A dinuclear iron(III) complex with a bridging urea anion: implications for the urease mechanism†

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A novel complex $[\mathrm{Fe^{III}_2(\mu\text{-}O)\{\mu\text{-}OC(NH_2)NH\}(tpa)_2](ClO_4)_3}$ [tpa = tris(2-pyridylmethyl)amine] has been synthesized and fully characterized; its formation from urea and the $[\mathrm{Fe^{III}_2(\mu\text{-}O)(tpa)_2(OH)(H_2O)}]^{3+}$ precursor in aqueous acetonitrile is a reversible multistep process.

Dinuclear metallohydrolases have been recognized as a wide class of enzymes which utilize two metal cations (Zn, Mg, Mn, Fe, Co, Ni) for the activation of different organic substrates for hydrolysis.[1] Urease, which catalyses urea hydrolysis, is probably the best known enzyme in this class. Ureases isolated from various species of plants, fungi and bacteria have invariably contained two $\mathrm{Ni^{II}}$ ions in their active site.[1,2] However, relevant substrates such as ethylurethane can be hydrolyzed by enzymes utilizing other metals. A urethanase with four $\mathrm{Fe^{III}}$ ions per molecule has been reported.[3]

The mechanism of urease action remains under debate. Initially it was proposed that urea binds monodentately to one $\mathrm{Ni^{II}}$ ion and is attacked by a hydroxide which is attached to the second $\mathrm{Ni^{II}}$ ion.[1,2,4] A new proposal for the urease mechanism suggests that both urea and hydroxide initially form bidentate bridges between the $\mathrm{Ni^{II}}$ atoms.[5] Two model nickel compounds with bridging urea anions have been recently characterized.[6] Here, we report the first dinuclear iron complex with bridging ureate having distinct metal sites.

Our approach was based on the rich chemistry of diiron oxo-bridged complexes with tris(2-pyridylmethyl)amine (tpa).[7] The $[\mathrm{Fe^{III}_2(\mu\text{-}O)(tpa)_2(OH)(H_2O)}]^{3+}$ precursor **1** reacts with various ligands HL to form $[\mathrm{Fe^{III}_2(\mu\text{-}O)(\mu\text{-}L)(tpa)_2}]^{n+}$ complexes.[7c,d,8] Compound **1** can also activate acetonitrile for hydrolysis.[7c,d]

The complex $[\mathrm{Fe_2(\mu\text{-}O)\{\mu\text{-}OC(NH_2)NH\}(tpa)_2}]^{3+}$ **2** can be prepared either from **1** and urea in MeCN solution or directly from $\mathrm{Fe(ClO_4)_3}$, tpa, urea and $\mathrm{Et_3N}$ in MeOH.‡ The spectral characteristics of **2** obtained by either method are identical.† The UV–VIS and ^{1}H NMR spectra of **2** are similar to those of relevant acetamidate and acetate bridging complexes, $[\mathrm{Fe^{III}_2(\mu\text{-}O)\{\mu\text{-}OC(Me)NH\}(tpa)_2}]^{3+}$ and $[\mathrm{Fe^{III}_2(\mu\text{-}O)(\mu\text{-}MeCO_2)(tpa)_2}]^{3+}$.[7] A detailed mass spectrometric study allowed the unambiguous assignment of **2** as a ureate complex. The peak with *m/z* 965 {corresponding to $[\mathbf{2}\mathrm{(ClO_4)_2}]^+$} is the most prominent in both the ESMS$^+$ and FAB$^+$ spectra. The peak is shifted to *m/z* 967 in the spectra of the sample of $\mathbf{2}\mathrm{(ClO_4)_3}$ prepared from urea-$^{15}\mathrm{N_2}$. Satellite peak intensities and exact mass determinations confirm the compositions of the *m/z* 965 and 967 ions as $[\mathbf{2}\mathrm{(ClO_4)_2}]^+$ and $[(\mathbf{2}\text{-}^{15}\mathrm{N_2})\mathrm{(ClO_4)_2}]^+$, respectively.

