Protein Models

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Models for Cytochromes *c*′: Observation of an Extremely Labile Spin State in Monoimidazole Complexes of Saddle-Shaped Iron(III) Porphyrinates**

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Cytochromes *c*′ have a quite unique structure in the sense that they are pentacoordinate with a histidyl imidazole group as the fifth ligand. In the oxidized state, these proteins exhibit a unique spin state, a mixed intermediate- ($S = 3/2$) and high-spin ($S = 5/2$) state that ranges from a mainly $S = 5/2$ to a mainly $S = 3/2$ state depending on the bacterial sources and spectroscopic methods applied for the measurements.[1] To reveal the ambiguous spin state of these proteins, both structural and spectroscopic studies with synthetic monoimidazole complexes are necessary. Although we have reported the spin states of a wide variety of $[Fe(TMP)L]^+$ model complexes, we were unable to obtain good crystals for structural studies.[2,3] In fact, the isolation of monoimidazole complexes is quite difficult because of the formation of stable bisadducts. To date, the only structurally characterized complex is $[Fe(OEP)(2\text{-MeIm})]ClO_4$, reported by Scheidt et al.[4] This complex maintains the $S = 5/2$ state, at least above 60 K. In this paper, we report that the monoimidazole complexes $[Fe(oetpp)L]ClO_4$, in which L is HIm (**1**), 2-MeIm (**2**), or 2-MeBzIm(**3**), exhibit extremely labile spin states, on the basis of ^1H NMR, ^{13}C NMR, EPR, and Mössbauer spectroscopy and X-ray crystallography.

Complexes **1**–**3** were prepared by the addition of HIm, 2-MeIm, or 2-MeBzIm (1.0 equiv), respectively, to CH_2Cl_2 solutions of $[Fe(oetpp)(thf)_2]ClO_4$. The black crystal thus obtained was recrystallized from $CHCl_3$/cyclohexane. The molecular structures of **1** and **2** were determined by X-ray crystallography at 123 and 120 K, respectively. Figure 1 shows the molecular structure of **2**; the molecular structure of **1** is given in the Supporting Information. Both **1** and **2** exhibit a highly saddled porphyrin core. The average $Fe{-}N_p$ bond lengths are 1.969(3) and 1.966(2) Å for **1** and **2**, respectively; these values are quite close to the average $Fe{-}N_p$ bond length in $S = 3/2$ $[Fe(oetpp)]ClO_4$, that is, 1.963(7) Å,[5] and are much shorter than that in $S = 5/2$ $[Fe(OEP)(2\text{-MeIm})]ClO_4$, that is, 2.038(6) Å.[4] In contrast, the $Fe{-}N_{axial}$ bond length in **2** is longer than that in **1** by 0.013 Å. The axial 2-MeIm ligand is aligned along the N–Fe–N axis.

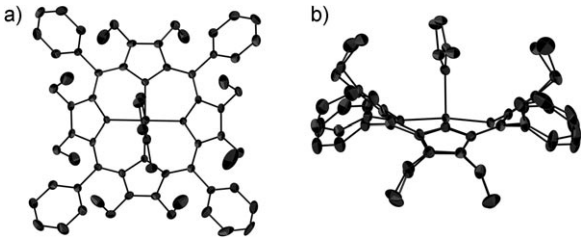

Figure 1. The molecular structure of **2**. ORTEP diagram viewed from a) the top and b) the side. Thermal ellipsoids are drawn to enclose 50% probability.

Table 1: Structural comparison of five-coordinate iron(III) porphyrinates.

