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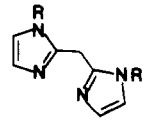
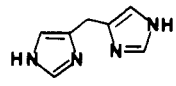
Iron-Catalyzed Oxidation of Bis(imidazol-2-yl)methane into Bis(imidazol-2-yl) Ketone

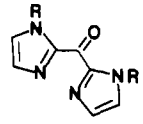
Polyimidazole and -benzimidazole ligands are commonly used to mimic the metal binding sites of various metalloproteins.[1] Among these, bis(imidazol-4-yl)methane (4-BIM) and bis(imidazol-2-yl)methane (2-BIM) have been very little studied[2,3] and very few data are available about the properties of their iron complexes.[2] We have found that in methanol, in the presence of air and of a nitrogenous base, tris[bis(imidazol-2-yl)methane-N,N']iron(III), $[Fe^{III}(2\text{-BIM})_3]^{3+}$ (**1**(III)), is quantitatively converted into the blue complex tris[bis(imidazol-2-yl) ketone-N,N']iron(II), $[Fe^{II}(2\text{-BIK})_3]^{2+}$ (**2**(II)).



R = H : 2-BIM
R = Me : Me_2 2-BIM



4-BIM



R = H : 2-BIK
R = Me : Me_2 2-BIK

The colorless complexes $[Fe^{II}(2\text{-BIM})_3]X_2$ (X = perchlorate, chloride, tetrafluoroborate)[5] are obtained in quantitative yield by addition of 2-BIM[4] to the corresponding iron(II) salts (3:1) in ethanol under an argon atmosphere. ^1H NMR spectroscopy shows that, in DMSO or methanol, the same single paramagnetic complex $[Fe^{II}(2\text{-BIM})_3]^{2+}$ (**1**(II)) is formed either from 2-BIM and the iron(II) salts (3:1) or from 2-BIM and the iron(III) salts (4:1) (nitrate, perchlorate, or chloride salt) under argon. The redox potential of **1**(III)/**1**(II) (cyclic voltammetry in CH_3CN) is 0.51 V (vs NHE). We have checked that **1**(III), prepared by electrochemical oxidation of **1**(II) in acetonitrile or methanol under argon, is readily reduced by 2-BIM into **1**(II).

A methanol or ethanol solution of **1**(II) slowly turns blue in the presence of air. Mass spectrometry (FAB) shows that this blue solution contains the four possible complexes with the 2-BIM and/or 2-BIK ligands.[7] The deep blue paramagnetic $[Fe^{II}(2\text{-BIK})_3]^{2+}$ complex (**2**(II))[8] is quantitatively obtained in ethanol at 50 °C after about 24 h in the presence of air, starting either from Fe(II) (perchlorate, chloride, or tetrafluoroborate salt) (3–4 mM) and 3 equiv of 2-BIM or, preferably, from Fe(III) (perchlorate, nitrate or chloride salt) and 4 equiv of 2-BIM. ^1H NMR monitoring of these reactions confirms the MS observation of the four possible Fe(II) complexes containing 2-BIM and/or 2-BIK,[9] which are finally transformed into **2**(II). The redox potential of **2**(III)/**2**(II) (cyclic voltammetry in CH_3CN) is 1.11 V.[10] **2**(III) generated electrochemically from **2**(II) is readily reduced by 2-BIM and more slowly by ethanol into **2**(II).