Two different solvated complexes, $\mathbf{2}\mathrm{(ClO_4)_3 \cdot 2H_2O}$ and $\mathbf{2}\mathrm{(ClO_4)_3 \cdot 0.75CO(NH_2)_2 \cdot 0.25H_2O}$, were crystallized and characterized by X-ray diffraction. The molecular dimensions of the complex dinuclear cation **2** in both are very similar (Fig. 1). Bond distances and angles in the asymmetric (tpa)Fe–O–Fe(tpa) unit of **2** are close to those in the relevant carboxylate bridged $[\mathrm{Fe_2(\mu\text{-}O)(\mu\text{-}RCO_2)(tpa)_2}]^{3+}$ complexes [α(Fe–O–Fe) = 130(1)°],[7] which is in agreement with the isoelectronic nature and similar sizes of the delocalized carboxylate ($-\mathrm{CO_2}^-$) and amidate groups ($-\mathrm{CONH}^-$). The structural parameters of the planar ureate anion in **2** [*d*(C–O) = 1.238(12), *d*(C–NH) = 1.311(13), *d*(C–NH$_2$) = 1.439(15) Å] are close to those found in the $[\mathrm{Ni^{II}_4\{\mu_3\text{-}OC(NH)NH_2\}(L)_2}]^{4+}$ complex [*d*(C–O) = 1.254, *d*(C–NH) = 1.311, *d*(C–NH$_2$) = 1.427 Å][6b] and those calculated by an *ab initio* method for $\mathrm{OC(NH_2)NH^-}$ [*d*(C–O) = 1.260, *d*(C–NH) = 1.324, *d*(C–NH$_2$) = 1.488 Å].[9] The bent (tpa)Fe–O–Fe(tpa) core has two non-equivalent vacant positions at the potentially hexacoordinate $\mathrm{Fe^{III}}$ atoms, one with a tertiary aliphatic N atom and the other with a pyridine N atom as *trans* donors. The ureate anion in **2** coordinates with its oxygen atom *trans* to the pyridine N atom, which apparently allows for a stronger π-interaction along the $\mathrm{O_{ureate}}$–Fe–$\mathrm{N_{pyr}}$ axis *versus* the possible $\mathrm{N_{ureate}}$–Fe–$\mathrm{N_{pyr}}$ alignment.[10]

The equilibrium and kinetics of formation of **2** from the precursor **1** and urea were studied in aqueous acetonitrile (0.05–1.3 M H$_2$O) at 25 °C. In the presence of excess urea, **2** is stable in these solutions for weeks.

Spectrophotometric titrations gave the equilibrium constant K = 650 ± 100 M for reaction (1) confirming the release of two molecules of water.

$$\mathbf{1} + \mathrm{CO(NH_2)_2} \rightleftarrows \mathbf{2} + \mathrm{2H_2O} \qquad (1)$$

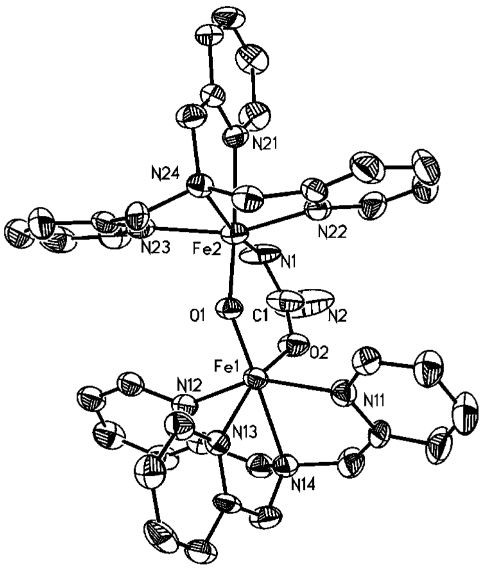

Fig. 1 Molecular structure of the complex cation in $\mathbf{2}\mathrm{(ClO_4)_3 \cdot 0.75CO(NH_2)_2 \cdot 0.25H_2O}$. Selected distances (Å) and angles (°): Fe1–O1 1.791(5), Fe1–O2 2.001(6), Fe2–O1 1.812(5), Fe2–N1 1.984(8); Fe1–O1–Fe2 129.9(3), O2–C1–N1 125.1(11), O2–C1–N2 115.2(11), N1–C1–N2 119.7(11).

