Experimental and theoretical vibrational spectroscopy studies of acetohydroxamic acid and desferrioxamine B in aqueous solution: Effects of pH and iron complexation

DAVID C. EDWARDS, STEEN B. NIELSEN, ANDRZEJ A. JARZECKI, THOMAS G. SPIRO, and SATISH C. B. MYNENI

Abstract—The deprotonation and iron complexation of the hydroxamate siderophore, desferrioxamine B (desB), and a model hydroxamate ligand, acetohydroxamic acid (aHa), were studied using infrared, resonance Raman and UV-vis spectroscopy. The experimental spectra were interpreted by a comparison with DFT calculated spectra of aHa (partly hydrated) and desB (reactive groups of unhydrated molecule) at the B3LYP/6-31G* level of theory. The ab initio models include three water molecules surrounding the deprotonation site of aHa to account for partial hydration. Experiments and calculations were also conducted in D2O to verify spectral assignments. These studies of aHa suggest that the cis-keto-aHa is the dominant form, and its deprotonation occurs at the oxime oxygen atom in aqueous solutions. The stable form of iron-complexed aHa is identified as Fe(aHa), for a wide range of pH conditions. The spectral information of aHa and an ab initio model of desB were used to interpret the chemical state of different functional groups in desB. Vibrational spectra of desB indicate that the oxime and amide carbonyl groups can be identified unambiguously. Vibrational spectral analysis of the oxime carbonyl after deprotonation and iron complexation of desB indicates that the conformational changes between anion and the iron-complexed anion are small. Enhanced electron delocalization in the oxime group of Fe-desB when compared to that of Fe(aHa), may be responsible for higher stability constant of the former.

1. INTRODUCTION

The chemistry of biomacromolecules influences the dissolution of minerals, solubility and redox cycling of metals, and the acquisition of metals by organisms (Neilands, 1981; Hersman et al., 1995; Holmen et al., 1997; Barbeau et al., 2001). Of the different macromolecules, siderophores are important because of their common occurrence in terrestrial and marine systems, and their high metal complexation characteristics. Bacteria secrete siderophores to obtain iron for metabolic purposes in iron-limiting situations (Neilands, 1981; Bossier et al., 1988; Matzanke et al., 1989). Because of their strong binding ability, siderophores can also affect the ligand-promoted dissolution of iron(III) hydrox(oxides) and alter the geochemical cycling of iron in the environment (Stumm and Salzburger, 1992; Hersman et al., 1995; Holmen et al., 1997; Kraemer et al., 1999; Kalinowski et al., 2000). The high stability constants of siderophore-metal complexes also make them amenable for applications in the extraction, concentration and remediation of metal contaminated soils and sediments at waste repositories (Brainard et al., 1992), and in the treatment of siderosis (Bergeron and Brittenham, 1994). Information on the coordination environment and the chemical state of Fe-siderophore complex is central to the evaluation of the role of siderophores in the aforesaid processes. A detailed understanding of the molecular behavior of siderophores in aqueous solutions and at mineral-water interfaces is necessary to assess their role in different biogeochemical processes in the environment. Here we present an experimental and theoretical investigation of the functional group chemistry of a hydroxamate siderophore (desferrioxamine B, desB) and its iron complexation behavior in aqueous solutions.

Characterization of siderophores in conditions representative of natural systems has been difficult due to their low concentration and interference from other organic species. Recently, researchers began isolating siderophores from natural samples and characterizing their crystaline Fe complexes using X-ray crystallography (vanderHelim and Poling, 1976; Hossain et al., 1983; Hossain et al., 1998; Dhungana et al., 2001). In addition, researchers are using smaller molecules, which contain the same functional groups as siderophores, to understand the behavior of siderophores (Santos et al., 1993; Farkas et al., 1999). Several studies have also indicated that infrared spectroscopy is an ideal tool to examine the functional group chemistry of organic molecules and their metal complexes (Holmen et al., 1997; Boily et al., 2000; Loring et al., 2000; Duckworth and Martin, 2001; Strathmann and Myneni, 2004). However, the structure of the metal complex is difficult to assess when the complexed ligand has multiple functional groups with overlapping infrared absorption transitions. To overcome this situation, we used resonance Raman spectroscopy, a technique sensitive to the coordination environment around the metal in a metal-siderophore complex.

