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# 2-Methylimidazole Does Not Bind to (Octaethylporphinato)iron(III) Chloride in the Presence of Methanol: A Resonance Raman Study

Tadayuki Uno,*[1a] Keiichiro Hatano,[1b] and
Yoshifumi Nishimura[1c]

*Faculty of Pharmaceutical Sciences*
*University of Tokushima*
*Shomachi, Tokushima 770, Japan*
*Faculty of Pharmaceutical Sciences*
*Nagoya City University*
*Tanabedori, Mizuho-ku, Nagoya 467, Japan*
*Graduate School of Integrated Science*
*Yokohama City University*
*Seto, Kanazawa-ku, Yokohama 236, Japan*

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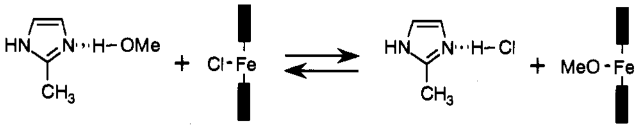
In many heme proteins, the heme moiety is bound to the protein through the nitrogen atom of a histidyl imidazole. To elucidate the mode of action of the iron porphyrin center in the heme proteins, many studies have been done on the imidazole complexes of iron porphyrins.[2–12] The earlier work by Walker's group[3] has revealed that two imidazole bases coordinate to the iron simultaneously and/or in a stepwise manner and the bis-ligated complex is an ultimate product. In studying the binding property of 2-MeImH[13] to Fe(OEP)(Cl) in $CH_2Cl_2$, however, we have found a curious phenomenon: the product was not Fe(OEP)(2-MeImH) nor Fe(OEP)(2-MeImH)$_2$, but was the Fe(OEP)(OMe) complex. The methoxide ligand originated from methanol, which is present as a stabilizer[14] in our commercial $CH_2Cl_2$. Employing visible-absorption and resonance Raman technique, here we report the formation of the Fe(OEP)(OMe) complex by titrating Fe(OEP)(Cl) with 2-MeImH in the presence of a trace amount of methanol.[15]

In Figure 1 are shown visible spectral changes upon increasing the amount of 2-MeImH. Since spectrum 1 is almost identical with the spectrum of Fe(OEP)(Cl), methanol does not bind directly to the iron in the absence of 2-MeImH. On addition of 2-MeImH, absorption bands at 580 and 467 nm increased (spectra 2–6) with one set of isosbestic points. This indicates that the reaction proceeds with an equilibrium between Fe(OEP)(Cl) and the product species. It should be noted that spectrum 6 is quite similar to the spectrum of Fe(OEP)(OMe) we have reported.[17,20] The absorbance change was well explained by 1 mol of 2-MeImH equilibrating with 1 mol of Fe(OEP)(Cl). The apparent binding constant was estimated to be about 100 M$^{-1}$.

In Figure 2 are shown resonance Raman spectral changes under parallel conditions with the absorption spectral experiment. The Raman lines in the 1300–1700-cm$^{-1}$ region are a sensitive probe for the iron states of porphyrin complexes.[21–24] In the absence of both methanol and 2-MeImH (A), $\nu_4$, $\nu_3$, $\nu_2$, and $\nu_{10}$ modes[25] of the starting complex, Fe(OEP)(Cl), were observed at 1377, 1494, 1582, and 1629 cm$^{-1}$, respectively. These frequencies are in good agreement with those reported[20,26] for 5-coordinate ferric high-spin complexes. The higher frequency modes were insensitive to the presence of methanol (B) and the successive addition of 2-MeImH (C–E). In the lower frequency region, however, a new line at 541 cm$^{-1}$ gains intensity on addition of 2-MeImH.

As shown in Figure 3, the 541-cm$^{-1}$ line clearly downshifted to 528 cm$^{-1}$ by substituting $CH_3OH$ with $CD_3OD$, although it did not shift with $CH_3OD$ substitution (data not shown). The frequency well coincides with the $\nu$(Fe–$OCH_3$) stretch in the Fe(OEP)(OMe) complex in $CH_2Cl_2$ solution (541 cm$^{-1}$)[17] and in the crystalline state (542 cm$^{-1}$),[27] the structure of which is confirmed by an X-ray study.[27] In both cases, the $\nu$(Fe–$OCD_3$) stretch was detected at about 530 cm$^{-1}$. Therefore, the present resonance Raman results have established unequivocally that, in the presence of traces of methanol, 2-MeImH does not coordinate to the iron but promotes the formation of the 5-coordinate ferric high-spin product, Fe(OEP)(OMe).

The possible acid–base chemistry may be drawn schematically as below:



The added 2-MeImH may engage in hydrogen bonding with methanol and promote the formation of methoxide. The chloride ligand then will exchange with the methoxide to produce the Fe(OEP)(OMe) complex and 2-MeImH$_2^+$Cl$^-$. The apparent binding constant may be the estimate of this equilibrium reaction. Since addition of ImH or 1-MeIm to Fe(OEP)(Cl)[16] and even to Fe(OEP)(OMe)[17] produced bis-coordinated complex, the methyl substituent at the 2-position may offer sterical hindrance to the binding of 2-MeImH.

It has been established by NMR[28–30] and X-ray[31] studies that [Fe(OEP)(2-MeImH)$_2$]$^+$ is produced when Fe(OEP)(Cl) is

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(13) Abbreviations used: ImH, imidazole; 2-MeImH, 2-methylimidazole; 1-MeIm, 1-methylimidazole; OEP, dianion of octaethylporphyrin; OMe, methoxide anion.

