Study of the Kinetics of Reduction of an Iron(III) Porphyrin by Hexaammineruthenium(II). Estimate of the Self-Exchange Rate for a High-Spin Iron Porphyrin Complex

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Abstract: The reduction of tetrakis(4-*N*-methylpyridyl)porphineiron(III) ($Fe^{III}TMpyP$) by $Ru(NH_3)_6^{2+}$ has been studied as a function of pH. The rate constant for the reduction of the aquo complex is 1.6×10^5 M^{-1} s^{-1} while, for the monohydroxo complex, the rate constant is 5.1×10^6 M^{-1} s^{-1}. These results lead to the determination of the self-exchange rate constant for the two forms as 1.2×10^6 M^{-1} s^{-1} for the former and $>10^9$ M^{-1} s^{-1} for the latter. This very large difference in self-exchange rate is suggested to correlate with a smaller Franck–Condon barrier for low-spin than for high-spin iron porphyrins.

The iron-prosthetic group plays a vital role in a variety of biological processes. Among these is the mitochondrial-based electron-transfer activity of the cytochromes as part of the oxidative phosphorylation network. The vital biological activity and ready availability of cytochrome *c* have combined to make this protein the target of considerable chemical investigation. Several of these studies have focused on the oxidation–reduction pathways of cytochrome *c* with a variety of inorganic reactants. Reports include the kinetics of reduction of ferricytochrome *c* by chromium(II),[1,2] $Fe(EDTA)^{2-}$,[3] $Ru(NH_3)_6^{2+}$,[4] and dithionite[5,6] and the oxidation of ferrocytochrome *c* by $Fe(CN)_6^{3-}$ [7] and by $Co(phen)_3^{3+}$.[8,9] For most of these systems, and especially at or near physiological pH, the electron-transfer mechanism is almost certainly of the outer-sphere type and the Marcus theory[10] has been applied with some success to the kinetic results.[11] According to this theory, for an outer-sphere electron-transfer reaction in which the decrease in free energy is not very large:

$$k_{12} = (k_{11}k_{22}K_{12})^{1/2} \quad (1)$$

where k_{12} and K_{12} are the rate and equilibrium constants, respectively, for the electron-transfer reaction and k_{11} and k_{22} are the appropriate self-exchange rate constants. The value of the self-exchange rate constant for cyt *c* has been determined[12] as 5×10^4 M^{-1} s^{-1}, a value which has been rationalized as being the product of the self-exchange rate constant for the iron porphyrin unit (estimated at $\sim 10^8$ M^{-1} s^{-1})[11] and a steric factor which corrects the above rate for the fact that the heme group occupies only a small fraction of the surface area of the cytochrome *c* molecule.[13] The estimate of $k_{11} \geq 10^8$ M^{-1} s^{-1} for iron porphyrins is derived from the $Fe(phen)_3^{2+}/Fe(phen)_3^{3+}$ system and, thus, it has been assumed that the three 1,10-phenanthroline molecules with their highly delocalized electron densities provide a ligand environment sufficiently similar to the porphyrin ligand that the self-exchange rates are comparable. An experimental justification for this approach was obtained when it was shown that the self-exchange rates for $Co(phen)_3^{2+/3+}$ and a cobalt porphyrin are virtually identical.[14] However, the validity of this assumption may well depend on whether the spin state of the metal in the model system (in this case, the trisphenanthroline complex) is the same as in the metalloporphyrin complex.

It is now widely accepted that for most iron(III) porphyrin complexes, the coordination number of the iron atom and its position relative to the porphyrin plane correlate with its spin state.[15] Thus, different inner-shell reorganization energies would be expected for high-spin iron porphyrin complexes in which the iron atom is significantly out of the porphyrin plane than for low-spin complexes in which the iron atom is coplanar with the four pyrrole-type nitrogen atoms. This difference in the Franck–Condon barriers would result in different values for the self-exchange rate constants for the two spin states. The value of $k_{11} \gtrsim 10^8$ M^{-1} s^{-1} may then be taken as an estimate of the rate constant for low-spin iron porphyrins but no model is available for the self-exchange of high-spin iron porphyrin complexes. It has previously been noted that although the free-energy change for the oxidation of ferrocytochrome *c* is less favorable by some 3 kcal/mol than the oxidation of deoxygenated hemoglobin, the reaction with $Fe(CN)_6^{3-}$ is 200 times faster for the former protein.[7,16] Although this difference presumably can be accounted for by differences between the self-exchange rate of low-spin iron (as in the cytochromes) and high-spin iron (as in deoxygenated hemoglobin and myoglobin), no direct corroboration of this suggestion has been offered.