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(7) Mass spectral data, *m/e*: 499 (M − 1; $Fe(2\text{-BIM})_3$; 513 (M − 1; $Fe(2\text{-BIM})_2(2\text{-BIK})$); 527 (M − 1; $Fe(2\text{-BIM})(2\text{-BIK})_2$); 543 (M + 1; $Fe(2\text{-BIK})_3$).
(8) Data for $[Fe^{II}(2\text{-BIK})_3](ClO_4)_2$. Anal. Calcd for $C_{21}H_{18}N_{12}O_3Fe(ClO_4)_2 \cdot 2H_2O$ (found): C, 32.43 (32.58); H, 2.83 (2.72); N, 21.62 (21.54); Cl, 9.14 (9.11); Fe, 7.21 (7.07). Mass spectral data (FAB): *m/e* 543 (M + 1), 380 (M − (2-BIK)). UV (EtOH), λ_{max}, nm (ε, M^{-1} cm^{-1}): 570 (2000), 330 (46 000), 322 (46 400), 293 (26 500), 283 (23 500). IR (KBr): $\nu_{C=O}$ 1630 cm^{-1}. ^1H NMR (250 MHz, DMSO-d_6), δ, ppm: 7.4, $C_{4/5}H$; 21.1, $C_{4/5}H$; 36.8, N_1H (1:1:1). ^{13}C NMR (DMSO-d_6) (without decoupling), δ, ppm (*J*, Hz): 492.3, C_α, s; 290.1, $C_{4/5}$, d (J_{CH} = 200); 186.6, $C_{4/5}$, d (J_{CH} = 185); 167.4, C_2, s (1:2:2:2). Preliminary magnetic susceptibility measurements by Evans' method[6] gave μ_B = 2.0, 2.8, 4.0, 4.4, and 4.7, respectively, at 230, 251, 278, 296, and 307 K, the latter value indicating a nearly pure high-spin state. Comparison of these results with the temperature dependence of the visible spectra shows that, from 293 to 193 K, a MeOH solution of **2**(II) exhibits a red shift (λ_{max}, 570 → 588 nm) and an increase of the molar absorptivity (ε, 2000 → 6000 M^{-1} cm^{-1}), suggesting that the intensification of the blue color is related to the population of the low-spin isomer. Complex $[\mathbf{2}(II)](ClO_4)_2$ was identical with an authentic sample prepared from iron(II) and 3 equiv of 2-BIK, the latter being prepared from 1-(diethoxymethyl)imidazole by the same procedure as in ref 15: mp 282 °C dec (lit.[4] mp 285 °C dec); λ_{max} (EtOH) 326 nm (ε = 19 700) 286 nm (ε = 10 250).
(9) Reference solutions containing Fe(II), 2-BIM, and 2-BIK in the ratios 1:3:0, 1:2:1, 1:1:2, and 1:0:3 have been prepared and analyzed by ^1H NMR and UV spectroscopy and cyclic voltammetry (CV). Their respective absorbances at 330 nm were found to be proportional to the amount of bound 2-BIK. CV shows two shoulders on the oxidation wave to **2**(III) with varying intensities according to the proportions of the intermediate complexes.
(10) Whereas **1**(III)/**1**(II) gives a quasi-reversible redox reaction, the electrochemical behavior of **2**(III)/**2**(II) implies a chemical transformation of **2**(III) whose products after reduction give back the **2**(II) complex.

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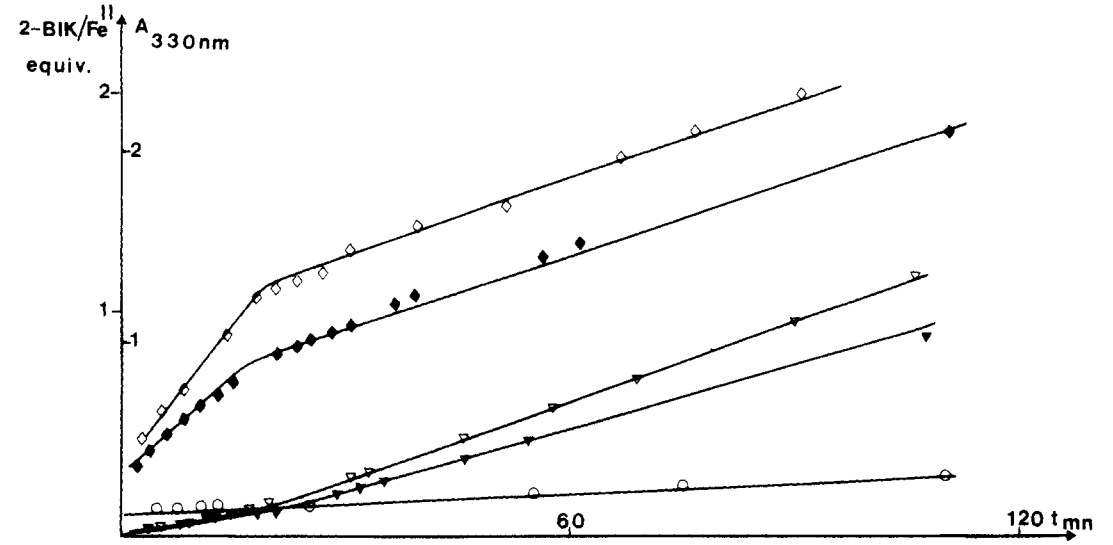