Among the different siderophores in the environment, desB is particularly interesting because of its ubiquity (Fig. 1). The concentration of desB varies in natural environments, with concentrations as high as micromolar levels in the soil rhizosphere, and nanomolar concentrations in seawater (Powell et al., 1980; Bergeron and Pegram, 1988). In aqueous solution,
desB has four proton dissociation constants (pKₐ) at 8.3, 9.0, 9.46, and 10.84 (measured in KCl solutions of ionic strength, 0.1 mol/dm³), which correspond to successive deprotonation of the 3-hydroxamate groups (NOH), and the amine group (NH₃⁺) (Farkas et al., 1999), respectively. The interactions of desB with Fe(III) in solution and at particle interfaces are poorly understood in the environmental pH range of 3 to 9.

A small hydroxamate siderophore analog, acetohydroxamic acid (aHa), is also used to elucidate the molecular structure of desB in aqueous solutions. Acetohydroxamic acid is an oxime that exists in either an enol or keto form and has a pKₐ of 9.37 (Schwarzenbach and Schwarzenbach, 1963; Fig. 1). Theoretical studies indicate that the keto-form is dominant in aqueous solution with the cis conformation being more stable than the trans conformation (Hadzi and Prevorsek, 1957; Bagno et al., 1994). However, the deprotonation site of aHa in aqueous solutions is debated because of its two acidic protons (N-H, NO-H), and discrepancies that exist between the NMR predicted and theoretically derived structures (Fitzpatrick and Ma geswaran, 1989; Ventura et al., 1993; Bagno et al., 1994; Yazal and Pang, 1999). An NMR study of aHa in aqueous solution revealed that NO-H is the primary site of deprotonation (Bagno et al., 1994), while calculations on anhydrous gas-phase aHa predicted N-deprotonation (Ventura et al., 1993; Bagno et al., 1994; Yazal and Pang, 1999). However, calculations also indicated that the addition of solvated water molecules stabilized O-deprotonated species more than the N-deprotonated species (Ventura et al., 1993). Complexation of aHa with Fe(III) shows that the Fe(aHa), is the dominant species in the pH range of 4–10 (Holmen et al., 1997; Farkas et al., 1998), with aHa forming three five-membered rings around Fe (Monzyk and Crumbliss, 1979; Brink and Crumbliss, 1984; Crumbliss, 1990).

The goal of this research is to evaluate: 1) the stable structures of aHa and desB in aqueous solution, 2) the nature of Fe(III) complexation with aHa and desB, and 3) the influence of Fe(III) complexation on the amide and amine groups of desB that are not involved in complex formation. By combining experimental (UV-vis, IR and resonance Raman) and ab initio (DFT) analysis, the functional group chemistry of aHa and desB and the structures of their Fe(III) complexes are evaluated. This work represents part of a larger effort to characterize molecular interactions between metal ions and organic molecules in aqueous solutions and at mineral-water interfaces, and their role in various biogeochemical reactions.