Complexes	$Fe{-}N_p$ [Å]	$Fe{-}L_{axial}$ [Å]	Δ [Å]	Reference
$[Fe(OEP)(2\text{-MeIm})]ClO_4$	2.038(6)	2.068(4)	0.36	[4]
$[Fe(oetpp)]Cl$	2.031(5)	2.2418(23)	0.47	[6]
$[Fe(oetpp)]ClO_4$	1.963(7)	2.059(6)	0.25	[5]
$[Fe(oetpp)(HIm)]ClO_4$ (**1**)	1.969(3)	2.066(3)	0.24	this work
$[Fe(oetpp)(2\text{-MeIm})]ClO_4$ (**2**)	1.966(2)	2.079(2)	0.26	this work

The deviation of the Fe^{III} ion from the 4N mean plane is rather small (0.26 Å), which is again quite close to that in $[Fe(oetpp)]ClO_4$ (0.25 Å).[5] Table 1 lists the structural parameters of **1** and **2**, together with those of some analogous complexes mentioned above.[4–7] The similarity of the structural parameters of **1** and **2** to those of $[Fe(oetpp)]ClO_4$ suggests that **1** and **2** adopt the $S = 3/2$ state.

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The ^{1}H NMR spectra of **1**–**3** in a dry degassed CD_2Cl_2 solution at 298 K are shown in the Supporting Information. Chemical shifts of the major signals in these complexes are listed in Table 2. Curie plots of the signals for **1**–**3** exhibited a good linearity, as shown in the Supporting Information, which suggests that these complexes maintain a single spin state in the temperature range 298–173 K. The negative δ-(*meta*)−δ(*para*) values, together with the negative Curie slopes of the *meta* signals, indicate that the *meso* carbon atoms have negative spin,[7,8] which in turn indicates that **1**–**3** adopt the $S = 3/2$ state; the $S = 5/2$ Fe^{III} complexes have positive spin at the *meso* carbon atoms and thus give positive δ-(*meta*)−δ(*para*) values, as in the case of [Fe(oetpp)]Cl.[6,9] The solution magnetic moment of **2** determined by the Evans method was 4.2 μ_B at 298 K and was invariant down to 173 K. This value is close to the spin-only value expected for the $S = 3/2$ state.

The ^{13}C NMR spectra of **1**–**3** are also given in the Supporting Information. The signals were assigned by the conventional method. Chemical shifts of the major signals are listed in Table 2. The α- and β-pyrrole signals appeared more upfield than those in [Fe(oetpp)]Cl, which should be ascribed to the vacant $d_{x^2-y^2}$ orbital. Considerable upfield shifts of the *meso* signal, $\delta = -79$, -54, and 11 ppm in **1**, **2**, and **3**, respectively, satisfy the condition required for saddle-shaped intermediate-spin complexes.[8,10] A large upfield shift was also observed for the CH_2 signals. The average chemical shifts of these signals are −48, −45, and −45 ppm for **1**, **2**, and **3**, respectively; these values are close to the corresponding chemical shift in [Fe(oetpp)]ClO_4. Thus, the ^{13}C NMR results also suggest that **1**–**3** adopt the $S = 3/2$ state in CD_2Cl_2 solution. As listed in Table 2, the *meso*-C atom in **3** showed two signals, at $\delta = 7.7$ and 13.6 ppm at 298 K, which suggests that ligand rotation about the Fe–N_{axial} bond is slow on the NMR timescale, even at ambient temperature.[11] This signal broadened and coalesced at 338 K (as shown in the Supporting Information), from which the activation free energy ($\Delta G^{\neq}$) for rotation was estimated to be 64 kJ mol^{-1}. To our knowledge, this is the highest rotational barrier for an axial imidazole ligand in an Fe^{III} porphyrin.[11] A similar phenomenon was observed in **2** at a lower temperature; the $\Delta G^{\neq}$ value was 35 kJ mol^{-1} at 197 K. In contrast, **1** maintained a single *meso*-C signal, even at 183 K. The extremely high barrier to rotation in **3** should be ascribed to the fact that the bulky 2-MeBzIm ligand is placed in the cavity created by the saddled porphyrin ring, as illustrated for **2** in Figure 1 b.

