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An Active-Site Model of Nitrile Hydratase: Axially Coordinate Non-Heme Iron Complexes in the Low-Spin Ferric State

Sir:

Recently, nitrile hydratase isolated from *Brevibacterium* R312[1] and *Pseudomonas chlororaphis* B23[2] was found to be a new iron-containing enzyme that catalyzes the hydration of aliphatic nitriles to the corresponding amides: $RCN + H_2O \rightarrow RCONH_2$. The active site of this enzyme was proposed to have a typical low-spin ferric coordination structure,[3] similar to those of hemoproteins such as cytochrome P-450. The axial positions of the non-heme iron site in the resting state of the enzyme are concluded to be occupied by thiolato and aquo ligands.[3]

During investigations on bis(thilato) metalloporphyrin complexes,[4] we found that a non-heme iron(III) complex of a square-planar type binds thiolato ligands in the axial positions and forms bis(thiolato)–non-heme iron complexes that are similar to bis(thiolato) metalloporphyrin complexes. We report here the detection and characterization of the bis(thiolato)–non-heme iron(III) complexes by ESR (electron spin resonance) spectrometry and propose the active-site structure of native nitrile hydratase in the monomer form, postulating an axial thiolato–iron(III)–imidazole coordination mode on the basis of analyses of the ESR parameters.

Experimental Section. (*N,N'*-Disalicylideneethylenediaminato)iron(III) *d*-camphor-10-sulfonate (Fe(salen)(*d*-camphor-10-sulfonate), $FeC_{26}H_{29}N_2O_6S$) was prepared and purified by a reported procedure.[5] Ethyl thioglycolate (TGE), imidazole (Im), and tetrabutylammonium hydroxide (Bu_4NOH (TBAH), 10% solution in methanol) were products of Wako Pure Chemicals. Acetone and methanol (MeOH) were distilled before use. All samples for analyses of ESR and electronic absorption spectra were prepared in air at 20 °C. ESR measurements were carried out in a JES-FE1XG spectrometer with 100-kHz field modulation at 77 K. Li–TCNQ (g = 2.002 52) and Mn(II) doped in MgO were used as standards. Electronic absorption spectra were recorded in air on a Union Giken SM 401 high-sensitivity spectrometer at 20 °C. The crystal field parameters of tetragonality ($|\mu/\lambda|$) and rhombicity ($|R/\mu|$) were computed from three ESR g values due to low-spin ferric states by Bohan's method.[6]

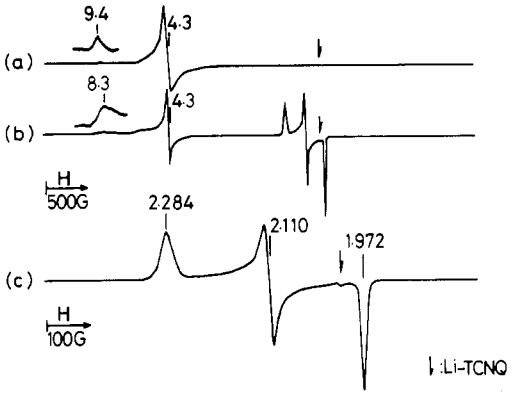

Figure 1. ESR spectra of Fe(salen) complexes in acetone at 77 K: (a) Fe(salen), 1 mM; (b) solution as in (a) + TGE, 100 mM; (c) solution as in (b) + TBAH, 50 mM.

