the well-known spectrum of bare monomeric deuteroheme in benzene and the one of coordinated deuteroheme in ethanol[16] or in pure 2-propanol,[28] the spectrum obtained in phosphate buffer (6.2×10^{-3} M, pH 7.2)/2-propanol (6.5 M) mixtures can be attributed to monomeric, partially coordinated deuteroheme molecules according to

$$DPFe^{II} + L \rightleftarrows DPFe^{II}\text{-}L \qquad (8)$$

where L stands for water or 2-propanol molecules. This result agrees with the very weak coordinating properties of alcohols and water reported elsewhere.[16]

The reaction of α-hydroxyisopropyl radicals with deuterohemin was investigated by giving a pulse of 0.5–1.7 krad to an argon-saturated solution of deuterohemin (5×10^{-5} to 2×10^{-4} M) in phosphate buffer (6.2×10^{-3} M, pH 7.2) containing 2-propanol (6.5 M) and acetone (3.4×10^{-2} M). The dose was adjusted so that the initial concentration in α-hydroxyisopropyl radicals (the main product of the solvent mixture irradiation as mentioned before) did not exceed 10% of the deuterohemin concentration.

The optical changes were observed with 1-mm and 1-cm path length cells in the Soret and the visible regions, respectively. A typical result is given in Figure 1a. The optical changes obey first-order kinetics as shown by the plot of log $(OD_t - OD_\infty)$ vs. time (see Figure 1c) where OD_∞ is the optical density after the end of the reaction and OD_t is the optical density at time t of the reaction. The first-order rate constant is proportional to the deuterohemin concentration (Figure 2) and the second-order rate constant is found to be 3.7×10^8 M^{-1} s^{-1}. As shown in Figure 1b little or no other change can be seen up to 0.5 s after the pulse. The same results were obtained at other wavelengths.

In Figure 3 is reported the absolute difference spectrum calculated as $(OD_\infty - OD_0)/C$ where OD_0 is the optical density before the pulse and C is the concentration of deuterohemin which has reacted. C is calculated as described in the Experimental Section taking into account the small fraction

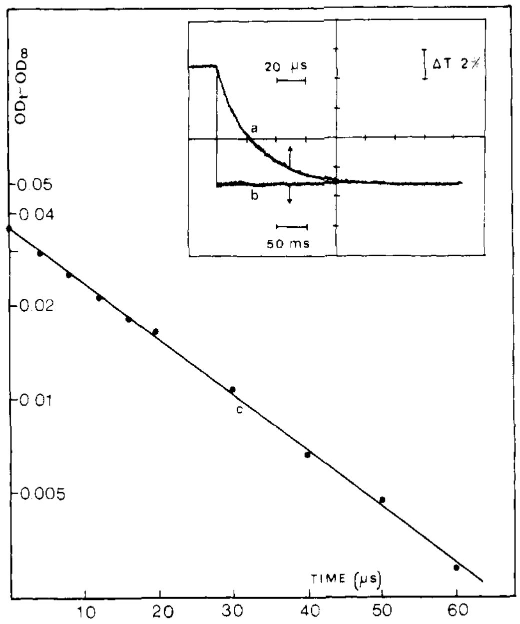

Figure 1. Reduction of deuterohemin by α-hydroxyisopropyl radicals. Solvent phosphate buffer (6.2×10^{-3} M, pH 7.2)/2-propanol (6.5 M)/acetone (3.4×10^{-2} M); deuterohemin concentration 10^{-4} M; dose 1.7 krad. (a, b) Transmission changes (λ 407 nm). (c) Semilogarithmic plot of ($OD_t - OD_\infty$) vs. time.

of α-hydroxyisopropyl radicals which react with each other. The difference spectrum thus obtained agrees very well with the one obtained from conventional reduction methods. A small discrepancy is observed in the Soret region owing to the low spectral resolution of the monochromator used in the radiolysis setup.