The EPR spectrum of **2** at 60 K with dry and degassed CH_2Cl_2 as the solvent is shown at the top of Figure 2 a. We expected that **2** should give signals at $g = 4.0$ and 2.0, as for a typical $S = 3/2$ complex.[12] Actually, however, **2** exhibited the major signal at $g = 5.9$, together with a minor signal at $g = 4.0$, which clearly indicates that a major proportion of **2** adopts the $S = 5/2$ state at 60 K. As the temperature was further lowered, the signal at $g = 4.0$

Table 2: ^{1}H and ^{13}C NMR chemical shifts of [Fe(oetpp)]Cl, [Fe(oetpp)]ClO_4, and [Fe(oetpp)L]$^+$ (**1**–**3**) in CD_2Cl_2 at 298 K.

Axial ligand	Spin state	^{1}H NMR: δ [ppm]						^{13}C NMR: δ [ppm]					Ref.
		CH_2	CH_3	*o*	*m*	*p*	ligand	CH_2	CH_3	α	β	*meso*	
Cl^-	$S=5/2$	20.1, 32.1, 34.8, 49.0	1.8, 3.2	9.1, 11.5	12.2, 12.4	7.5	–	53, 61	116, 163	525, 642	973	456	[d]
ClO_4^-	$S=3/2$	13.0, 42.7	0.7	13.4	7.1	9.8	–	−66	220	195	248	−47	this work
HIm (**1**)	$S=3/2$	9.4, 18.3, 24.3, 52.4	−1.0, 1.7	11.8, 14.8	6.9, 7.4	10.0	42.9, 78.3, 93.7	−49, −47	173, 227	275, 378	270, 308	−79	this work
2-MeIm (**2**)	$S=3/2$	9.0, 18.4, 20.6, 54.4	−1.1, 1.6	11.3, 14.6	7.0, 7.6	9.8	65.0, 104.1	−50, −40	169, 229	240, 349	279, 314	−54	this work
2-MeBzIm (**3**)	$S=3/2$	7.9, 8.3, 18.6, 19.5, 20.1, 57.0[a]	−1.0, −0.3, 1.0[b]	10.7, 13.9	7.4, 7.9	9.4		−55, −54, −41, −31	164, 174, 233, 236	289, 298, 311, 316, 331[c]		7.7, 13.6	this work

[a] The signals at $\delta = 19.5$ and 57.0 ppm correspond to 4 H. Other signals correspond to 2 H. [b] The signals at $\delta = -1.0$, −0.3, and 1.0 ppm correspond to 6 H, 6 H, and 12 H, respectively. [c] Either α or β signals. [d] ^{1}H NMR: reference [9]; ^{13}C NMR: this work.

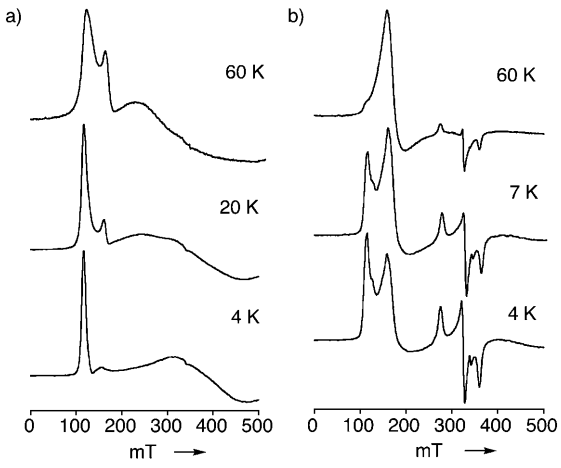

Figure 2. Temperature-dependent EPR spectra of a) **2** and b) **1**, recorded in frozen CH_2Cl_2 solution.

decreased in intensity and it completely disappeared at 4 K. Thus, **2** exists as a pure $S=5/2$ complex at 4 K. The process was reversible because the original spectrum was completely reproduced when the temperature was raised back up to 60 K. The NMR and EPR spectra can best be explained in terms of a spin transition from the $S=3/2$ to the $S=5/2$ state.[13] **3** exhibited a spectral change quite similar to that of **2**.

