$[Fe^{III}(2\text{-BIM})_3]Cl_3$, 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.

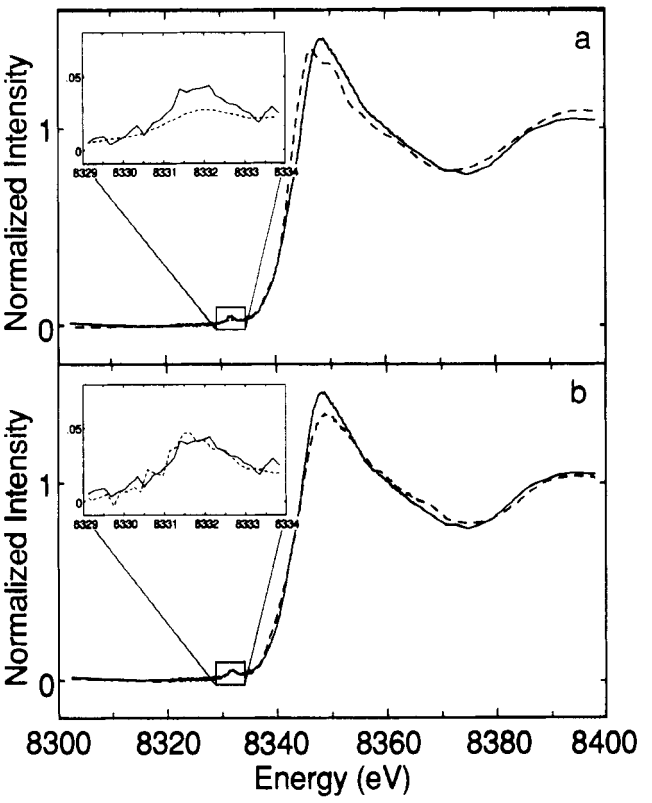

Figure 1. Comparison of the Ni K-edge X-ray absorption spectral region for (a) native urease (—) and $[Ni(en)_3]Cl_2 \cdot 2H_2O$ (- - -) and (b) native (—) and 2-ME-treated urease (- - -). The insets show an expanded view of the region near the 8332-eV 1s → 3d transition.

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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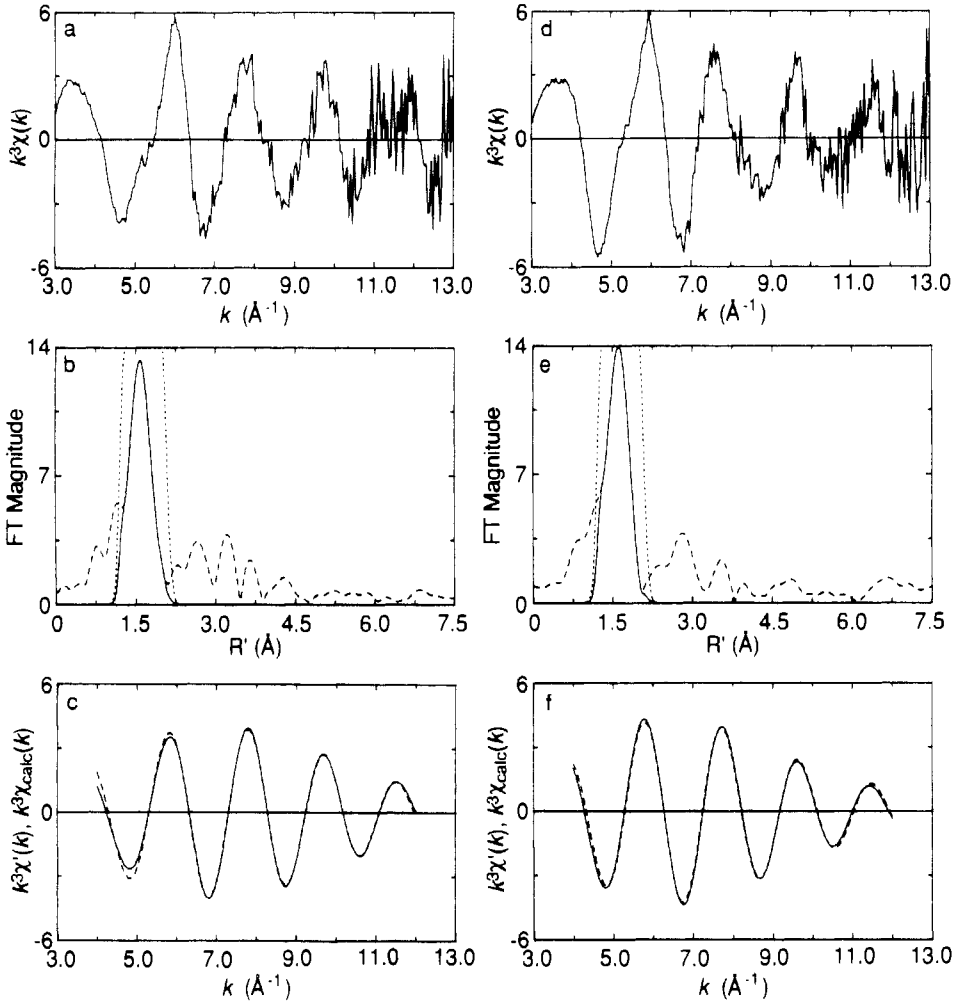