The present report deals with this issue and involves the reduction of tetrakis(4-*N*-methylpyridyl)porphineiron(III) ($Fe^{III}TMpyP$)[17] by the rather mild, outer-sphere reducing agent, hexaammineruthenium(II) ($Ru(NH_3)_6^{2+}$). Fe^{III}-TMpyP has the advantages of being water soluble over an extensive pH range and of having its acid/base behavior well understood.[18a] The following equilibria account for the spectrophotometric titration data obtained for this metalloporphyrin:

$$H_2O + FeP(H_2O)^{5+} \rightleftarrows FeP(OH)(H_2O)^{4+} + H^+ \qquad pK_{a1} = 4.7$$

$$FeP(OH)(H_2O)^{4+} \rightleftarrows FeP(OH)_2^{3+} + H^+ \qquad pK_{a2} = 6.5 \quad (2)$$

$$2FeP(OH)_2^{3+} \rightleftarrows O\text{-}(FePOH)_2 + H_2O \qquad K_D \simeq 9 \times 10^5\ M^{-1}$$

The experiments to be described in this report were conducted at and below pH 4.5 so that the predominant metalloporphyrin species present in solution is $FeP(H_2O)^{5+}$ with some monohydroxo present as well. The aquo form of the porphyrin involves high-spin, pentacoordinated iron(III). Relaxation experiments suggest that the monohydroxo iron porphyrin exists in solution in two forms in rapid equilibrium: a high-spin pentacoordinated form and, as shown in eq 2, a low-spin hexacoordinated form.[18a]

At pH 4.5 $Fe^{III}TMpyP$ forms a low-spin diliganded adduct with imidazole, the equilibrium constant at 25 °C being:[18b]

$$Fe^{III}TMpyP + 2Im \rightleftarrows Fe^{III}TMpyP(Im)_2 \qquad \beta_2 = 2.5 \times 10^7\ M^{-2}$$

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It thus proves possible by conducting experiments on the reduction of the hexacoordinated, bisimidazole adduct of the iron(III) porphyrin by $Ru(NH_3)_6^{2+}$ to compare results obtained for the reduction of the metalloporphyrin in its high-spin and low-spin states.

Experimental Section

$[Fe^{III}TMpyP](ClO_4)_5$ was prepared, purified, and converted to the chloride form according to published procedures.[18a] Aqueous metalloporphyrin solutions were freshly prepared and protected from visible and fluorescent light.

Trifluoromethanesulfonic acid was purchased from the 3M Co., Minneapolis, Minn., and was doubly distilled. Hexaammineruthenium(III) chloride ($Ru(NH_3)_6Cl_3$) was purchased from Matthey Bishop, Inc., Malvern, Pa., and purified by literature methods.[19] Europium(III) chloride ($EuCl_3 \cdot 6H_2O$), purchased from Ventron, Alfa Products, Beverly, Mass., and all other chemicals were reagent grade and were used without further purification.

All experiments were conducted at 25 °C and the spectral and most stopped-flow experiments at an ionic strength of 0.05 M. The temperature-jump experiments and the stopped-flow experiment dealing with the bisimidazole adduct were conducted at $\mu = 0.5$ M. Potassium phthalate buffer (0.01 M) was used in all solutions with $3 < pH < 5$. All solutions were prepared with doubly distilled water.

Solutions of europium(II) were prepared by reducing europium(III) with amalgamated zinc under an argon atmosphere.[19] A carefully measured and deficient amount of the europium(II) solution was transferred to a solution of hexaammineruthenium(III) using anaerobic syringe techniques. The resulting hexaammineruthenium(II) solutions were used within 20 min to reduce the $Fe^{III}TMpyP$ solutions.

Spectral measurements were made with a Cary 14 spectrophotometer and reaction rates were determined with a Durrum Model D110 stopped-flow and a temperature-jump apparatus previously described.[20] The temperature-jump cell was modified by placing a strip of Gooch tubing over the opening so as to permit experiments to be conducted anaerobically. The cell was placed on an argon gas line for 15 to 30 min and then filled using standard syringe techniques.