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† Electronic supplementary information (ESI) available: selected data for **2** kinetic data and rotatable 3-D crystal structure diagram in CHIME format. See http://www.rsc.org/suppdata/cc/b0/b000286k/

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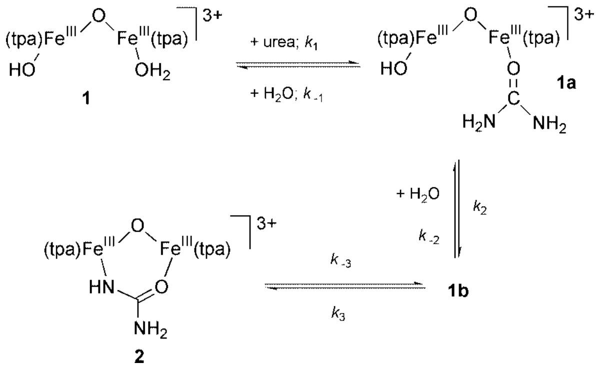
The kinetics of reaction (1) were studied by a stopped-flow technique under concentration conditions, which made the formation of **2** almost irreversible (yield > 96%). The excess of urea (8–60 fold) and water (> 100 fold) also provided pseudo-first order conditions. Two distinct steps are observed in reaction (1). In the first step, there is a nearly instantaneous (within the mixing time of the stopped-flow instrument, 1–2 ms) change in the optical spectrum when the solutions of **1** and urea are mixed. The dependence of this initial absorbance change on $[H_2O]$ and $[CO(NH_2)_2]$ corresponds to the formation of intermediate **1a** in a preequilibrium with $K_1 = 9 \pm 3$ (Scheme 1). It has been shown that the O-bonded urea is usually the first kinetic product of complex formation.[11] Thus, **1a** is most likely the $[Fe_2(\mu\text{-}O)(tpa)_2(OH)\{\eta_1\text{-}OC(NH_2)_2\}]^{3+}$ complex.



Scheme 1

The following step is observed as a nearly single exponential change of optical absorbance with the rate constant k_{obs} (0.1–1.8 s^{-1} under our experimental conditions) and corresponds to the formation of **2** from the preequilibrium mixture of **1** and **1a**. The process is accelerated by urea and decelerated by water. The simplest model that can account for the concentration dependences is shown in Scheme 1 and eqn. (2) (see also ESI).†

$$k_{obs} = \frac{k_2(k_3/k_{-2})}{(k_3/k_{-2}) + [H_2O]} \cdot \frac{K_1[CO(NH_2)_2]}{K_1[CO(NH_2)_2] + [H_2O]} \qquad (2)$$

Optimization of this model by non-linear least squares methods gave the parameters $K_1 = k_1/k_{-1} = 8.9$, $k_2 = 2.6\ s^{-1}$ and $k_3/k_{-2} = 0.185$ M. Combination of the kinetic data with the equilibrium constant $K = 650$ M for the overall process (1) gave $k_{-3} = 6.6 \times 10^{-3}\ s^{-1}$ (Scheme 1).

The results of this study help us to understand the potential advantages and drawbacks of the new bidentate mechanism of urease activity[5] when compared to the previously accepted monodentate mechanism.[2,4] It is believed that the hydrolytic stability of the urea molecule is due to high resonance stabilization energy, which may be reduced on coordination to metal ion(s).[2,12] Bidentate N,O-coordination of urea leads to partial loss of resonance as witnessed by a significant elongation of the C–NH$_2$ bond in the μ-OC(NH$_2$)NH$^-$ ligand of **2** [d(C–NH$_2$) = 1.44 Å] *versus* uncoordinated urea [d(C–NH$_2$) = 1.34 Å].[13] In the neutral μ-OC(NH$_2$)NH$_2$ ligand both C–NH$_2$ bonds are elongated [$d(C\text{–}NH_2)_{endo} = 1.42$, $d(C\text{–}NH_2)_{exo} = 1.37$ Å] as was found in a Co^{II} complex.[14] In contrast, monodentate coordination of urea (typically *via* the O atom) is known to preserve its metric parameters [d(C–NH$_2$) = 1.34(1) Å].[15] Thus, the loss of resonance stabilization on bidentate N,O-coordination might be a key factor in urea activation by two metal ions in urease.