Results and Discussion. Aerobic addition of TBAH to a solution containing Fe(salen)[7] and TGE in acetone, which shows a typical ESR signal at g = 4.3 due to the mononuclear non-heme Fe(salen) complex (Figure 1a), resulted in the appearance of new signals due to a rhombic ferric complex in the low-spin state, similar to those of ferric porphyrin complexes,[4] concomitant with a signal at g = 4.3 (Figure 1b,c).[8] The present complex prepared in MeOH gave a similar ESR spectrum, suggesting that the MeOH molecule does not bind to the axial positions of Fe(salen). These results indicate that two thiolato groups of the deprotonated TGE ($TGE(S^-)$) coordinate axially to the square-planar Fe(salen) complex to form the bis(thiolato) Fe(salen) complex. Indeed, the estimated g values (g_1 = 2.284, g_2 = 2.110, and g_3 = 1.972) differ distinctly from those of a typical non-heme iron–sulfur cluster (g_1 = 2.05, g_2 = 1.94, and g_3 = 1.88).[9] It is, thus, very interesting that the ESR features of the present ferric low-spin complex are very similar in both spectral pattern and g values to those of native nitrile hydratase R312 (g_1 = 2.284, g_2 = 2.140, and g_3 = 1.971),[3] which is proposed to be a non-heme iron protein with a typical mononuclear ferric low-spin coordination structure.

Table I summarizes the ESR g values and crystal field parameters of the ferric low-spin Fe(salen) and iron(III) proto-

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(7) Fe(salen) dissolved in acetone or methanol shows a signal at g = 4.5 at 77 K.
(8) The initial spectrum of the solution containing Fe(salen) (1 mM) and TGE (100 mM) shows the absorption bands at 433 (sh) and 476 nm. On addition of TBAH (40–130 mM) to the initial solution, the absorption bands changed to 403 and 482 nm.
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Table I. ESR Spectral Data on Ferric Low-Spin Complexes with Various Types of Axial Coordination Modes

syst[a]	solvent[b]	axial coord mode	ESR g value (77 K)			cryst field param		no. in Figure 2	ref
			g_1	g_2	g_3	$\lvert\mu/\lambda\rvert$	$\lvert R/\mu\rvert$		
Fe(salen)/TGE(S^-)	A	S^-–Fe–S^-	2.284	2.110	1.972	13.39	0.54	1	
	M	S^-–Fe–S^-	2.282	2.110	1.971	13.29	0.55	2	
Fe(salen)/Im	A	N–Fe–N	2.394	2.146	1.911	8.18	0.60	3	
Fe(salen)/Im^-	A	N^-–Fe–N^-	2.364	2.150	1.924	8.23	0.65	4	
Fe(salen)/TGE(s^-)/Im^-	A	S^-–Fe–N^-	2.304	2.140	1.960	11.36	0.58	5	
Fe(salen)/TGE(S^-)/H_2O	AW		2.269	2.106	1.973	13.73	0.55	6	
nitrile hydratase									
native R312	pH 7.2		2.284	2.140	1.971	10.62	0.63	7	3
propionitrile-bound R312	pH 7.2		2.230	2.163	1.982	10.97	0.33	8	3
isobutyronitrile-bound R312	pH 7.2		2.207	2.124	1.984	13.10	0.48	9	3
mercury-treated B23	pH 7.2		2.286	2.141	1.969	10.47	0.62	10	3
propionitrile-bound B23	pH 7.2		2.227	2.161	1.984	11.19	0.33	11	3
Fe(PPIX)/TGE(S^-)	AW	S^-–Fe–S^-	2.285	2.223	1.959	8.17	0.23	12	4c
Fe(PPIXDME)/TGE(S^-)	A	S^-–Fe–S^-	2.289	2.228	1.957	8.01	0.22	13	4c
Fe(PPIX)/TGE(S^-)/H_2O	AW	S^-–Fe–O	2.317	2.239	1.949	7.47	0.26	14	4c
Fe(PPIXDME)/TGE(S^-)/MeOH	A	S^-–Fe–O	2.345	2.248	1.940	7.01	0.24	15	4c
Fe(PPIX)/TGE(S^-)/Im	AW	S^-–Fe–N	2.347	2.234	1.934	7.04	0.34	16	4c
Fe(PPIXDME)/TGE(S^-)/Im^-	A	S^-–Fe–N^-	2.359	2.242	1.930	6.81	0.34	17	4c
Fe(PPIX)/Im^-	AW	N^-–Fe–N^-	2.713	2.255	1.700	3.95	0.65	18	4c