The EPR spectra of **1** (Figure 2b) are more unusual. This complex exhibited a major signal at $g=4.0$ at 60 K, although some weak signals were observed at a higher field. As the temperature was lowered, the signal at $g=4.0$ decreased in intensity while the signals at $g=6.0$, 2.5, and 2.1 increased. At 4 K, **1** existed as a mixture of three species with different spin states: an $S=5/2$ species with $g_{\perp}=6.0$, an $S=3/2$ species with $g_{\perp}=4.0$, and an $S=1/2$ species with $g=2.47$, 2.10, and 1.55. Formation of the $S=1/2$ and $S=5/2$ complexes could be ascribed to the disproportionation of $[Fe(oetpp)(HIm)]ClO_4$ to form $[Fe(oetpp)(HIm)_2]ClO_4$ and $[Fe(oetpp)]ClO_4$. This possibility is, however, completely ruled out because $[Fe(oetpp)]ClO_4$ is well characterized as an essentially pure $S=3/2$ complex.[5] Furthermore, the g values of $[Fe(oetpp)(HIm)_2]ClO_4$ determined under the same conditions are 2.72, 2.37, and 1.64,[14] which are quite different from those of the $S=1/2$ complex observed at 4 K. Thus, it is reasonable to consider that the $S=5/2$ and $S=1/2$ complexes are spin isomers and that they exist as an equilibrium mixture with the $S=3/2$ complex.

The existence of the three kinds of spin isomer indicates that there are three different structures in **1** at extremely low temperatures. The spin transition from the $S=3/2$ to the $S=1/2$ state at lower temperatures induces the contraction of the $Fe-N_{axial}$ and $Fe-N_{equatorial}$ bonds.[10b] A similar spin transition was reported quite recently by Scheidt and co-workers in the five-coordinate iron(II) porphyrinate $[Fe(TPP)(CN)]^-$.[15] This complex showed a decrease in both the Fe–CN bond length and the out-of-plane distance of the iron(II) ion, by 0.23 and 0.28 Å, respectively, as the structural consequence of the spin transition from the $S=2$ to the $S=0$ state. In contrast, the spin transition from the $S=3/2$ to the $S=5/2$ state should induce an out-of-plane displacement of the iron(III) ion toward the axial imidazole ligand. We have observed a similar spin transition in the five-coordinate saddle-shaped monoaqua complex $[Fe(OMTArP)(H_2O)]^+$.[13a] With **2** and **3**, which are carrying hindered imidazole ligands, the approach of the axial ligand toward the porphyrin ring should be hampered due to steric repulsion between the axial ligand and the porphyrin core.[3] Note that the $Fe-N_{axial}$ bond of **2** is longer than that of **1** by 0.013 Å. A space-filling model of the crystal structure of **2** (shown in the Supporting Information) also indicates that the further approach of the ligand toward the porphyrin ring is impossible because of steric repulsion between the ligand methyl and the *meso*-phenyl groups. For this reason, **2** and **3** exhibit only one type of spin transition, $S=3/2$ to $S=5/2$, in contrast to the case of **1**.

As mentioned, ^1H NMR and ^{13}C NMR spectroscopy, as well as the effective magnetic moments measured in CD_2Cl_2 solution, have revealed that **1**–**3** maintain the $S=3/2$ state in the temperature range 298–173 K. In addition, the X-ray molecular structures of **1** and **2** determined at approximately 120 K have revealed that these complexes still maintain the $S=3/2$ state at this temperature. However, the EPR spectra measured in a frozen CH_2Cl_2 solution in the temperature range 70–4 K show a quite rare spin transition, that is, the spin transition from the $S=3/2$ to the $S=5/2$ state in **2** and **3** or from the $S=3/2$ state to a mixture of the $S=5/2$ and $S=1/2$ states in **1**. The spin states of these complexes have been determined by various methods at various temperatures, so it is important to determine the spin states by means of a single type of equipment over a wide range of temperatures, that is, 300–4 K. Among the possible types of measurement, Mössbauer spectroscopy satisfies this requirement because it can clearly discriminate the spin states of the iron(III) porphyrinates over a wide range of temperatures.[16] Figure 3 shows the Mössbauer spectra of **1** and **2** taken for the microcrystalline