These results clearly show that deuterohemin is reduced by α-hydroxyisopropyl radicals leading to deuteroheme according to

$$(CH_3)_2\dot{C}OH + DPFe^{III} \xrightarrow{k_9} DPFe^{II} + (CH_3)_2CO + H^+ \tag{9}$$

The apparent reaction rate constant being proportional to the deuterohemin concentration ($k_{app} = k_9[DPFe^{III}]$), α-hydroxyisopropyl radicals do not react with any solvent impurity.[25] Thus, the reduction of deuterohemin by these radicals appears to be a very clean and convenient method.

The reaction rate constant, $k_9 = 3.7 \times 10^8$ M^{-1} s^{-1}, compares well with the values reported for the reaction of α-hydroxyethyl radicals with protohemin dissolved in water/alcohol mixtures ($k = 9 \times 10^8$ M^{-1} s^{-1} at pH 13; see ref 10) or intercalated in sodium dodecyl sulfate micelles ($k = 5.6 \times 10^8$ M^{-1} s^{-1} at pH 9.2, ref 12) or with the reaction of α-hydroxyisopropyl radicals with hemin *c* (2.8×10^9 M^{-1} s^{-1}, ref 11).

The reduction of deuterohemin necessarily involves several steps, the reduction of the iron being followed by protonation or release of the counterion and attainment of the equilibrium state according to relationship (8). Our results showing a single-step process indicate that the rearrangement of the coordination sphere must proceed with a rate constant $k \geq 5 \times 10^5$ s^{-1}. In contrast to our results, in the case of hemin intercalated in micelles,[12] the hydroxyl counterion is slowly converted to a water molecule ($k = 8 \times 10^2$ s^{-1}). This difference could be due to the restricted access of water to the inner coordination sphere of the intercalated iron.[12]

Reaction of Deuterohemin and Deuteroheme with Trichloromethyl Radicals. According to Köster and Asmus[29] and Willson and Slater[30] α-hydroxyisopropyl radicals react with CCl$_4$ leading to ·CCl$_3$ radicals:

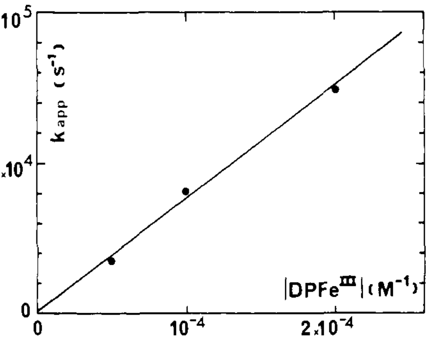

Figure 2. Reduction of deuterohemin by α-hydroxyisopropyl radicals. Solvent phosphate buffer (6.2×10^{-3} M, pH 7.2)/2-propanol (6.5 M)/acetone (3.4×10^{-2} M). Plot of k_{app} vs. deuterohemin concentration.

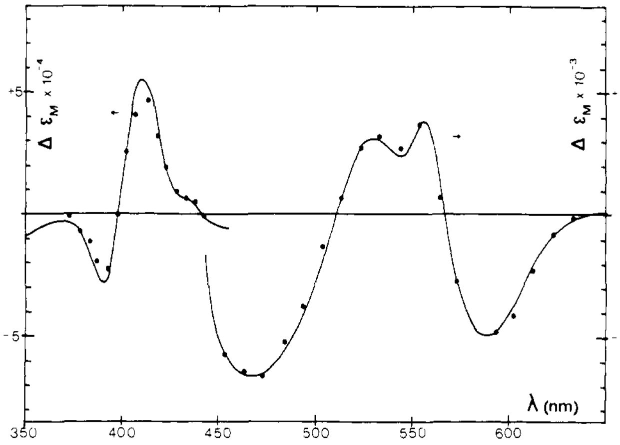

Figure 3. Reduction of deuterohemin by α-hydroxyisopropyl radicals. Solvent phosphate buffer (6.2×10^{-3} M, pH 7.2)/2-propanol (6.5 M)/acetone (3.4×10^{-2} M). Difference spectra (DPFe^{II} minus DPFe^{III}) obtained from conventional reduction methods (—) and from pulse radiolysis study (●).

$$(CH_3)_2\dot{C}OH + CCl_4 \xrightarrow{k_{10}} \cdot CCl_3 + (CH_3)_2CO + H^+ + Cl^- \tag{10}$$

These authors respectively report the reaction rate constant to be 1×10^8 and 7×10^8 M^{-1} s^{-1}, which is comparable with the rate constant for the deuterohemin reduction.