Results

Spectral and Thermodynamic Considerations. Previous investigation of the solution properties of $Fe^{III}TMpyP$ permits identification of the various forms of the metalloporphyrin present in solution during the redox experiments.[18a] At pH 2.65, where 99% of the $Fe^{III}TMpyP$ is in a high-spin five-coordinate aquo form, addition of excess (25-fold or greater) $Ru(NH_3)_6^{2+}$ results in a red shift of the Soret and visible bands: from 401 to 444 nm and from 520 to 560 nm, respectively. These results correlate well with those obtained earlier in which the reduction was effected electrochemically.[21] For a fixed metalloporphyrin concentration, the intensity of the 444-nm Soret band increases with increasing $Ru(NH_3)_6^{2+}$, indicating the reaction does not go to completion under the conditions of these experiments. An isosbestic point was observed for the aquo forms of $Fe^{II}TMpyP$ and $Fe^{III}TMpyP$ at 422 nm. A value of $\epsilon_{1/2}^0 = -0.081$ V was extrapolated from the data of Meyer and Taube[22] for the reaction $Ru(NH_3)_6^{2+} \rightleftarrows e^- + Ru(NH_3)_6^{3+}$ at an ionic strength of 0.05 M while $\epsilon_{1/2}^0 = +0.15$ V[21] for $FeP(H_2O)^{5+} + e^- \rightleftarrows FeP(H_2O)^{4+}$. The calculated equilibrium constant for the reaction $FeP(H_2O)^{5+} + Ru(NH_3)_6^{2+} \rightleftarrows FeP(H_2O)^{4+} + Ru(NH_3)_6^{3+}$ is:

$$K_{FeP(H_2O)} = [FeP(H_2O)^{4+}][Ru(NH_3)_6^{3+}]/[FeP(H_2O)^{5+}][Ru(NH_3)_6^{2+}] = 15$$

Spectral experiments for the reduction of $Fe^{III}TMpyP$ by excess $Ru(NH_3)_6^{2+}$ were conducted in a pH range from 2.65 to 4.50. At this highest pH the iron porphyrin is approximately 60% in the aquo and 40% in the hydroxo form. Yet the spectral properties of the reduction product are identical throughout the entire pH region, indicating that the stable product is $FeP(H_2O)^{4+}$. These spectral results suggest that $FeP(H_2O)^{4+}$ is a weaker acid than $Fe(H_2O)^{5+}$ and, therefore, although $\epsilon_{1/2}^0$ is known only for the reduction of $FeP(H_2O)^{5+}$, it seems certain that $\epsilon_{1/2}^0$ for the reduction of $FeP(OH)(H_2O)^{4+}$ will be less favorable, i.e., $< +0.15$ V.[21]

In the presence of sufficient imidazole at pH 4.5, the diliganded, low-spin complex $FeP(Im)_2^{5+}$ is formed.[18b] This metalloporphyrin complex has absorption maxima at 422, 515, and 635 nm. Reduction of the imidazole adduct of $Fe^{III}TMpyP$ by excess $Ru(NH_3)_6^{2+}$ yields a product having a broad Soret centered at about 445 nm and visible absorption bands at 543 and 570 nm. The stability constant for $FeP(Im)_2^{4+}$ has not been measured, but it is known from other systems that stability constants for imidazole ligation are larger for the iron(III) than for the iron(II) derivative of a given porphyrin.[23] We anticipate that $\epsilon_{1/2}^0 < +0.15$ V for $FeP(Im)_2^{5+}$ and, thus, for the reduction of $Fe^{III}TMpyP$ species by $Ru(NH_3)_6^{2+}$, the equilibrium constants are related by $K_{FeP(H_2O)} > K_{FeP(OH)(H_2O)}, K_{FeP(Im)_2}$.

Treatment of $Fe^{III}TMpyP$ by $Ru(NH_3)_6^{2+}$ at pH 9, where the porphyrin is primarily in the dimer form,[18a] results in no reaction. Apparently $Ru(NH_3)_6^{2+}$ is not a strong enough reducing agent to reduce the dimer. This is consistent with results obtained by Wilson and Neri that $\epsilon_{1/2}^0 \simeq 0.02$ V for the dimer form of the porphyrin.[21]

When oxygen is bubbled through reduced metalloporphyrin solutions within 20 min of reduction, the Soret spectrum returns to its original position and profile. At low pH (~2) there is essentially no loss of intensity, but at higher pHs the Soret becomes broadened and shows a decrease in intensity. When the reduced metalloporphyrin is allowed to remain unoxygenated for longer periods of time (30–60 min) in the presence of excess reducing agent, the Soret band further broadens and decreases in intensity, the reaction being somewhat more rapid at the higher pHs. These spectral effects are probably due to the slow attack on the porphyrin ring system itself, catalyzed by hydroxide, following the initial, reversible reduction of the metal center.