2. MATERIALS AND METHODS

2.1. Preparation of Solutions

Aqueous solutions of acetohydroxamic acid (Aldrich), desferrioxamine mesylate (Sigma), FeCl₃ · 6H₂O (Fisher Scientific), NaOH (Fisher Scientific), and HCl (Fisher Scientific) were prepared with high purity 18 MW cm⁻¹ water (Milli-Q Plus, Millipore) or 99.99% D₂O (Cambridge Isotope Lab). Aqueous solutions were prepared on a gravimetric basis, and a density of 1.00 g/cm³ was assumed for the solution when converting to a molar scale. The concentrations of aHa and desB were kept constant at 47 (± 2) mM, and 25 (± 2) mM, respectively, in all the vibrational spectroscopy experiments. However, dilute solutions were used for collecting UV-vis spectra (0.4 mM aHa and 0.13 mM FeCl₃, and 0.045 mM desB and 0.045 mM FeCl₃), The pH of aHa and desB were adjusted by adding 0.01 M NaOH or HCl. Iron complexation with these ligands was examined for [aHa]/[FeCl₃] of 3, and [desB]/[FeCl₃] of 1. The pH measurements were made using an Orion 525A pH meter fitted with a semimicro pH probe. In acidic solutions, changes larger than one pH unit occurred for aHa solutions when they were stored at room temperature overnight. To minimize such pH variations, samples were stored at 4°C except when used for spectroscopic analysis. The pH (or pD) of D₂O solutions were measured with the same pH meter and then corrected it by adding 0.41 to the measured pH value (Gary et al., 1964).

2.2. UV-vis Spectroscopy

UV-vis spectra of aqueous aHa, desB, and their Fe (III) complexes were measured on a Hewlett Packard 8452A diode array spectrophotometer. The solutions were placed in quartz cuvettes and scanned in the range of 190–820 nm with water as the background. The resolution of this instrument was 2 nm.

2.3. Vibrational Spectroscopy

Aqueous solutions of aHa and desB and their Fe(III) complexes were analyzed on a Bruker IFS 66 v/s FT-IR spectrometer, using 45°/110° ZnSe detector: Hg-Cd-Te (MCT), number of scans: 500. A reference spectrum of deionized water was collected after each sample, and subtracted from the sample spectrum to reduce the absorption from water at 1636 cm⁻¹. For aHa and desB samples containing D₂O, a
reference spectrum of D₂O together with an aliquot of sample (without the ligands) was used as background. The spectrum of each sample is an average of five independent acquisitions (this was done to reduce the background variations caused by detector drifts). The spectral analysis was performed using the software program Grams 5.2.

Resonance Raman spectra were obtained using an excitation wavelength of 407 nm (Spectra Physics krypton laser) and a 270° backscattering sample geometry. The laser beam (power 20 mW) was focused with a cylindrical lens onto a NMR tube containing the sample, and the scattered light was collected and focused onto a single spectrograph (Chromex) equipped with a CCD (Princeton Instruments). This is an in-house built Raman spectrometer. All samples were scanned 20 times (each 30 s) and averaged. Raman bands of N,N-dimethylformamide at 1662, 1439.7, 1406.6, and 1092.4 cm⁻¹ were used to calibrate all samples. The Raman spectrum of water was used to subtract water contributions from sample.

2.4. Ab Initio Calculations

The geometries of aHa and desB (only part of desB is considered because of size limitations) in different coordination environments were optimized and their vibrational frequencies were calculated at the DFT B3LYP/6-31G* level, using the Gaussian 92 package (Frisch et al., 1992). Three water molecules were placed around the deprotonation site of aHa to mimic an aqueous environment. The intensities of IR and Raman bands were evaluated from the analytical dipole moment and numerical polarizability derivatives, respectively. Calculations performed for the Fe(aHa)₃ complex were within C₃ symmetry constraints. A Lorentzian curve with a fixed half-width of 5 cm⁻¹ was used to represent each calculated vibrational band, and its height corresponds to the calculated intensity. A single scale factor of 0.956 was applied to all calculated harmonic frequencies to account for any systematic errors due to basis set truncation or incomplete treatment of electron correlation (Baker et al., 1998). This factor was obtained from a linear least-squares fit of calculated (this study) and experimental (Lin-Vien, 1991) wave numbers for acetic acid and acetamide. These compounds were chosen based on their functional group similarities to aHa and desB.