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(15) Fe(OEP)(Cl)[16] (Aldrich) and 2-MeImH[17] were recrystallized as described previously. $CH_2Cl_2$ was doubly distilled over $CaH_2$. Aliquots of 2-MeImH solution were added to about 0.1 mM Fe(OEP)(Cl) dissolved in $CH_2Cl_2$ containing 1% $CH_3OH$ (Nakarai Chemicals, SP grade), $CH_3OD$ (99.5 atom % D, Aldrich), or $CD_3OD$ (99 atom % D, Merck), and visible[17] and resonance Raman[18,19] spectra were recorded.

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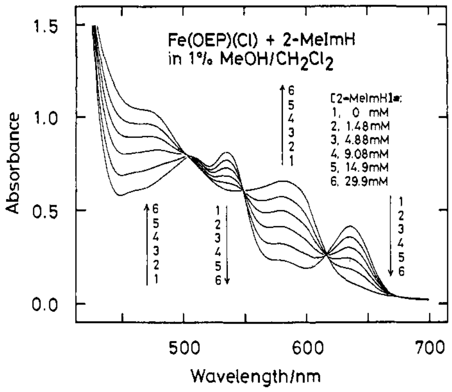

**Figure 1.** Visible spectral changes upon addition of 2-MeImH to Fe(OEP)(Cl). Aliquots of 2-MeImH solutions were added to Fe(OEP)(Cl) in $CH_2Cl_2$ containing 1% $CH_3OH$. Concentrations of 2-MeImH (mM): (1) 0; (2) 1.48; (3) 4.88; (4) 9.08; (5) 14.9; (6) 29.9.

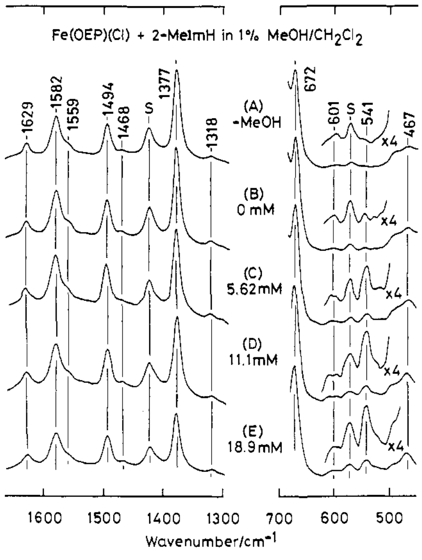

**Figure 2.** Resonance Raman spectral changes of Fe(OEP)(Cl) upon addition of 2-MeImH. Spectra were obtained for Fe(OEP)(Cl) dissolved in $CH_2Cl_2$ in the absence (A) or presence of 1% $CH_3OH$ with (B) 0, (C) 5.62, (D) 11.1, (E) 18.9 mM 2-MeImH. The Raman lines with S denote those of the solvent. Excitation: 406.7 nm. Laser power: 10 mW. Slit width: 5 cm$^{-1}$. Data acquisition time: 200 s.

reacted with 2-MeImH. Indeed, by the addition of 2-MeImH in great excess (about 1 M), an absorption spectrum of $[Fe(OEP)(2\text{-}MeImH)_2]^+$ was obtained even when methanol was present. Therefore, the $[Fe(OEP)(2\text{-}MeImH)_2]^+$ complex is the ultimate product but the addition of 2-MeImH does not necessarily mean its binding, at least at the first stage. It should be noted that the addition of 2-MeImH in the presence of ethanol, which is contained in chloroform as a stabilizer, also produced spectral changes similar to those in Figure 1.

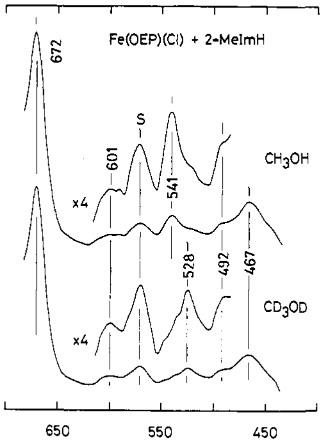

**Figure 3.** Effect of methanol isotopes on the resonance Raman spectrum of Fe(OEP)(Cl) in the presence of 18.9 mM 2-MeImH. The solution of Fe(OEP)(Cl) was measured in $CH_2Cl_2$ containing 1% $CH_3OH$ (upper) or 1% $CD_3OD$ (lower). Spectral conditions were the same as those in Figure 2.

Recently, photoreduction of iron porphyrin was reported[14,32] in a system quite similar to that in our present study. A trace amount of methanol was indispensable to the photoreduction,[14] as we have already reported.[33] The starting adduct, having a $\nu_{10}$ line at 1630 cm$^{-1}$ before photoreduction, was assigned to a 6-coordinate ferric intermediate-spin complex,[14] $[Fe(OEP)(2\text{-}MeImH)(CH_3OH)]^+$. The $\nu$(Fe–methanol) stretching frequency (524 cm$^{-1}$)[14] is interestingly close to the $\nu$(Fe–OMe) stretch in the presence of a higher amount (20%) of methanol (527 cm$^{-1}$),[17] where the hydroxide moiety of bulk methanol hydrogen bonds to the methoxide ligand. Therefore, the starting adduct may have a methoxide ligand, which is in good agreement with the recent proposal that the primary process in the photoreduction consists of an electron transfer from the axially ligated alkoxide to iron.[34]

In the community of porphyrin chemists, imidazoles have been widely believed to coordinate directly to the iron. However, our present results clearly indicate that 2-MeImH does not necessarily bind to the Fe(OEP)(Cl) in the presence of a trace amount of methanol. Our finding will stimulate the reconsideration of the product species thus far reported for the reactions of iron porphyrins and imidazoles and may provide a clue for a more detailed understanding of porphyrin chemistry.

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