Figure 2. Comparison of raw EXAFS data and analyses for native urease (left column) and urease + 2-ME (right column). Plots a and d are the raw $k^3\chi(k)$ data for the two samples; plots b and e are the Fourier transforms (k^3-weighted, k = 3.0–13.0 Å^{-1}) (– –) of the data in plots a and d, respectively, showing the windows (- - -) used for the first-shell filters, which are shown as solid lines in plots c and f, respectively. The best curve fits to the first-shell filtered data are shown as the dashed lines in plots c and f and correspond to fits 1 and 6 in Table II, respectively.

of the specific activity based on A_{280} and the specific activity based on Ni content (maximum value 1.33×10^{11} IU/Ni) indicates the urease samples had ca. two Ni atoms per subunit. The XAS samples were run as frozen glasses in 50–60% glycerol. Nickel concentrations (Table I) were determined by atomic absorption spectroscopy. Urease samples exposed to the X-ray beam retained >90% of their original activity.

The nickel X-ray absorption edge spectrum of native urease is compared in Figure 1a with the edge spectrum of the approximately octahedral NiN_6 compound $[Ni(en)_3]Cl_2 \cdot 2H_2O$ (en = 1,2-ethylenediamine). The generally featureless edge shape is characteristic of pseudo-octahedral Ni(II) geometry.[8] The slightly enhanced intensity of the 8332-eV 1s → 3d transition in the native urease edge is indicative of a slight distortion from the ideally centrosymmetric octahedral geometry.[8] Curve-fitting analysis of the Ni EXAFS region (Figure 2, Table II) confirms the presence of five or six (N,O)-containing ligands at an average Ni–(N,O) distance of 2.06 Å. These results are in substantial agreement with an earlier XAS determination of the native urease Ni(II) site structure (using lower quality EXAFS data)[10] and with magnetic[4] and spectroscopic[11,12] evidence for a pseudooctahedral Ni(II) site.

Addition of 2-ME to native urease causes changes in the Ni X-ray absorption edge and EXAFS spectra as shown in Figures 1b and 2d, respectively. The slight shift of the edge to lower energy and decrease in the edge height are both consistent with an increased covalency of the Ni(II) site expected from coordination of a sulfur-containing ligand.[8] The curve-fitting results (Table

Table II. Curve-Fitting Results for the First Coordination Spheres of Jack Bean Urease and Its 2-Mercaptoethanol Complex[a]

		Ni–(N,O)			Ni–S			
sample	fit	N	R, Å	$\Delta\sigma^2$, Å^2	N	R, Å	$\Delta\sigma^2$, Å^2	f'[b]
oxidized	1	6[c]	2.06	–0.0013				0.021
	2	5	2.06	–0.0026				0.020
	3	5	2.06	–0.0026	1	2.10	0.6066	0.020
	4	3	2.06	–0.0034				0.020
		3	2.07	+0.0018				
oxidized + 2-ME	5	6	2.08	–0.0012				0.034
	6	5	2.07	–0.0020	1	2.29	–0.0000	0.021
	7	3	2.02	–0.0060				0.024
		3	2.14	–0.0065				

[a] N is the number of scatterers per nickel; R is the nickel–scatterer distance; $\Delta\sigma^2$ is a relative mean square deviation in R, $\Delta\sigma^2 = \sigma^2$(sample) – σ^2(reference), where the reference is $[Ni(en)_3]Cl_2 \cdot 2H_2O$[15] at 4 K for Ni–(N,O) and $[(C_6H_5)_4P]_2[Ni(SC_6H_5)_4]$[16] at 4 K for Ni–S. All fits were over the range k = 4.0–12.0 Å^{-1}. Errors in R and $\Delta\sigma^2$ are estimated to be ±0.03 Å and +0.0050/–0.0025 Å^2 for Ni–(N,O) and ±0.02 Å and +0.0019/–0.0013 Å^2 for Ni–(S,Cl).[17] [b] f' is a goodness-of-fit statistic normalized to the overall magnitude of the $k^3\chi(k)$ data:

$$f' = \frac{\{\sum[k^3(\chi_{obsd}(i) - \chi_{calcd}(i))]^2/N\}^{1/2}}{(k^3\chi)_{max} - (k^3\chi)_{min}}$$

[c] Coordination numbers were not varied during optimization.

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II) confirm the requirement of one (S,Cl)-containing ligand (at a Ni-(S,Cl) distance of 2.29 Å) in addition to approximately five (N,O)-containing ligands to simulate the first-shell Ni EXAFS of the 2-ME-bound urease derivative. Although the improvement in f' upon addition of a Ni-(S,Cl) shell to the simulation for the 2-ME-bound derivative (fit 6, Table II) is less than a factor of 2 and could result from simply the increase in the number of parameters, we have attempted other fits, the results of which suggest that the (S,Cl)-containing ligand is required to fit the data. For example, providing the same additional parameters to fit the oxidized urease data does not result in improvement in f', whether this is from a (S,Cl) shell (fit 3) or an additional (N,O) shell (fit 4). Another two-shell fit of the data for the 2-ME-bound derivative (fit 7) using different (N,O) shells does result in f' improvement (suggesting that two separate shells are required for this derivative but not for the oxidized derivative), but the resulting Ni-(N,O) distance for the second shell (2.14 Å) is chemically unreasonable.