[a] TGE(S^-) and Im^- represent $^-SCH_2COOC_2H_5$ and imidazolate, respectively. [b] Abbreviations: A = acetone; M = methanol; W = water.

porphyrin complexes with various types of axial coordination modes in comparison with those of nitrile hydratase. Titration of the bis(thiolato) Fe(salen) complex with TBAH resulted in no remarkable changes of ESR g values (g_1 = 2.284–2.281, g_2 = 2.110–2.109, and g_3 = 1.973–1.972), but the intensity of the signals decreased with an increase in the amount of TBAH, probably due to binding of hydroxide to Fe(salen) by substitution of bis(thiolato) ligands. The ESR spectrum of the bis(imidazole) complex of Fe(salen) showed ESR features (g_1 = 2.394, g_2 = 2.146, and g_3 = 1.911) more anisotropic than those of the bis(thiolato) complex, but addition of TBAH changed the g values to those of an anisotropic complex (g_1 = 2.364, g_2 = 2.150, and g_3 = 1.924) smaller than the bis(imidazole) complex, due to the coordination of bis(imidazolato) (Im^-) with Fe(salen). When the bis(imidazolato) complex was titrated with TGE in the presence of TBAH, less anisotropic ESR features were observed (g_1 = 2.304, g_2 = 2.140, and g_3 = 1.960). Comparison of the g values indicates that the axial positions of the Fe(salen) complex are simultaneously coordinated by a thiolato group of TGE and an imidazolato ligand. Similar observations have been made for the iron(III) protoporphyrin complexes, in which the complexes are stabilized by axial coordination of thiolato and imidazolato ligands.[4c]

The crystal field parameters tetragonality ($|\mu/\lambda|$) and rhombicity ($|R/\mu|$) proposed originally by Blumberg and Peisach[10] and calculated by Bohan's method[6] for various types of non-heme Fe(salen) and iron(III) protoporphyrin complexes and for nitrile hydratases are plotted in Figure 2. A similar crystal field diagram for low-spin ferric porphyrin complexes has been used successfully to deduce the axial coordination mode of cytochrome P-450 in the resting state.[4c] As seen in Figure 2, the parameters for the coordination modes of N^-–Fe–N^-, S^-–Fe–N^-, and S^-–Fe–S^- in the non-heme Fe(salen) (A) and iron(III) protoporphyrin complex (B) systems are approximately linearly correlated. The region (points 14 and 15) for the S^-–Fe–O(H_2O or MeOH) coordination mode of iron protoporphyrin complexes as the active center of cytochrome P-450 in the resting state is clearly located between the regions of S^-–Fe–N^- and S^-–Fe–S^- (Figure 2B), while the crystal field point for the native nitrile hydratase (point 7) is located between the parameters for N^-–Fe–N^- and S^-–Fe–N^- coordination modes in the non-heme Fe(salen) systems (Figure 2A). From these observations, it seems unlikely that the axial positions in the native nitrile hydratase are occupied by thiolato and aquo ligands as proposed in the literature.[3]

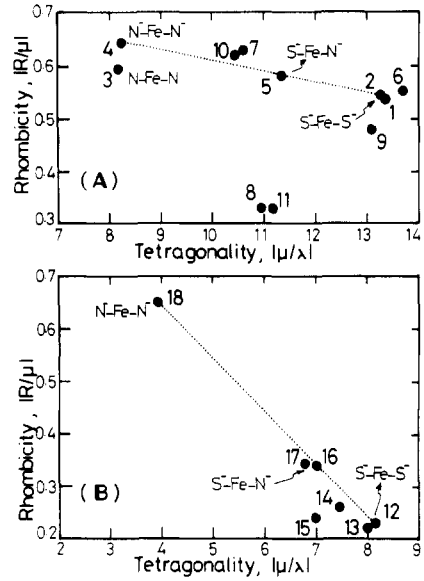

Figure 2. Crystal field diagram for low-spin ferric complexes and nitrile hydratases: (A) non-heme Fe(salen) complexes and nitrile hydratases; (B) iron(III) protoporphyrin complexes and their dimethyl ester derivatives. Numbers refer to the systems in Table I.