Figure 1. Oxidation of the 2-BIM ligand in $[Fe^{II}(2\text{-BIM})_3](ClO_4)_2$ ($[\mathbf{1}(II)](ClO_4)_2$) and $[Fe^{III}(2\text{-BIM})_3](ClO_4)_3$ ($[\mathbf{1}(III)](ClO_4)_3$) 1 mM in CH_3OH under an oxygen atmosphere ($[O_2]$ = 5 mM). The absorbance at 330 nm, which is proportional to the amount of 2-BIK bound to Fe^{II},[9] is recorded as a function of time: (▼) **1**(II); (▽) **1**(II) + 1 mM 1-MeIm; (○) **1**(III); (♦) **1**(III) + 1 mM 1-MeIm; (◇) **1**(III) + 3 mM 1-MeIm.

1(III) prepared by electrochemical oxidation of **1**(II), 4mM in methanol, is stable under air for a few hours at room temperature. If, to this solution of **1**(III), one equivalent of 1-methylimidazole (1-MeIm) is added, a complete Fe(III) reduction is observed within 20 min (cyclic voltammetry) and a deep blue color develops within 30 min, indicative of the formation of the BIK complexes. This reaction is faster with piperidine than with 1-MeIm.

The oxidations of both **1**(III) and **1**(II), under a dioxygen atmosphere, in the presence or absence of 1-MeIm, have been followed by monitoring, at 330 nm, the appearance of 2-BIK bound to Fe(II)[9] (Figure 1). The **1**(III) to **2**(II) conversion in the presence of 1-MeIm is clearly biphasic. In the first step, there is a rapid increase of absorbance up to a value corresponding roughly to the formation of one 2-BIK ligand per initial **1**(III). This step is dependent on the base concentration whereas the slower second step is not. The oxidation of **1**(II) into **2**(II) starts very slowly and then proceeds at a rate comparable to that of the second step of the oxidation of **1**(III). It shows a small dependence on the presence of base. It is noteworthy that the reaction starting from **1**(II) is slower under an air atmosphere than under dioxygen atmosphere whereas this is not the case for the reaction starting from **1**(III).

The air oxidation of 2-BIM into 2-BIK can be run with ratios of ligand to iron(III) larger than 3. However, this catalytic reaction is not very efficient. With 20mM 2-BIM and 0.4mM $FeCl_3$ or $Fe(ClO_4)_3$ (50:1) in ethanol at 50 °C, the reaction does not proceed beyond 50% formation of 2-BIK after 3 days.

It was reported some years ago that, under a dioxygen atmosphere, Cu^{2+}—but not Fe^{2+} or Fe^{3+}—catalyzed the oxidation of bis(1-methylbenzimidazol-2-yl)methane and bis(2-pyridyl)-methane into the corresponding ketones.[11,12] An oxidation reaction comparable to that of **1**(II) into **2**(II) has been observed with Fe(II) complexes containing bis(β-diimine) macrocyclic ligands to give tetraenedione complexes. The following mechanism was postulated: Fe^{II} generation of the superoxide ion, $O_2^{\bullet-}$ abstraction of a proton from the β-diimine methylene followed by Fe^{III} oxidation of the anion, and coupling of the resulting radical with $HO_2^{\bullet}$, giving the hydroperoxide precursor of the keto group.[13] For the Co(III) complex of an analogous (β-diimine) macrocyclic ligand, the base-induced generation of a Co(II)–methine radical intermediate was demonstrated and proposed to be part of the path of the ligand oxygenation.[14]

Our results suggest that both mechanisms are valid for the oxidation of 2-BIM into 2-BIK depending on the oxidation state of the iron in the starting $(2\text{-BIM})_3$ complex. The fact that the more efficient reaction starting from **1**(III) requires a nitrogenous base supports a base-assisted Fe(III)-catalyzed oxidation of the first 2-BIM ligand. The slowdown of the oxidation reaction after the formation of one 2-BIK ligand per molecule of **1**(III) is probably dependent on the rate of the dioxygen oxidation of $[(2\text{-BIM})_{3-n}(2\text{-BIK})_n]Fe^{II}$ to the Fe(III) complexes ($0 < n < 2$).