Kinetics. Stopped-flow kinetics experiments for the reactions of $Fe^{III}TMpyP$ with excess $Ru(NH_3)_6^{2+}$ show a multiphasic kinetic pattern in the pH range from 2 to 4. The several kinetic effects are sufficiently different in rate that they can be analyzed independently; both the Guggenheim[24] and Swinebourne[25] methods were used for this purpose. The first, fastest phase involves most of the color change observed in the stopped-flow experiments and can be ascribed to the reduction of the metal center. This assignment is made on the basis of the following evidence. (1) It is only the first step which shows a dependence on the $Ru(NH_3)_6^{2+}$; the slower phases for which $k_{obsd}^{slow} \simeq 1\text{–}10\ s^{-1}$ show little if any dependence on the concentration of the reducing agent. (2) The change in absorbance at various wavelengths for the fastest effect correlates with the spectral experiments described earlier. Unlike the fastest process, the slower processes have wavelength dependence different from those obtained in the static experiments. The main difference between the static and stopped-flow experiments is the lower level of oxygen achievable for the former. It is well known that iron(II) porphyrins are extremely sensitive to oxygen and we believe that the slower processes observed in the kinetic experiments are related to higher oxygen levels on the stopped-flow apparatus. When $Fe^{III}TMpyP$ solutions not previously deoxygenated were used for reduction experiments on the stopped-flow, the magnitude of the slow effects increased relative to the initial fast reduction step. The half-life of the fast effect remained the same but that of the slower step decreased significantly. (3) Kinetics experiments conducted on the temperature-jump apparatus where lower levels of oxygen can be maintained show only one uncoupled relaxation effect. This effect was in the time range of the fast

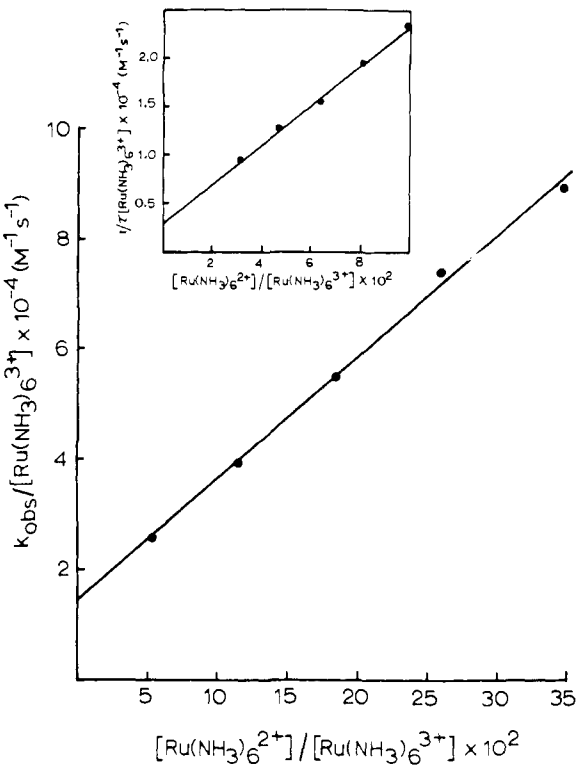

Figure 1. A plot of $k_{obsd}/[Ru(NH_3)_6^{3+}]$ vs. $[Ru(NH_3)_6^{2+}]/[Ru(NH_3)_6^{3+}]$ at pH 2.65 using stopped-flow results. The resulting rate constants are $k_f = 2.2 \times 10^5$ M^{-1} s^{-1} and $k_r = 1.5 \times 10^4$ M^{-1} s^{-1}. The insert shows temperature-jump results at the same pH yielding $k_f = 1.8 \times 10^5$ M^{-1} s^{-1}.

effect obtained with the stopped-flow. The relaxation time is independent of wavelength but the direction of the effect is opposite at 401 nm (the reactant peak) from the one obtained at 444 nm (the product peak). (4) The rate of the fastest effect obtained with the stopped-flow is independent of wavelength. The rate of the reaction determined from the rate of disappearance of the reactant peak (401 nm) is the same as that determined from the appearance of the product peak (444 nm). If the fast effect leads to an intermediate which reacts slowly to form the final product, the rates measured at the two wavelengths would be different. (5) The fast effect was no longer observed when kinetic experiments were conducted at the isosbestic point between reactant and product determined from spectral experiments. The slower effects were still observable. (6) Lastly, as will be discussed later, the equilibrium constant obtained from the kinetic experiments for the fast effect is in good agreement with that calculated from electrochemical experiments.