The simplest explanation of these curve-fitting results involves the average $Ni(N,O)_5(S,Cl)$ coordination sphere discussed above. The Ni-(S,Cl) Debye-Waller factor derived from EXAFS curve-fitting is similar to that of the $[(C_6H_5)_4P]_2[Ni(SC_6H_5)_4]$ model only for a coordination number of 1 (not 0.5), supporting the binding of one 2-ME thiolate to each nickel or one thiolate simultaneously binding both. The similar intensity of the 8332-eV peak in the native and 2-ME-bound derivatives (Figure 1b) indicates the same slight distortion from octahedral symmetry, suggesting that 2-ME binding is a simple ligand-exchange reaction.

The diamagnetism observed upon 2-ME treatment of urease[4] thus correlates with direct binding of 2-ME to Ni(II) through the thiolate sulfur. Square-planar or substantially tetragonally distorted ligand fields are required for mononuclear Ni(II) compounds to adopt low-spin (diamagnetic) electronic configurations. Our edge data preclude such a large distortion from octahedral symmetry for the urease Ni(II) ions since the transition at 8336 eV, the characteristic signature of tetragonal geometries,[8] is not observed.

The alternative explanation for the diamagnetic ground state involves creation of strong antiferromagnetic coupling between the two high-spin ($S = 1$) Ni(II) ions upon 2-ME binding. Our structural results are fully consistent with the 2-ME thiolate sulfur bridging the two Ni(II) ions and mediating this antiferromagnetic exchange interaction.[13] In such a model, the competitive nature of the 2-ME inhibition would imply a substrate-binding site involving both Ni(II) ions. Future XAS studies will target identification of the Ni···Ni scattering expected from such an inhibitor-bridged binuclear site.

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Comparing the Acidity of Hydride and η^2-Dihydrogen Complexes of Transition Metals

The need for the measurement of the acidity of metal hydride complexes has been stressed, and the pK_a values for a number of carbonyl metal hydride complexes have been determined.[1] There are scattered reports that the proton of the dihydrogen ligand is acidic: for example, in $[CpRu(\eta^2\text{-}H_2)(dmpe)]^+$ (dmpe = $PMe_2CH_2CH_2PMe_2$),[2] $[IrH(\eta^2\text{-}H_2)(bq)(L)_2]^+$ (bq = 7,8-benzoquinolinate; L = PPh_3, PCy_3),[3] $[MH(\eta^2\text{-}H_2)(dppe)_2]^+$ (M = Ru, Fe; dppe = $PPh_2CH_2CH_2PPh_2$),[4] $[FeH(\eta^2\text{-}H_2)(dmpe)_2]^+$,[5] $[Cp^*Ru(CO)_2(\eta^2\text{-}H_2)]^+$,[6] and $[Cp^*Re(CO)(NO)(\eta^2\text{-}H_2)]^+$.[6] The η^2-dihydrogen ligand is known to be deprotonated in preference to the terminal hydride in the complex $[IrH(\eta^2\text{-}H_2)(bq)(L)_2]^+$[3] and in the mixture of complexes $[CpRu(\eta^2\text{-}H_2)(dmpe)]^+$ and $[CpRu(H)_2(dmpe)]^+$.[2] We report here a simple method for the ranking of the acidity of a range of η^2-dihydrogen and dihydride compounds $[CpRu(\eta^2\text{-}H_2)(dppm)]^+$ (dppm = $PPh_2CH_2PPh_2$),[7] $[CpRuH_2(dppe)]^+$,[7,8] $[CpRu(H)_2(dppp)]^+$ (dppp = $PPh_2CH_2CH_2CH_2PPh_2$),[7] $[CpRu(H)_2(PPh_3)_2]^+$,[9] and $[MH(\eta^2\text{-}H_2)(dppe)_2]^+$ (M = Fe, Ru, Os).[10] We also describe how approximate pK_a values can be obtained.

The method involves the determination of the equilibrium constant K_{eq} for the following reaction by NMR spectroscopy:

$$MH + M'H_2^+ \rightleftharpoons MH_2^+ + M'H \qquad (1)$$

We choose dichloromethane as the solvent because it is noncoordinating and it dissolves the neutral and ionic metal complexes without reaction. Although CH_3CN is the preferred solvent for hydride pK_a determinations,[1,2] it displaces H_2 from most dihydrogen complexes including the ones described in this work. In a typical experiment appropriate amounts of a neutral compound and an ionic complex were loaded into an NMR tube and then CD_2Cl_2 was added. After a period was waited to let the system reach equilibrium,[11] a ^1H NMR spectrum was recorded; a typical example is shown in Figure 1. By measuring the intensity of the hydride resonances, one can calculate the relative

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