Thus the reaction of ·CCl$_3$ radicals with deuteroheme and deuterohemin can be investigated using aqueous solutions of deuterohemin containing 2-propanol, acetone, and carbon tetrachloride whose concentration is chosen in order to favor the simultaneous formation of deuteroheme and ·CCl$_3$ radicals or the exclusive formation of the latter species. Deuterohemin and CCl$_4$ being in large excess relative to the α-hydroxyisopropyl radical concentration, deuteroheme and ·CCl$_3$ radicals are produced under pseudo-first-order conditions. The ratio of the concentrations of these species is given[25] by

$$\frac{[\cdot CCl_3]}{[DPFe^{II}]} = \frac{k_{10}[CCl_4]}{k_9[DPFe^{III}]}$$

However, the reaction of ·CCl$_3$ radicals with the solvent or residual oxygen and their dimerization must be considered. The reaction with 2-propanol has been shown to be very slow[31] and need not be taken into account. On the other hand, the rate constant $2k_{11}$ for the dimerization reaction

$$2 \cdot CCl_3 \xrightarrow{2k_{11}} C_2Cl_6 \tag{11}$$

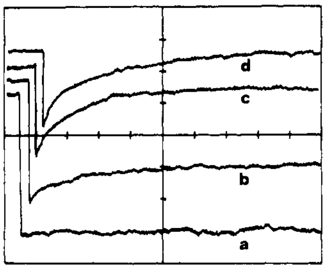

Figure 4. Transmission changes (λ 412 nm) after pulse irradiation (dose = 1.9 krad) of phosphate buffer (6.2×10^{-3} M, pH 7.2)/2-propanol (6.5 M)/acetone (3.4×10^{-3} M) solutions containing deuterohemin (10^{-4} M) and various carbon tetrachloride concentrations. Scales: vertical, ΔT = 2%/div; horizontal, time = 10 ms/div. Carbon tetrachloride concentration: (a) 0; (b) 0.25×10^{-4} M; (c) 0.7×10^{-4} M; (d) 10^{-4} M.

has been reported to range between 10^8 M^{-1} s^{-1} in nonpolar solvents[32] and 7.4×10^8 M^{-1} s^{-1} in water.[33] An intermediate value might be expected in our system. In addition to the precautions taken against oxygen contamination, the good agreement between the kinetic scheme and results of experiments performed even with high doses seems to rule out any important contribution of the reaction of $\cdot CCl_3$ radicals with residual oxygen.

Reaction of Deuterohemin with Trichloromethyl Radicals. This reaction was studied using a solution of deuterohemin (10^{-4} M) in phosphate buffer (6.2×10^{-3} M, pH 7.2) containing purified 2-propanol (6.5 M) and an excess of carbon tetrachloride (5×10^{-3} M). In this solution, hydrated electrons are scavenged by carbon tetrachloride, leading to the trichloromethyl radicals,[22] as are most of the α-hydroxyisopropyl radicals ($k_{10}[CCl_4] \gg k_9(DPFe^{III})$. In these conditions, we were unsuccessful in recording any spectral change.

