Intramolecular Hydrogen-Bonding from Heme Carboxylic Acid Side Chains to Axial Ligands

Kyoungja Woo and D. A. Sweigart*

Department of Chemistry, Brown University, Providence, Rhode Island 02912

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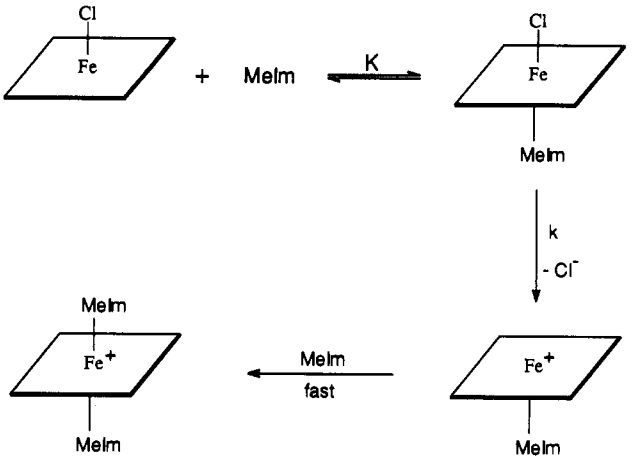
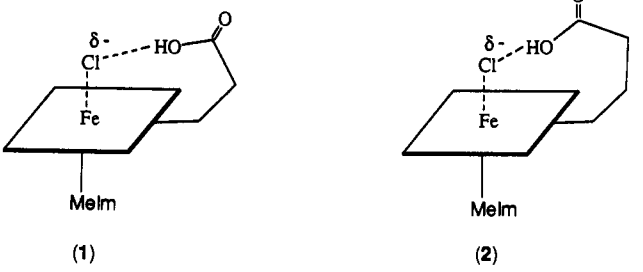
Hydrogen-bonding to or from the axial ligands of the iron porphyrin moiety is known to play an important mechanistic role in reactions mediated by heme proteins. Several types of such interactions have been discussed. "Proximal" type H-bonding involves an interaction between the N–H group of a coordinated imidazole (histidine) and a basic site in the protein and has been proposed as a means by which the protein regulates ligand binding and reactivity, redox potentials, and electron-transfer rates.[1,2] Such effects have been observed with simple metalloporphyrin model systems.[3-6] "Distal" type H-bonding refers to an external group acting as the H-donor to an axially coordinated ligand. With oxyhemoglobin, oxymyoglobin, and appropriate model hemes, there is strong evidence that H-bonding from a distal imidazole to the O_2 ligand significantly influences oxygen-binding thermodynamics and kinetics, and possibly CO/O_2 affinity ratios.[7-10] An analogous interaction in peroxidase enzymes may facilitate the heterolytic cleavage of the peroxide O–O bond to generate Compound I.[1,3,11]

Another type of H-bonding akin to the distal type mentioned above involves the heme propionic acid side chains. The propionic acid groups can have pK_a values from 4.5 to greater than 9, depending on their locations in the protein (exposed to solvent or buried).[12] It has been suggested[13] that variation of the pK_a with metal oxidation state is a mechanism for redox potential modulation in monoheme cytochromes; the interaction affecting the redox potential may be purely electrostatic or it may involve H-bonding *from* $-CO_2H$ or *to* $^-CO_2^-$, depending on the pK_a. Cleavage of the O–O bond in peracids coordinated to model hemes is catalyzed by an H-bonding interaction between a heme propionate group and the axial ligand.[3]