The protonation state of the bridging urea ligand is another important issue which was overlooked in the bidentate mechanism of urease action.[5] In the monodentate mechanism it did not arise,[2,4] because monodentate O-coordinated urea in model complexes has almost the same high pK_a as the free ligand (13 *cf.* 13.5).[16] However, N-coordination (which can be achieved in kinetically inert model complexes) is known to change the acidity of urea protons dramatically, down to pK_a = 3(1) in aqueous solution.[16] Quantitative formation of ureate complex **2** in the Et_3N/Et_3NH^+ buffer in MeOH confirms the enhanced acidity of urea on bidentate N,O-coordination. Because of the lower polarizing power of the Ni^{II} ions, a μ-OC(NH$_2$)NH$_2$ ligand coordinated to them will probably be less acidic than that coordinated to Fe^{III}. However, formation of a bridging urea anion still should be considered in urease under its optimal pH 4–8.[4] Relatively slow formation of the bidentate ureate and its hydrolytic stability as seen in complex **2** suggest that μ-OC(NH$_2$)NH$^-$ is unlikely to be the actual activated form of urea in the enzyme. An alternative possibility is that urease modulates the pK_a of the μ-OC(NH$_2$)NH$_2$ ligand to avoid its premature deprotonation.

Notes and references

‡ Precursor $\mathbf{1}(ClO_4)_3$ was prepared by a published procedure.[7d]

Synthesis of **2**: urea (0.03 g, 0.5 mmol), tpa (0.29 g, 1 mmol) and Et_3N (0.152 g, 1.5 mmol) were dissolved in 40 ml of MeOH and added to a solution of $Fe(ClO_4)_3{\cdot}9H_2O$ (0.516 g, 1 mmol) in 10 ml of MeOH. Compound $\mathbf{2}(ClO_4)_3{\cdot}H_2O$ crystallized as a brown solid from the resulting yellow solution on standing overnight in a refrigerator. Elemental analysis: calc. for $C_{37}H_{43}Cl_3Fe_2N_{10}O_{16}$: C, 40.33; H, 3.93; N, 12.71; Fe, 10.14. Found: C, 40.27; H, 3.55; N, 12.46; Fe, 10.37%. Single crystals of $\mathbf{2}(ClO_4)_3{\cdot}2H_2O$ formed on recrystallization from MeCN. When the recrystallization was carried out in the presence of an added excess of urea, single crystals of $\mathbf{2}(ClO_4)_3{\cdot}0.75CO(NH_2)_2{\cdot}0.25H_2O$ were obtained.

Crystal data for **2** $(ClO_4)_3{\cdot}2H_2O$: $C_{37}H_{43}Cl_3Fe_2N_{10}O_{16}$, $M_w = 1101.86$, monoclinic, space group $P2_1/c$, $a = 12.178(3)$, $b = 10.329(5)$, $c = 39.179(10)$ Å, $\beta = 94.65(2)°$, $V = 4912(3)$ Å^3, $Z = 4$, μ(Mo-Kα) = 0.829, $T = 293(2)$ K, 5489 reflections measured, $R1[I > 2\sigma(I)] = 0.070$, $wR2 = 0.28$ for all reflections. For $\mathbf{2}(ClO_4)_3{\cdot}0.75CO(NH_2)_2{\cdot}0.25H_2O$: $C_{37.5}H_{42.5}Cl_3Fe_2N_{11.5}O_{15}$, $M_w = 1115.38$, monoclinic space group $P2_1/c$, $a = 12.1610(15)$, $b = 10.4451(17)$, $c = 38.758(10)$ Å, $\beta = 97.137(16)°$, $V = 4885.1(16)$ Å^3, $Z = 4$, μ(Mo-Kα) = 0.834, $T = 293(2)$ K, 6383 reflections measured, $R1[I > 2\sigma(I)] = 0.067$, $wR2 = 0.206$ for all reflections. CCDC 182/1607. See http://www.rsc.org/suppdata/cc/b0/b000286k/ for crystallographic files in .cif format.

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