Furthermore, when the bis(thiolato) Fe(salen) complex was titrated with water, an 8.3-G broadening of the g = 2.284 signal was clearly observed, as in the case with the nitrile hydratase.[3] Nevertheless, the crystal field parameters (point 6 in Figure 2A) for the bis(thiolato) Fe(salen) complex in the presence of water were located near the coordination mode of S^-–Fe–S^-. Thus, on the basis of analyses of these crystal field parameters of model complexes and nitrile hydratase, we propose that thiolato and imidazolato ligands coordinate to the non-heme iron active center of the enzyme.

In the present study, we used the Fe(salen) complex, having four equatorial nitrogen ligands. In order to construct better models of nitrile hydratase, non-heme iron complexes consisting of different sets of equatorial ligands should be used in the future. However, on the basis of the results of the present investigation, the four equatorial nitrogen ligands, probably from imidazoles of histidine residues and/or from amino groups of asparaginate, glutamate, or arginate residues, are proposed for the candidates.

In conclusion, the present ESR study reveals that (1) Fe(salen) complexes having suitable axial coordination ligands are good models of nitrile hydratase and (2) a thiolato group and an im-

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idazole or imidazolato group from a cysteine and histidine residue, respectively, of the protein are the most favorable candidates for the axial ligands in the active site of native nitrile hydratase in the ferric low-spin state.

Further characterization of the present model complexes is under way by Mössbauer, EXAFS, and X-ray structure analysis methods.

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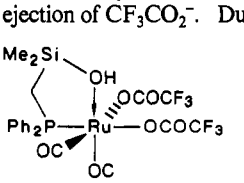
Siloxyl-Bridged Diruthenium(II) Complexes: Structural Models for Cluster-Derived, Silica-Supported Ruthenium Catalysts

Sir:

Transition-metal catalysts dispersed on high-surface-area oxides are essential to the operation of many industrial processes, and accordingly the structure and performance of such materials is an area of continuing speculation and study. Understanding surface geometry, in particular the way in which reactive metal centers are bound to a support, is regarded as a primary objective;[1] however, owing to the rarity of suitable molecular models, the current state of knowledge concerning metal–support interactions rests almost entirely on indirect characterization using various spectroscopic techniques.[2] Recently the X-ray crystal structure of the *triangulo*-triosmium derivative $[Os_3(CO)_{10}(\mu\text{-H})(\mu\text{-OSiEt}_3)]$ (**1**) was projected as "the first molecular analogue of the silica surface cluster"[3] and has since been related specifically to EXAFS analysis of alumina-supported osmium clusters.[4] We have used X-ray diffraction to determine the structures for two new siloxyl-bridged diruthenium complexes, providing reference parameters for oxide-supported ruthenium catalysts, which are numerous[5] but for which no suitable molecular models have previously been described. In either compound two low-valent Ru atoms are held adjacent but are not bonded to each other (as are the Os centers[3] in **1**): such a configuration is likely to parallel the result of cluster fragmentation[4] that accompanies catalyst activation.

NMR evidence for enhanced acidity of the silanol hydrogen in the unusual complex[6] **2** vs that in $Ru(n\text{-cym})Cl_2(PPh_2CH_2SiMe_2OH)$ (**3**; cym = *p*-cymene; the silanol O is not attached to Ru[7]) suggested that treatment of **2** with H^- might generate a reactive, coordinatively unsaturated siloxy–Ru species via hydrogen loss and ejection of $CF_3CO_2^-$. During an appropriate experiment gas evolution was indeed observed (KH, 1 mol equiv;