Assuming that the reaction of deuterohemin with 10% of the $\cdot CCl_3$ radicals initially produced could be detected, and taking into account the self-reaction of $\cdot CCl_3$ radicals, makes it possible to determine via a relationship similar to eq 7 an upper limit for the rate constant of the reaction

$$DPFe^{III} + \cdot CCl_3 \xrightarrow{k_{12}} \text{products} \qquad (12)$$

$$k_{12} \leq 10^6 \text{ M}^{-1} \text{ s}^{-1}$$

Reaction of Deuteroheme with Trichloromethyl Radicals. Optical changes following the irradiation of solutions of deuterohemin (10^{-4} M) in phosphate buffer (6.2×10^{-3} M, pH 7.2) containing 2-propanol (6.5 M), acetone (3.4×10^{-2} M), and various amounts of carbon tetrachloride are given in Figure 4. The amplitude of the initial optical change, which is related to the concentration of deuteroheme formed via reaction 9, is seen to decrease as the carbon tetrachloride concentration is increased, exemplifying the competition between reactions 9 and 10. The initial optical change was found to be reduced by half when the carbon tetrachloride concentration was about 10^{-4} M, which means that $k_9 \sim k_{10}$.

A subsequent slow recovery of the initial absorption was observed in the Soret region. This recovery can be characterized as a two-step process by changing the dose. As depicted in Figures 5a,b, the slower step is found to obey first-order kinetics independent of the dose used. On the other hand, fast scans (see Figures 5c,d) clearly show that a rapid process occurs between the deuterohemin reduction and the slow unimolecular decay to the final products. The related optical changes depend on the dose used, which strongly suggests that this step is the bimolecular reaction between deuteroheme and $\cdot CCl_3$ radicals:

$$DPFe^{II} + \cdot CCl_3 \xrightarrow{k_{13}} [DPFe^{II}\text{-}CCl_3] \qquad (13)$$

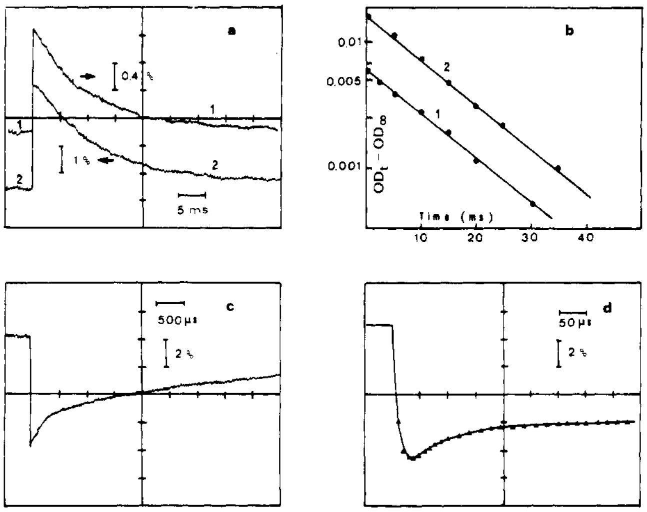

Figure 5. Transmission changes after pulse irradiation of phosphate buffer (6.2×10^{-3} M, pH 7.2)/2-propanol (6.5 M)/acetone (3.4×10^{-2} M) solutions containing deuterohemin (10^{-4} M) and carbon tetrachloride (10^{-4} M): (a) λ 387 nm, dose = 0.67 (1) and 2.2 krad (2); (b) same experiment, semilogarithmic plots of ($OD_t - OD_\infty$) vs. time; (c) λ 412 nm, dose = 5.8 krad; (d) λ 412 nm, dose = 6.2 krad. ▲, experimental; —, theoretical curve calculated with $k_{13} = 2 \times 10^9$ M^{-1} s^{-1} (see text).

In theory, the above considerations make it possible to predict the change in the transient optical density as a function of time elapsed after the pulse. Figure 5d shows the good agreement between the experimentally observed transient shape and the calculated one assuming $k_9 = 3.7 \times 10^8$ M^{-1} s^{-1}, $k_{10} = 3.7 \times 10^8$ M^{-1} s^{-1}, and $k_{13} = (2 \pm 1) \times 10^9$ M^{-1} s^{-1}.

The reaction rate constant for the slow unimolecular decay

$$[DPFe^{II}\text{-}CCl_3] \xrightarrow{k_{14}} \text{products} \qquad (14)$$

was found to be $k_{14} = 70$ s^{-1}. The transient absorption spectrum recorded 50 ms after the pulse shows maximums at about 390 and 540 nm. As will be seen below, it looks like the one observed in presence of dichloromethane. As no subsequent redox processes are expected in the present system, reaction 14 is believed to be a structural reorganization of the [$DPFe^{II}$-CCl_3] intermediate or of its coordination sphere.