We have observed a similar and faster oxidation reaction in the case of bis(*N*-methylimidazol-2-yl)methane giving the blue complex tris[bis(*N*-methylimidazol-2-yl) ketone-*N*,*N*']iron(II).[15,16] A detailed analysis of the mechanism is under way.

It is noteworthy that bis(imidazol-4-yl)methane[2] (4-BIM) gives the colorless $[Fe^{II}(4\text{-BIM})_3]^{2+}$ (**3**(II)) and the yellow $[Fe^{III}(4\text{-BIM})_3]^{3+}$ (**3**(III)) complexes (redox potential 0.31 V in CH_3CN), when added respectively to iron(II) and iron(III) salts. **3**(II) in methanol is slowly oxidized into **3**(III) in the presence of air, but no oxidation of the ligand is observed, even in the presence of 1 equiv of 1-MeIm. The lower acidity of the 4-BIM methylene compared to that of 2-BIM[17] may explain why it is not oxidized when bound to Fe(III).

The **1**(III) to **2**(II) oxidation reaction, in the presence of base, presumably via an intermediate hydroperoxide, can be compared to the oxidation of the 1,4-pentadienyl units of fatty acids by the Fe(III) of lipoxygenases.[18,19]

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Registry No. $[Fe^{II}(2\text{-BIM})_3](ClO_4)_2$, 124756-01-0; $[Fe^{II}(2\text{-BIM})_3]Cl_2$, 124756-02-1; $[Fe^{II}(2\text{-BIM})_3](BF_4)_2$, 124756-03-2; $[Fe^{III}(2\text{-BIM})_3](NO_3)_3$, 124756-05-4; $[Fe^{III}(2\text{-BIM})_3](ClO_4)_3$, 124779-89-1;

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$[Fe^{III}(2\text{-BIM})_3]Cl_3$, 124756-06-5; $[Fe^{II}(2\text{-BIK})_3](ClO_4)_2$, 124756-08-7; $[Fe^{III}(2\text{-BIK})_3]^{3+}$, 124756-09-8; 2-BIM, 64269-81-4; 1-MeIm, 616-47-7.

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X-ray Absorption Spectroscopic Evidence for Binding of the Competitive Inhibitor 2-Mercaptoethanol to the Nickel Sites of Jack Bean Urease. A New Ni–Ni Interaction in the Inhibited Enzyme

Jack bean urease (EC 3.5.1.5), the first nickel-containing metalloenzyme identified,[1] catalyzes the hydrolysis of urea to carbon dioxide and ammonia. The enzyme consists of a hexamer of identical subunits, each containing two nickel ions and one catalytic site.[2] While the biochemical properties of urease have been characterized,[3] detailed physical studies of the nickel active site have been undertaken only recently. In particular, magnetic susceptibility measurements have now indicated a weak magnetic exchange interaction between the two paramagnetic Ni(II) ions, providing evidence for a binuclear Ni(II) active site in urease.[4] Further, competitive inhibitors have been shown to dramatically affect the ground-state electronic properties of the urease Ni(II) ions. On addition of the competitive inhibitor 2-mercaptoethanol (2-ME; $K_i = 0.72 \pm 0.26$ mM at 25 °C[3]), near-UV absorption bands arise that have been assigned as thiolate→Ni(II) charge-transfer transitions, suggesting direct binding of the thiolate to the nickel ion(s) ($K_d = 0.95 \pm 0.05$ mM at 25 °C[3]). Binding of 2-ME to urease also causes the Ni(II) ions to become diamagnetic.[4] Reported herein are the results of a preliminary structural investigation using X-ray absorption spectroscopy (XAS) of the nickel sites of urease in its native and 2-ME-bound forms. This work confirms the direct binding of 2-ME to Ni(II) through the thiolate sulfur.