An expression for the observed rate constant, k_{obsd}, for the reversible reduction of $Fe^{III}TMpyP$ (eq 3) in the presence of excess $Ru(NH_3)_6^{2+}$ and $Ru(NH_3)_6^{3+}$ is given by eq 4.

$$Fe^{III}TMpyP + Ru(NH_3)_6^{2+} \underset{k_r}{\overset{k_f}{\rightleftharpoons}} Fe^{II}TMpyP + Ru(NH_3)_6^{3+} \quad (3)$$

$$k_{obsd} = k_f[Ru(NH_3)_6^{2+}] + k_r[Ru(NH_3)_6^{3+}] \quad (4)$$

A plot of $k_{obsd}/[Ru(NH_3)_6^{3+}]$ vs. $[Ru(NH_3)_6^{2+}]/[Ru(NH_3)_6^{3+}]$ is shown in Figure 1 for the experiments conducted at pH 2.65 on the stopped-flow. The insert in the figure shows the results of the temperature-jump experiments at the same pH. The higher $Ru(NH_3)_6^{2+}$ concentrations required to obtain relaxation effects in a convenient time range make the value of the intercept less precise for the temperature-jump experiments than for the stopped-flow experiments. The slopes

Table I. Kinetic Results for the Reduction of $Fe^{III}TMpyP$ by $Ru(NH_3)_6^{2+}$ (25 °C; μ = 0.05 M)

pH	k_f, M^{-1} s^{-1}	k_r, M^{-1} s^{-1}	$K_{kinetic}$
2.65	2.2×10^5	1.5×10^4	15
3.15	2.9×10^5	7.4×10^3	39
3.62	4.4×10^5	2.03×10^4	22
3.80	7.9×10^5	3.50×10^4	23
3.97	1.0×10^6	4.22×10^4	24
4.04	9.6×10^5	5.40×10^4	18

of the lines give values for k_f at pH 2.65. From the stopped-flow experiments $k_f = 2.2 \times 10^5$ M^{-1} s^{-1} while for the temperature-jump experiments $k_f = 1.8 \times 10^5$ M^{-1} s^{-1}. Temperature-jump experiments at pH 4 showed relaxation effects having times in fair agreement with the results of the fast effect of the stopped-flow experiments at that pH. A limiting aspect in the precision and reproducibility of the temperature-jump data at this higher pH is the necessity of incubating the metalloporphyrin with the reducing agent for extended periods before the relaxation experiments can be performed. As discussed in the section on Spectral and Thermodynamic Considerations, extended contact of the reagents at high pH leads to changes in the porphyrin spectrum.

Plots of the stopped-flow results of $k_{obsd}/[Ru(NH_3)_6^{3+}]$ vs. $[Ru(NH_3)_6^{2+}]/[Ru(NH_3)_6^{3+}]$ lead to determinations of k_f and k_r at each pH. From these results, kinetically determined values for the equilibrium constants, $K = k_f/k_r$, for reaction 4 are obtained and these values are listed in Table I. The equilibrium constants are independent of pH over the range studied and average to a value of 23 in good agreement with the value of 15 obtained from extrapolations of electrochemical data.

The rate constants, k_f and k_r, show a pH dependence interpreted as arising from two forms of the porphyrin, $FeP(H_2O)^{5+}$ and the $FeP(OH)(H_2O)^{4+}$. Using standard kinetic techniques we obtain that:

$$k_f = \frac{k_1 + k_2K_{a1}/[H^+]}{1 + K_{a1}/[H^+]}$$

$$k_r = \frac{k_{-1} + k_{-2}K_{a1}'/[H^+]}{1 + K_{a1}'/[H^+]} \quad (5)$$

where k_1 and k_2 are the forward rate constants for the reduction of $FeP(H_2O)^{5+}$ and $FeP(OH)(H_2O)^{4+}$, respectively, and K_{a1} is the acid dissociation constant of $FeP(H_2O)^{5+}$, equal to 2.0×10^{-5} under the conditions of these experiments.[18a] In the expression for k_r, k_{-1} and k_{-2} are the rate constants for the oxidation of the $FeP(H_2O)^{4+}$ and $FeP(OH)(H_2O)^{3+}$, respectively, and $K_{a1}' = [FeP(OH)(H_2O)^{3+}][H^+]/[FeP(H_2O)^{4+}]$. A plot of $k_f(1 + K_{a1}/[H^+])$ vs. $1/[H^+]$ yields a straight line whose slope $k_2K_{a1} = 1.0 \times 10^2$ s^{-1} and intercept $k_1 = 1.6 \times 10^5$ M^{-1} s^{-1} (cf. Figure 2). From the known value of K_{a1} we obtain that $k_2 = 5.1 \times 10^6$ M^{-1} s^{-1}. This type of analysis was not possible for k_r because the value for K_{a1}' is not known. However, by assuming that $K_{a1}'/[H^+] \ll 1$ and plotting k_r vs. $1/[H^+]$, we obtain that $k_{-1} = 4.5 \times 10^3$ M^{-1} s^{-1} and $k_{-2}K_{a1}' = 4.3$ s^{-1} (cf. Figure 3).