Reaction of Deuteroheme with Dichloromethyl Radicals. This reaction was studied using a solution of deuterohemin (10^{-4} M) in phosphate buffer containing purified 2-propanol (6.5 M) and chloroform (1.2×10^{-3} M). In this solution, hydrated electrons are scavenged by chloroform[22] and most of the α-hydroxyisopropyl radicals by deuterohemin leading respectively to $\cdot CHCl_2$ formation and deuterohemin reduction. With the exception of the unimolecular slow decay step (rate constant $\sim 10^3$ s^{-1}) the optical changes following the pulse appear to be, in all points, similar to those reported in the above experiments.

Reaction of Deuteroheme with Monochloromethyl Radicals. As previously reported,[8,16] dichloromethane was found to be inert toward ferrous porphyrins. Thus, the direct reaction between chemically reduced deuteroheme and monochloromethyl radicals produced by irradiation can be investigated. Deuterohemin (10^{-4} M) was reduced by sodium dithionite (10^{-4} M) in phosphate buffer/purified 2-propanol mixtures. Dichloromethane (4×10^{-2} M) was then added. Following the pulse, a fast absorbance change is observed over 100 μs. This transient absorption change is a two-exponential process characterized by rate constants of 2×10^5 and 4×10^4 s^{-1}, respectively. By bubbling the solution with N_2O or by adding acetone, only the slower process was observed.

In both cases, the yield of deuteroheme consumption was found to be $G \simeq 6$. This result implies that hydrated electrons and α-hydroxyisopropyl radicals react with dichloromethane leading to $\cdot CH_2Cl$ radicals which is expected from the well-known reducing properties of alcohol radicals.[34]

Thus, in argon-saturated solution, the faster transient absorption change corresponds to the pseudo-first-order reaction of deuteroheme with the $\cdot CH_2Cl$ radicals produced by the fast scavenging of hydrated electrons. The slower one is the consequence of the reaction of deuteroheme with $\cdot CH_2Cl$ radicals whose formation results from the rate-limiting reaction

$$(CH_3)_2\dot{C}OH + CH_2Cl_2 \xrightarrow{k_{15}} \cdot CH_2Cl + (CH_3)_2CO + H^+ + Cl^- \qquad (15)$$

The reaction rate constant k_{15} was found to be $k_{15} \simeq 10^6$ $M^{-1}\ s^{-1}$, a value consistent with the upper limit reported by Willson and Slater for the reaction of α-hydroxyisopropyl radicals with chloroform.[30] The rate constant of the reaction

$$DPFe^{II} + \cdot CH_2Cl \xrightarrow{k_{16}} \text{products} \qquad (16)$$

$k_{16} = 2 \times 10^9\ M^{-1}\ s^{-1}$, was found to be similar to the one reported in the case of $\cdot CCl_3$ radicals.

The absorption spectrum of the intermediate species taken 100 μs after the pulse is reported in Figure 6. Slight spectral modifications are observed in the millisecond range (see Figure 6) suggesting some reorganization of the coordination sphere around the complex.

However, a question remains about the nature of the observed intermediate species because of a possible diffusion-controlled reduction of a $[DPFe^{II}\text{-}CH_2Cl]$ complex by excess deuteroheme or sodium dithionite leading to a carbene complex. We have therefore undertaken the pulse radiolysis study of solutions of deuterohemin (10^{-4} M) in phosphate buffer containing purified 2-propanol (6.5 M) and dichloromethane (4×10^{-2} M). In this system, $\cdot CH_2Cl$ radicals and deuteroheme molecules are produced without excess reducing agent. The optical changes were found similar to those observed with chloroform. The spectrum 4.5 ms after the pulse compares fairly well with that reported for the former experiment with chemically reduced deuteroheme (see Figure 6). This suggests that the spectrum can be reasonably attributed to a $[DPFe^{II}\text{-}CH_2Cl]$ complex.