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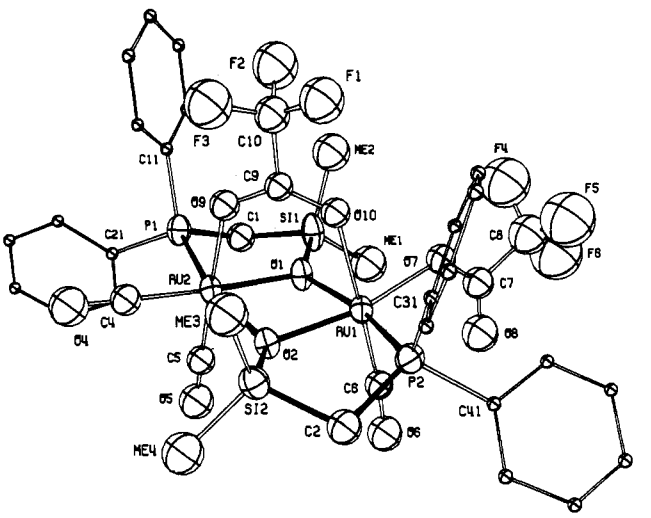

Figure 1. ORTEP drawing of compound **4**. Selected bond distances (Å) and angles (deg): Ru(1)–O(1), 2.135 (7); Ru(1)–O(2), 2.091 (6); Ru(2)–O(1), 2.087 (6); Ru(2)–O(2), 2.111 (7); Ru(1)–O(7), 2.085 (7); Ru(1)–O(10), 2.183 (7); Ru(2)–O(9), 2.118 (7); Ru(1)–P(2), 2.262 (3); Ru(2)–P(1), 2.321 (3); Ru(1)–O(1)–Ru(2), 100.5 (3); Ru(1)–O(2)–Ru(2), 101.2 (3); O(1)–Ru(1)–O(2), 77.8 (2); O(1)–Ru(2)–O(2), 78.4 (3); P(2)–Ru(1)–O(2), 89.3 (2); P(1)–Ru(2)–O(1), 87.3 (2). The two Ru centers lie 3.247 (1) Å apart.

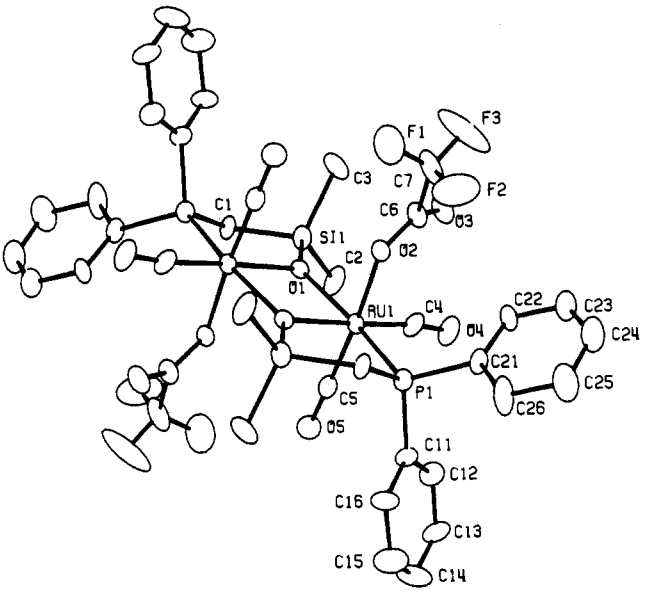

Figure 2. ORTEP drawing of compound **6**. Selected bond distances (Å) and angles (deg): Ru(1)–O(1), 2.135 (10); Ru(1)–O(2), 2.086 (12); Ru(1)–P(1), 2.325 (5); Ru(1)–O(1)–Si(1), 135.4 (6); O(1)–Ru(1)–P(1), 165.1 (3); P(1)–Ru(1)–C(4), 94.4 (5); Ru(1)–O(1)–R u(1)′ = 103.0 (4). The two Ru centers lie 3.313 (1) Å apart.

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