A study of the reduction of $FeP(Im)_2^{5+}$ by $Ru(NH_3)_6^{2+}$ at pH 4.5 was attempted using both temperature-jump and stopped-flow techniques. Relaxation data were obtained but were not reproducible, quite likely for reasons discussed earlier dealing with prolonged contact of metalloporphyrin and reducing agent at high pH. The stopped-flow experiments showed a large color change within or faster than the mixing time of the apparatus. Thus, for the conditions, $[Im]_{total} = 0.25$, $[Ru(NH_3)_6^{2+}] = 1.2 \times 10^{-3}$, $[Ru(NH_3)_6^{3+}] = 1.2 \times 10^{-3}$, and $[FeTMpyP]_{total} = 2.7 \times 10^{-5}$ (pH 4.5; $\epsilon_{1/2} \leq 1$ ms).

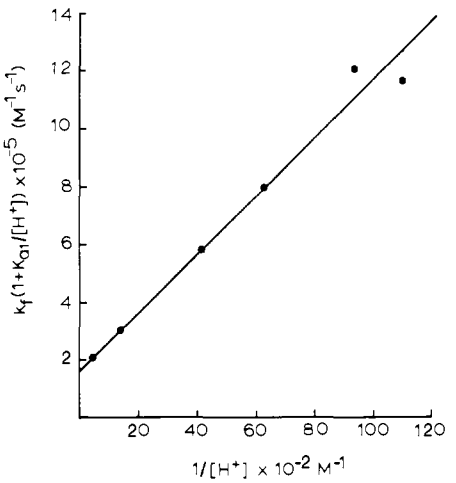

Figure 2. A plot of $k_f(1 + K_{a1}/[H^+])$ vs. $1/[H^+]$ from which we obtain that $k_1 = 1.6 \times 10^5$ M^{-1} s^{-1} and $k_2 = 5.1 \times 10^6$ M^{-1} s^{-1}.

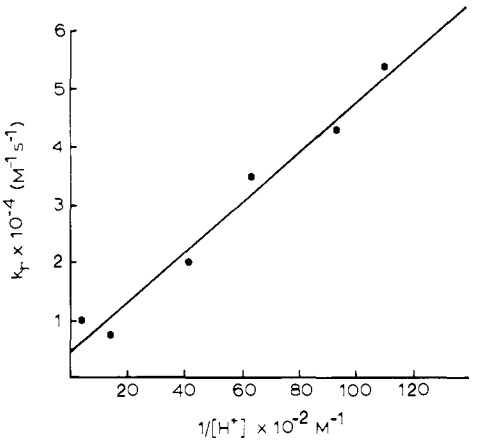

Figure 3. A plot of k_r vs. $1/[H^+]$ from which we obtain that $k_{-1} = 4.5 \times 10^3$ M^{-1} s^{-1} and $k_{-2}K_{a1}' = 4.3$ s^{-1}.

The rate constant for this redox reaction may be estimated as $k_{obsd} \geq 700$ s^{-1}.

Discussion

Electron transfer to metalloporphyrins can occur through axial ligands or through the porphyrin ring system with the preferred pathway quite likely dependent upon the nature of the axial ligands as well as on the metalloporphyrin and reducing agent. It has been suggested[11] that for electron-transfer processes proceeding via the porphyrin π system, the self-exchange rate for iron porphyrins may be approximated by a value derived from the $Fe(phen)_3^{2+/3+}$ system provided that the spin state of the metal is the same in both complexes. The hypothesis has been confirmed for CoTMpyP; the self-exchange rate constant for $Co(phen)_3^{2+/3+}$ is 21 M^{-1} s^{-1} [8,27] and a value of 20 M^{-1} s^{-1} was determined for the cobalt porphyrin complex.[14]