XAS has proven to be a useful structural probe of the active sites of nickel-containing enzymes,[5-7] the edge region yielding information about electronic structure (site symmetry, oxidation state, covalency)[8] and the extended X-ray absorption fine structure (EXAFS) region yielding the metrical details of the local nickel coordination environment. The urease Ni XAS data collection and reduction were accomplished as summarized in Table I.

Table I. X-ray Absorption Spectroscopic Data Collection and Reduction

sample(s)	urease (native and 2-Me-treated)	
	edges	EXAFS
SR facility	SSRL	SSRL
beam line	VII-3	II-2 (focused)
monochromator crystal	Si[400]	Si[111]
detection method	fluorescence	fluorescence
detector type	Ar ion chamber[a]	13-element solid-state array[b]
scan length, min	17	24
av no. of scans	3	13–14
metal concn, mM	2.1	1.4
temp, K	9	11
energy standard	Ni foil (1st inflcn)	Ni foil (1st inflcn)
energy calibration, eV	8331.6	8331.6
E_0, eV	8350	8350
precdge bkgd energy range, eV (polynomial order)	8020–8300 (2)	8370–9000 (2)[c]
spline bkgd energy range, eV (polynomial order)	8390–8732 (2)	8370–8531 (3) 8531–8750 (3) 8750–9000 (3)

[a] EXAFS Co., Seattle, WA. [b] Courtesy of S. P. Cramer, National Synchrotron Light Source, Brookhaven National Laboratory.[14] [c] The background was calculated from fitting this (EXAFS) region; then a constant was subtracted so that the background matched the data just before the edge.

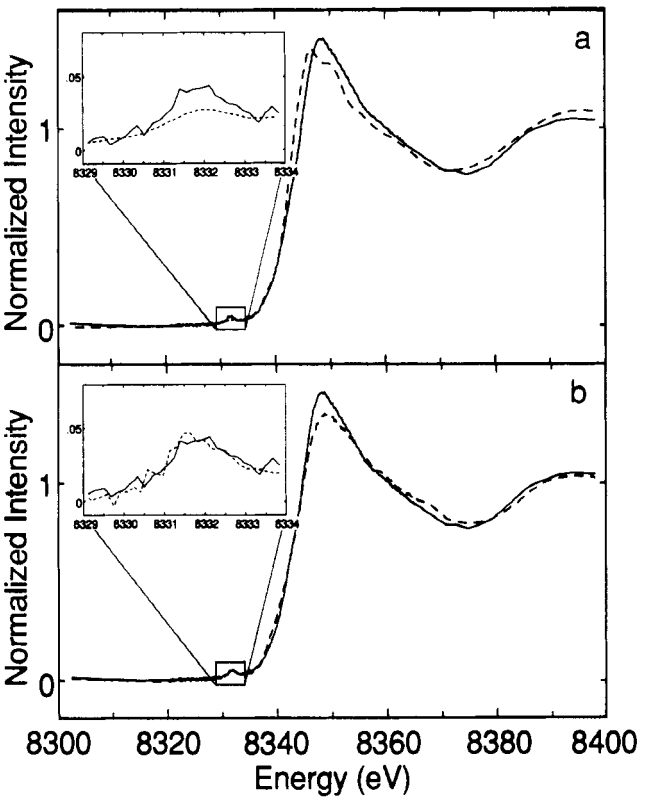

Figure 1. Comparison of the Ni K-edge X-ray absorption spectral region for (a) native urease (—) and $[Ni(en)_3]Cl_2 \cdot 2H_2O$ (- - -) and (b) native (—) and 2-ME-treated urease (- - -). The insets show an expanded view of the region near the 8332-eV 1s → 3d transition.

Urease was isolated, purified, and assayed as previously described.[4] 2-ME (15 mM) was added by equilibrium dialysis. The native and 2-ME-bound samples had specific activities >74% and >68% (determined after removal of the thiolate inhibitor), respectively, of the maximum reported[9] (2700 IU/mg), based on $\epsilon_{280} = 6.2 \times 10^4$ M^{-1} cm^{-1} $subunit^{-1}$; lower limits are reported because aggregation of jack bean urease results in increased absorbance at 280 nm due to light scattering and thus a lower specific activity based on protein concentration determined by A_{280}. Comparison

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