Conclusion and Biological Aspects

We have shown that ferrous porphyrins react with various chlorinated methyl radicals with rate constants ($\simeq 2 \times 10^9$ $M^{-1}\ s^{-1}$) near the diffusion limit. The resulting product is believed to be an example of unusual α-bonded carbon-iron complexes.[35]

This study can also help in understanding the toxicity of halogenated hydrocarbons, in particular carbon tetrachloride. Microsomal ferrous cytochrome P450 is believed to reduce this compound leading to $\cdot CCl_3$ radicals with concomitant oxidation of the hemoprotein.[4,5] Our results show that, if the protein is reduced again by the enzymic system, the scavenging of the radical by the ferrous cytochrome P450 will be very fast and in any case will not be a rate-limiting process. The ferrous cytochrome P450-radical complex is expected to have a sufficient lifetime to make possible further reduction leading to the dichlorocarbene complex. As reported elsewhere,[36] the carbene complex formation could be the way by which carbon tetrachloride is metabolized leading to the inert carbon dioxide molecule. Therefore, depending on the relative rates of reduction of ferric cytochrome P450 by cytochrome P450 reductase on one hand and of the reaction of $\cdot CCl_3$ radicals with the biological environment on the other hand, the metabolism of carbon tetrachloride could result in $\cdot CCl_3$-mediated toxicity or in detoxification.[37]

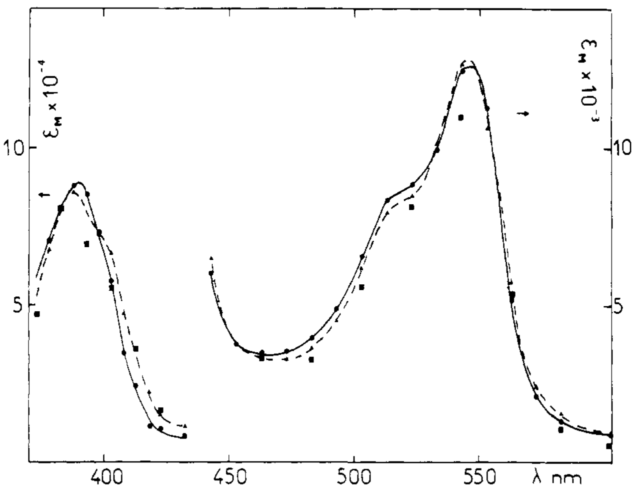

Figure 6. Reconstituted visible and near-UV spectra of transient species formed after pulse radiolysis of 10^{-4} M deuteroheme in phosphate buffer (6.2×10^{-3} M, pH 7.2)/2-propanol (6.5 M) mixture containing sodium dithionite (10^{-4} M) and dichloromethane (4×10^{-2} M): —, 100 μs after the pulse (●, experimental points); – –, 4.5 ms after the pulse (▲, experimental points). ■, Visible and near-UV spectra of the transient species observed 4.5 ms after pulse radiolysis of 10^{-4} M deuterohemin in phosphate buffer (6.2×10^{-3} M, pH 7.2)/2-propanol (6.5 M) mixture containing dichloromethane (4×10^{-2} M). The dose is 1.4 krad in both cases.

Acknowledgments. This work was supported in part by grants from the Cancer Research Campaign and the Medical Research Council. We wish to thank O. Delgado for his skillful help in the preparation and the purification of deuterohemin.

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Mechanistic Studies of the Homogeneous Catalysis of the Water Gas Shift Reaction by Rhodium Carbonyl Iodide