Scheme I



The kinetics of the reaction of the five-coordinate complex Fe(porphyrin)Cl with *N*-methylimidazole (MeIm) has been studied with a variety of porphyrins and proceeds according to Scheme I.[14,15] In this mechanism MeIm adds rapidly and reversibly to the open coordination site in Fe(porphyrin)Cl to give Fe(porphyrin)(MeIm)Cl, which undergoes rate-limiting chloride ionization. The ionization step in acetone solvent was found to be accelerated by the presence of low concentrations of H-bonders (imidazole, ethanol, phenol, water, chloroform, etc.), and this was ascribed to stabilization of the transition state by H-bonding to the departing chloride, $Fe\cdots Cl^{\delta-}\cdots H^{\delta+}—X$. Interestingly, it was found that the protoporphyrin IX complex, shown in Figure 1 as Fe(pro)Cl, reacts at a much greater rate than the corresponding dimethyl ester, Fe(prodme)Cl, and it was suggested[15] that this may be due to H-bonding from the propionic acid groups in Fe(pro)(MeIm)Cl, as depicted in structure **1**. If



true, this demonstrates that the propionic acid groups can significantly influence axial ligand reactivity. Molecular models show that the H-bonding in structure **1** would have to be weak because the Cl···O distance is restricted by the size of the porphyrin core to be at least 4 Å. However, an interaction of only ca. 6 kJ is required for a 10-fold change in a rate or equilibrium constant and this is easily within the realm of such long-range H-bonding.[9]

In order to test our hypothesis of H-bonding from a propionic acid side chain to the axial ligand in **1**, we studied the reaction of MeIm with the iron porphyrin having butyric acid groups (see Figure 1). The longer side chains in Fe(but)Cl compared to

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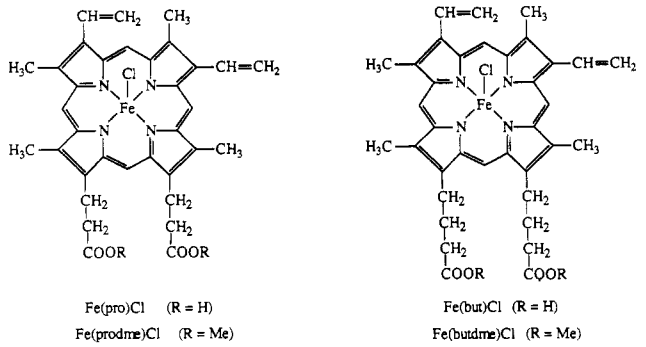

Figure 1. Structures of complexes studied.

Fe(pro)Cl should allow a stronger H-bonding interaction (structure **2**) and hence an increased chloride ionization rate. The corresponding esters, Fe(prodme)Cl and Fe(butdme)Cl, would be expected to react at similar rates. These expectations were realized, as described below.

Experimental Section

MeIm was distilled from KOH pellets under reduced pressure and stored over molecular sieves. Hemin chloride, Fe(pro)Cl, was purchased from Eastman Kodak. {Fe(prodme)}$_2$O was made from hemin chloride by standard methods[16] and converted to Fe(prodme)Cl by bubbling dry HCl through a methylene chloride solution and evaporating to dryness. The Grinstein method[16] was used to obtain metal-free protoporphyrin IX dimethyl ester from hemin chloride, and the methyl propionate side chains were then converted to methyl butyrate groups as described in the literature.[17] Some of this product was converted to the porphyrin with butyric acid side chains by base-catalyzed hydrolysis.[16] Insertion of iron by the ferrous sulfate method[16,18] yielded the complexes Fe(but)Cl and Fe(butdme)Cl, which have optical spectra virtually identical to those of the propionic acid and methyl propionate analogues.

Kinetic experiments were done in acetone at 25 °C with a Dionex stopped-flow spectrophotometer. Pseudo-first-order conditions were used, with the iron porphyrin complex at ca. 1.0×10^{-5} M and MeIm in at least 1000-fold excess. HPLC grade acetone was carefully dried immediately prior to use as previously described.[14a] All reactant solutions were prepared inside an inert-atmosphere glovebox so as to exclude adventitious water. Solutions were wrapped in aluminum foil to minimize exposure to light. Reproducibility was checked by repeating each kinetics experiment at least three times (on different days).