In the present study we considered the reaction of the low-spin iron(III) complex, $FeP(Im)_2^{5+}$, by $Ru(NH_3)_6^{2+}$ and concluded from the spectral results that under the conditions of our experiments a reduction of the metalloporphyrin does indeed take place. However, the reduction reaction is so rapid as to fall outside the stopped-flow range. If we consider $k_{obsd} \geq 700$ s^{-1} for the conditions described in the Results section, and knowing that $K_{FeP(Im)_2} < 15$ and that the self-exchange rate constant for $Ru(NH_3)_6^{2+/3+}$ is 8.2×10^2 M^{-1} s^{-1},[22] we calculate from the Marcus theory that $k_{11} \geq 10^7$ M^{-1} s^{-1} for low-spin iron in a porphyrin environment. This value is consistent with the estimate obtained from the $Fe(phen)_3^{2+/3+}$ system of $\geq 10^8$ M^{-1} s^{-1}.[11]

A better estimate for this self-exchange rate constant can be obtained from the results of the reduction of $FeP(OH)(H_2O)^{4+}$ by $Ru(NH_3)_6^{2+}$. Although $K_{FeP(H_2O)} > K_{FeP(OH)(H_2O)}$, the reaction of $FeP(OH)(H_2O)^{4+}$ with $Ru(NH_3)_6^{2+}$ is more than ten times faster than the reaction of $FeP(H_2O)^{5+}$:

$$FeP(H_2O)^{5+} + Ru(NH_3)_6^{2+} \xrightarrow{1.6 \times 10^5} FeP(H_2O)^{4+} + Ru(NH_3)_6^{3+}$$

$$FeP(OH)(H_2O)^{4+} + Ru(NH_3)_6^{2+} \xrightarrow{5.1 \times 10^6} FeP(OH)(H_2O)^{3+} + Ru(NH_3)_6^{3+}$$

$$FeP(OH)(H_2O)^{3+} \xrightarrow{+H^+ \,\|\, -H^+,\ \text{v. fast}} FeP(H_2O)^{4+}$$

On application of the Marcus theory we find that the self-exchange rate constant for $FeP(OH)(H_2O)^{3+/4+}$ is at least three orders of magnitude greater than that for $FeP(H_2O)^{4+/5+}$. We obtain that $k_{11} > 1 \times 10^9$ M^{-1} s^{-1} for $FeP(OH)(H_2O)^{3+/4+}$ and $k_{11} = 1.2 \times 10^6$ M^{-1} s^{-1} for $FeP(H_2O)^{4+/5+}$. We take the former value as being characteristic of a low-spin iron porphyrin complex and the latter value as that applicable to high-spin iron porphyrins.[18a] Substantial variations in self-exchange rate constants have been shown to occur for given metalloproteins when reacted with a variety of reducing agents.[28] This variability is dependent on the accessibility of the protein redox center to a given reagent. While this effect should not be a major factor in the simpler model system discussed here, it should be noted that the present estimate of the self-exchange rate constant for a high-spin iron porphyrin is based on a single cross-reaction and further studies with other reagents might change the value somewhat.

Thus, we conclude that there is essentially no Franck-Condon barrier to the self-exchange of low-spin iron porphyrin complexes in which the iron atom remains in the plane of the rather rigid porphyrin ligand; the process is near or at the diffusion-controlled limit. On the other hand, although the self-exchange of high-spin iron porphyrin complexes is about six orders of magnitude faster than for high-spin $Fe(H_2O)_6^{2+/3+}$,[29] there is still an appreciable free-energy barrier to the electron transfer taking place. This may reflect differences in the position of the iron atom relative to the porphyrin plane in the 2+ and 3+ oxidation states[15] which gives rise to a substantial inner-shell reorganization energy and hence to a significant activation energy barrier. Other factors being equal, we would anticipate reduction of low-spin iron porphyrin complexes to be from 30 to about 100 times more rapid than the reduction of high-spin iron porphyrin complexes.

Although this analysis is an oversimplification when applied to protein complexes because of differences in steric correction terms[11] and conformational requirements for electron transfer,[28,30] it does satisfactorily account if only qualitatively for the fact that the oxidation of ferrocytochrome *c* by $Fe(CN)_6^{3-}$ is a factor of 230 times faster than the oxidation of a heme group of deoxygenated hemoglobin by the same oxidizing agent, although free-energy considerations favor the oxidation of hemoglobin. We suggest that a major contribution to this difference in rate arises from the different spin states of the iron atom in the two metalloporphyrin-protein complexes and consequently the difference in the Franck-Condon barrier to oxidation.