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Abstract: The water gas shift reaction, $H_2O + CO = H_2 + CO_2$, is effectively catalyzed at low temperatures and pressures by a catalyst system based on rhodium carbonyl iodide. The results of semiquantitative kinetic studies and of quenching experiments are reported. In the temperature range 80–100 °C the catalytic reaction shows first-order dependence on the partial pressure of CO and inverse dependence on acid and iodide concentrations. The major species in solution are rhodium(III) carbonyls. However, below 65 °C the reaction is independent of CO pressure, and exhibits second-order dependence on iodide concentration and positive dependence on acid concentration. Rhodium(I) species now become predominant in solution. The temperature dependence of the rate shows corresponding behavior. Above 80 °C the Arrhenius plot is linear with a slope which yields an apparent activation energy of 9.3 kcal mol^{-1}. However, below 70 °C it is concave downwards with a slope at 55 °C corresponding to 25.8 kcal mol^{-1}. Limiting behavior is not reached on the low-temperature side. These results are explained in terms of a change in the rate-limiting step of the catalysis. At high temperature CO_2 production from reduction of Rh(III) species by CO is viewed as rate determining, while at lower temperature hydrogen production from oxidation of Rh(I) species by protons is thought to be limiting. Possible intermediates are discussed and a mechanistic scheme is proposed. A steady-state kinetic analysis and assumptions about rate-determining steps show the mechanism to be consistent with the observed behavior of the system.

Introduction

The water gas shift reaction

$$H_2O + CO = H_2 + CO_2 \qquad (1)$$

has been known and studied for over 75 years. Its significance derives from its role in the preparation of ammonia synthesis gas[1] and from its ability to increase the H_2:CO ratio in gaseous feedstock for methanation and Fischer–Tropsch synthesis.[2] Its importance for the reduction of nitrogen and sulfur oxides in the treatment of combustion exhausts has also been recognized.[3]

Thermodynamically, the reaction is favored and slightly exothermic under ambient conditions ($\Delta H°_{298} = +0.68$ kcal mol^{-1}; $\Delta G°_{298} = -4.76$ kcal mol^{-1}). But, like most reactions of this type, kinetic barriers are large and the reaction only proceeds at useful rates at elevated temperatures, even with the available catalysts. Commercial catalysts for (1) are based mainly on either Fe_3O_4 and related oxides or on copper and copper-zinc oxide mixtures.[1] Both types of systems are heterogeneous, and operate at temperatures of 300–350 and 200–250 °C, respectively. However, because the equilibrium constant for (1) exhibits a negative temperature dependence, there exist at these temperatures significant equilibrium concentrations of both reactants and products. Thus, catalysis of (1) at lower temperatures is a desirable objective.

Attempts to catalyze the shift reaction homogeneously can be traced back to studies by Hieber[4] in 1932 on iron carbonyl, carbonyl hydrides, and carbonyl hydride anions. The reaction of $Fe(CO)_5$ with aqueous base was observed to result in the oxidation of CO to CO_2 and the reduction of H^+ to coordinated hydride. Further reaction of the product CO_2 with base produced carbonate. Acidification of the $HFe(CO)_4^-$ produced resulted in the formation of an unstable dihydride which liberated hydrogen. Regeneration of the starting complex by CO addition completed the cycle, and the water gas shift reaction was achieved in principle. The problem, however, was that (2) required strong base which was consumed stoichiometrically in the catalytic cycle. Further studies[5] produced no solution to this problem until recently, when Ford and co-workers[6] were able to catalyze (1) homogeneously using $Ru_3(CO)_{12}$ in alkaline ethoxyethanol. The rates of the reaction are slow, but stoichiometric base consumption is avoided because the $KHCO_3$ produced is unstable in ethoxyethanol under the reaction conditions, and decomposes to give CO_2 and the starting base, KOH. Subsequent to Ford's initial report, he and others[7-9] have described successful catalysis of (1) using a variety of metal carbonyls and carbonyl cluster compounds in basic media. In addition Pettit and co-workers[8] and Laine[10] have observed hydroformylation with these same catalyst systems, and an earlier patent by Fenton[11] describes shift catalysis using ammonia and amines as the base under more forcing conditions (56 atm CO and 200 °C).

$$Fe(CO)_5 + 2OH^- \rightarrow HFe(CO)_4^- + HCO_3^- \qquad (2)$$

Our interest in homogeneously catalyzing the water gas shift reaction derives from several notions. First, the reaction is

0002-7863/80/1502-1020$01.00/0