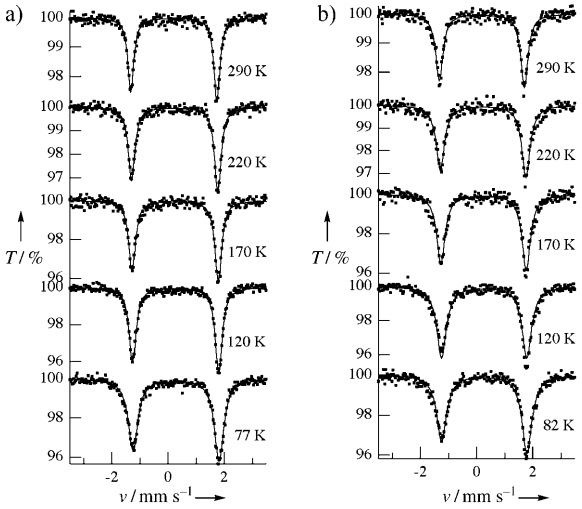

Figure 3. Temperature-dependent Mössbauer spectra of a) **1** and b) **2**.

samples in the temperature range 300–80 K. Mössbauer spectra taken at 4 K are shown in the Supporting Information. In contrast to the electronic structure in solution, **1** and **2** maintained the $S=3/2$ state between 4 and 300 K, as revealed from the quadrupole splitting (QS) and isomer shift (IS) values.[17] The results suggest that the solid samples do not exhibit the spin transition even at 4 K.

In summary, we have demonstrated that the saddle-shaped monoimidazole complexes $[Fe^{III}(oetpp)L]^+$ (**1**–**3**) are in an essentially pure intermediate-spin state between 298 and 173 K in CD_2Cl_2 solution, under the conditions at which the NMR spectra are taken. The EPR spectra of these complexes show, however, a spin transition below 70 K in the frozen CH_2Cl_2 solution. Whereas **1** exhibits an unprecedented spin transition from the $S=3/2$ state to a mixture of the $S=5/2$ and $S=1/2$ states below 60 K, **2** and **3**, which carry bulky imidazole ligands, show the spin transition from the pure $S=3/2$ to the pure $S=5/2$ state. The lack of a $S=1/2$ spin isomer in **2** and **3** has been ascribed to steric repulsion between the bulky imidazole ligand and the porphyrin core. In contrast to the magnetic behaviors in solution, the solid samples of **1**–**3** maintain the $S=3/2$ state in the temperature

range 300–4 K. These findings cast light on the long-standing mystery of the ambiguous spin state of cytochromes *c*′.

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[2] Abbreviations: HIm: imidazole; 2-MeIm: 2-methylimidazole; 2-MeBzIm: 2-methylbenzimidazole; THF:tetrahydrofuran; TMP, OEP, OMTArP, and oetpp: dianions of 5,10,15,20-tetramesitylporphyrin, 2,3,7,8,12,13,17,18-octaethylporphyrin, 2,3,7,8,12,13,17,18-octamethyl-5,10,15,20-tetraarylporphyrin, and 2,3,7,8,12,13,17,18-octaethyl-5,10,15,20-tetraphenylporphyrin, respectively.
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[17] The Mössbauer parameters: QS = 3.06, IS = 0.29 mm s^{-1} for **1** and QS = 3.04, IS = 0.27 mm s^{-1} for **2** at 80 K; QS = 3.07 and IS = 0.30 mm s^{-1} for **1** and QS = 3.04 and IS = 0.28 mm s^{-1} for **2** at 4 K.