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(17) FeTMpyP adds one or more axial ligands. When the ligand(s) are neutral, the charge is 5+ as in, for example, $FeP(H_2O)^{5+}$.
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Communications to the Editor

[5-Aspartic acid]-oxytocin: First 5-Position Neurohypophyseal Hormone Analogue Possessing Significant Biological Activity[1]

Sir:

After 25 years of extensive structure-activity studies,[2] starting with the original synthesis of oxytocin,[3] the first 5-position neurohypophyseal hormone analogue to retain a high degree of potency in the oxytocin-like activities is reported. The asparagine residue of oxytocin has been replaced by aspartic acid to yield [5-aspartic acid]-oxytocin.

Early structure-function correlations with oxytocin and vasopressin demonstrated that chemical modifications of the asparagine residue, present in position 5 of all nine naturally occurring neurohypophyseal peptides,[4] virtually abolished biological activity (see ref 5 for a recent review) and, in fact, it was not possible with analogues of such low specific activities to determine dose-response relationships.[6] The preferred solution conformation of oxytocin[7] offered an explanation of these results by assigning to the asparagine residue not only a key role in maintaining the preferred three-dimensional backbone structure, but, moreover, by assigning to the asparagine side chain an important function in the biologically "active site" of the hydrophylic surface of the hormone.[8,9] On the basis of this model it was speculated that an aspartic acid residue in position 5 could retain much of the hydrophilicity, appropriate steric requirements, and hydrogen-bonding capabilities of the asparagine residue (Figure 1).

The synthesis of [5-aspartic acid]-oxytocin has been achieved by stepwise solution techniques beginning with Z-Cys(Bzl)-Pro-Leu-Gly-NH_2[10] (2.5 g, 4.0 mmol). The benzyloxycarbonyl (Z) group was removed by treatment with 2 M HBr/AcOH and Boc-Asp(OBzl)-OH was coupled with dicyclohexylcarbodiimide[11] (DCC) mediated by 1-hydroxybenzotriazole (HBT)[12] in dimethylformamide/glyme (1:1). In this and succeeding steps the *tert*-butyloxycarbonyl (Boc) group was removed by CF_3CO_2H; glutamine was incorporated as Boc-Gln-ONp; Boc-Ile-OH, Boc-Tyr(Bzl)-OH, and Z-Cys(Bzl)-OH were coupled by preactivating a solution of the amino acid in glyme with DCC (1 equiv) in the presence of HBT (2 equiv). Completeness of coupling reactions was monitored by the semiquantitative ninhydrin test.[13] Each protected peptide intermediate was isolated and characterized by melting point, optical rotation, thin-layer chromatography (TLC), and elemental or amino acid analysis; the synthesis yielded 1.5 g (0.98 mmol) of Z-Cys(Bzl)-Tyr(Bzl)-Ile-Gln-Asp(OBzl)-Cys(Bzl)-Pro-Leu-Gly-NH_2.[14] All of the protecting groups were simultaneously removed by adding the nonapeptide (307 mg, 0.20 mmol) to a solution of Na in liquid NH_3;[15] cyclization of the dithiol intermediate was accomplished by oxidative disulfide bond formation with ICH_2CH_2I.[16] The product was purified by gel filtration on Sephadex G-15 (fine) in 50% AcOH and by partition chromatography[17] on Sephadex G-25 (block polymerisate, 100-200 mesh) in the system 1-BuOH/C_6H_6/H_2O containing 1.5% pyridine and 3.5% AcOH (6:1:7). Lyophilization gave 76 mg of [5-aspartic acid]-oxytocin: $[\alpha]^{25}_D$ − 14° (*c* 0.47, 1

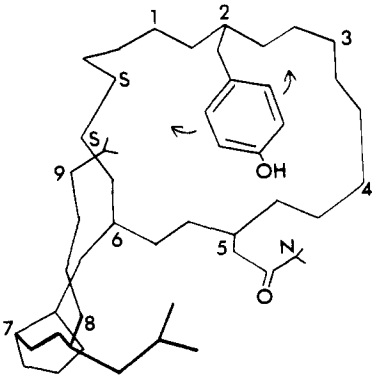

Figure 1. Schematic representation of the conformation of oxytocin thought to be optimal for the interaction of the hormone with the uterine smooth muscle receptor. Residues in positions 3, 4, 7, and 8, which are thought to contain "binding elements", are located at the corner position of the two β turns proposed for the hormone. The "active elements" are the hydroxy group of Tyr^2 and the carboxamide group of Asn^5. For details see ref 9.

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