Results and Discussion

Our previous studies[14,15] with a variety of Fe(porphyrin)Cl complexes, including Fe(pro)Cl and Fe(prodme)Cl, provide very strong support for the validity of the mechanism in Scheme I. In the present study, the reaction of MeIm with each of the four complexes in Figure 1 was found to be cleanly first order in iron porphyrin, with the observed rate constant given by eq 1. Plots

$$k_{obs} = \frac{kK[\text{MeIm}]}{1 + K[\text{MeIm}]} \qquad (1)$$

of k_{obs} versus [MeIm] were nonlinear, as illustrated in Figure 2 for Fe(pro)Cl and Fe(but)Cl. The equilibrium constant (K) and the chloride ionization rate constant (k) were obtained from linear plots of $1/k_{obs}$ versus $1/[\text{MeIm}]$. The constant k is also available from the directly observed limiting value of k_{obs} at high MeIm concentration. A summary of the results is given in Table I.

Before a discussion of these data, it must be noted that previous work established[15] that the carboxylic acid side chains in Fe(pro)Cl are not deprotonated by MeIm in acetone solvent. With this in mind, we ascribe the larger values of both k and K found for

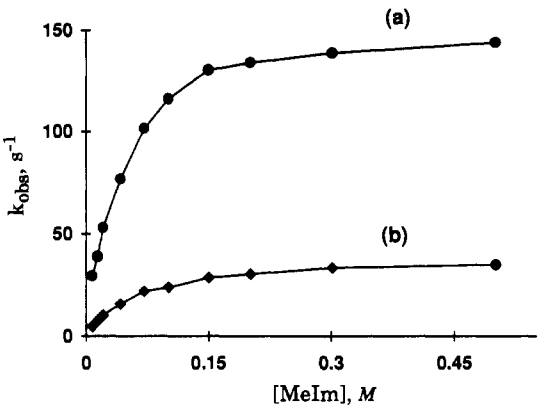

Figure 2. Plot of k_{obs} versus MeIm concentration for (a) Fe(but)Cl and (b) Fe(pro)Cl in acetone at 25 °C.

Table I. Rate Data for the Reaction of Fe(porphyrin)Cl with MeIm in Acetone at 25 °C[a]

complex	k, s^{-1}	K, M^{-1}	kK, M^{-1} s^{-1}
Fe(pro)Cl	36 ± 2	18 ± 1.8	650 ● 30
Fe(but)Cl	150 ± 10	25 ± 2.5	3750 ± 200
Fe(prodme)Cl	7.8 ± 1.2	2.0 ● 0.5	16 ± 2
Fe(butdme)Cl	10 ± 1.5	2.1 ± 0.5	21 ± 2

[a] See Scheme I for the mechanism.

Fe(pro)(MeIm)Cl and Fe(but)(MeIm)Cl compared to their corresponding esters as due to an H-bonding interaction with the axial chloride ligand (and possibly the pyrrole nitrogen of the MeIm ligand). The interaction must be intramolecular and not intermolecular because the concentration of metal complex was far too low to support significant association;[19] in agreement with this conclusion, k_{obs} was found to be invariant as the iron porphyrin concentration was varied over a factor of 5. Precedence for intramolecular H-bonding involving chloride can be found in the thermodynamic and spectral properties of *o*-chlorophenol, which clearly indicate an interaction of this type between the –OH and –Cl substituents.[19,20] It is most unlikely that the rate enhancements observed when the porphyrin substituents are changed from –COOMe to –COOH are due to a simple inductive effect; this view is supported by the lack of substantial substituent effects seen[14] with Fe(prodme)Cl and Fe(TPP)Cl, which react at similar rates yet have very different porphyrin substituents (TPP is the dianion of tetraphenylporphyrin).

The significant new result in this study is the observation that the acid Fe(but)Cl is much more reactive than Fe(pro)Cl, with the rate constant k greater by a factor of 4.2. Clearly, this cannot be ascribed to an inductive effect exerted by the extra methylene group in Fe(but)Cl compared to Fe(pro)Cl since the ester analogues Fe(butdme)Cl and Fe(prodme)Cl react at the same rate (Table I). We suggest that it is due to stronger H-bonding to the departing chloride in the transition state (structure **2** versus **1**) made possible by the greater carboxylic acid chain length in Fe(but)(MeIm)Cl. The two ester complexes cannot undergo H-bonding, and as expected, they react at similar rates. Acceleration of chloride ionization *via* H-bonding has precedent in organic and inorganic chemistry. Thus, H-bonding to the departing chloride is believed to be the major factor determining the *large* rate dependence on solvent of chloride ionization from *tert*-butyl chloride[21] and *cis*-[Pt(PEt$_3$)$_2$(*m*-MeC$_6$H$_4$)Cl].[22] Interestingly, we previously found[14] that the solvent dependence of chloride ionization from Fe(TPP)(MeIm)Cl is almost identical to that

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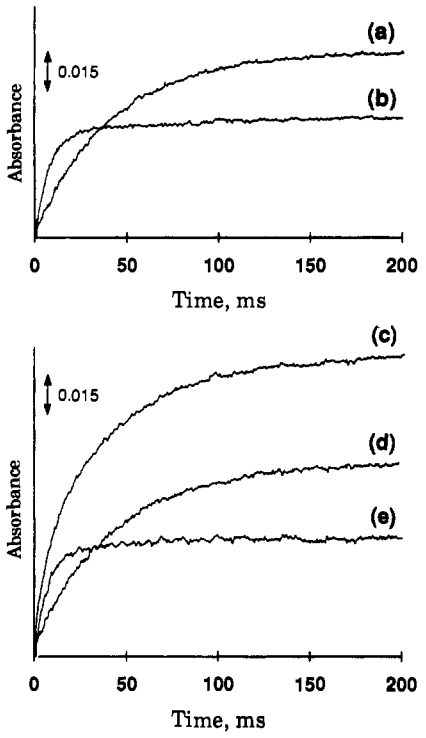

Figure 3. Absorbance versus time plots in acetone at 25 °C with [MeIm] = 0.10 M and λ = 450 nm. Top graph: (a) [Fe(pro)Cl] = 1.5×10^{-5} M; (b) [Fe(but)Cl] = 1.0×10^{-5} M. Bottom graph: (c) a mixture containing Fe(pro)Cl at 1.5×10^{-5} M and Fe(but)Cl at 1.0×10^{-5} M; (d) curve c minus curve b; (e) curve c minus curve a. All curves are set to a common absorbance origin (time = 0).

for the solvolysis of *tert*-butyl chloride, as reflected by a linear dependence of ln(k) on Gutmann acceptor numbers[23] (and hence H-bonding). The H-bonding interaction proposed in the present study, the reaction of MeIm with Fe(pro)Cl and Fe(but)Cl, is neither as large nor as simple as that observed in the solvent variation studies mentioned above because there is undoubtedly *competition* with H-bonding of the carboxylic acid side chains to solvent acetone and nucleophile MeIm as well as with intramolecular dimerization of the two side chains. Nevertheless, the present study allows one to infer that H-bonding from carboxylic acid side chains can significantly influence axial-ligand reactivity.

The reaction of MeIm with Fe(porphyrin)Cl complexes is very sensitive to impurities that can H-bond, and we considered the possibility that a trace protic impurity in Fe(but)Cl was responsible for the (reproducible) greater reactivity of Fe(but)Cl over Fe(pro)Cl. This, however, was ruled out by the observation that the reaction of MeIm with a 2:3 mixture of Fe(but)Cl and Fe(pro)Cl gave biphasic behavior that was virtually the exact superposition of the individual independent absorbance/time responses for the two complexes, as shown in Figure 3. We conclude that the observed rate enhancement is indeed due to intramolecular H-bonding from the carboxylic acid side chains. In terms of the activation free energy of the rate constant k, chloride ionization is easier from Fe(but)(MeIm)Cl compared to Fe(pro)(MeIm)Cl by 3.5 kJ. This figure is proposed to reflect *additional* H-bonding possible with the less sterically constrained Fe(but)(MeIm)Cl. Relative to that of Fe(butdme)(MeIm)Cl, the decrease in activation free energy for Fe(but)(MeIm)Cl is 6.7 kJ, which is an absolute measure of the H-bonding interaction and is similar to the estimate of the H-bond strength between the distal histidine and bound oxygen in oxymyoglobin and in model complexes.[9,10]

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