3. RESULTS

3.1. Theoretical Studies of aHa and desB

The hydrated species considered in this investigation were: neutral cis-aHa(H₂O)₃, O-deprotonated cis-aHa(H₂O)₃, O-deprotonated trans-aHa(H₂O)₃, and N-deprotonated cis-aHa(H₂O)₃ (Fig. 2). The calculated energies of these hydrated species indicate that O-deprotonated cis-aHa is the most stable anion (Table 1). Although the calculations of Ventura et al. (1993) predict that O-deprotonated aHa is stabilized more by the addition of water molecules when compared to the N-deprotonated aHa, their absolute energies indicate that the latter is the most stable species. The difference between the model used by Ventura et al. (1993) and this study is that they used four water molecules to mimic an aqueous environment. However, the bond distances predicted by Ventura et al. using different basis sets show the same trends with those reported here (Table 1). It is anticipated that hydrogen bonding between the NO-H and C=O in the case of N deprotonated aHa may make it more stable in the calculations conducted by Ventura et al. In the case of the Fe(aHa)₃ complex (Fig. 2), DFT calculations showed that the high-spin Fe(III) complex (S = 6) is energetically more favorable than the low-spin Fe(III) complex in the ground state. This is consistent with the experimental studies on the Fe(aHa)₃ complex (Monzyk and Crumbliss, 1979). All calculated normal modes fall into doubly degenerate (Eᵥ,₁), and total symmetric representations (Aᵥ) within the C₃ point group. There are 30 fundamentals (10 E, 10 A) in the energy region of 900–1700 cm⁻¹). Mass exchange calculations of hydrogen with deuterium on the nitrogen atom were also computed for the selected species. The resulting calculated wavenumbers agree with the measured values (Tables 2–4).

Because of constraints of the number of atoms (or the size of the molecule) that can be used in Gaussian 92, calculations for desB were conducted for part of the molecule, i.e., starting at the amine group and ending with a methyl group after the first amide functional group in desB (as shown in Figs. 1 and 2). This model includes all of the major functional groups of the molecule, and the calculated vibrational frequencies (shown in Table 5) may closely represent the frequencies of the entire molecule. Since the calculations
indicate that there are several vibrational bands from the bending vibrations of methylene groups in the fingerprint region of 900–1500 cm$^{-1}$ (Table 5), the vibrational spectral analysis focused on the amide I band and not on the amide II, amide III or $\nu(N-O)$. The calculated wavenumbers of the vibrational spectra are in good agreement with the experimental data. The magnitude of differences between them is the largest for amide I band of the amide group in the desB model (discrepancy of 85 cm$^{-1}$), and is the smallest for the $\nu(N-O)$ in the Fe(aHa)$_3$ model ($\sim$6 cm$^{-1}$). The large difference between the experimental and the calculated wavenumbers for the amide I band may be caused by the lack of solvated water molecules around the amide and oxime groups in desB. It should also be noted that it was difficult to simulate water solvation and a hydrogen bonding environment using 3 water molecules in these calculations. This can result in the noted differences in the calculated and experimental vibrations of aHa models. However, the Fe(aHa)$_3$ calculations agree closely with the experimental vibrational band energies because all functional groups in Fe(aHa)$_3$ complex are bound to Fe(III) and H-bonding is not an issue.

3.2. Spectroscopy Studies of aHa in Aqueous Solution

3.2.1. Protonation and deprotonation of aHa

Acetohydroxamic acid absorbs at 196 nm in the neutral state (pH $\approx$ 3.68), and this is attributed to the $\pi-\pi^*$ transition of the carbonyl group (Fig. 3). This absorption band shifts to 218 nm upon aHa deprotonation. The redshift is consistent with a weakening (lengthening) of the carbonyl bond strength (Rao, 1967). The loss of a proton causes excess charge on the oxime oxygen atom, leading to conjugation in the aHa CONC core (conjugation through the carbonyl oxygen, the carbonyl carbon, and the nitrogen) to distribute this excess charge (see hydroxyl deprotonation, Fig. 1). The following vibrational spectroscopy results also corroborate these results.

The calculated wavenumbers of the vibrational spectra are in good agreement with the experimental data. The magnitude of differences between them is the largest for amide I band of the amide group in the desB model (discrepancy of 85 cm$^{-1}$), and is the smallest for the $\nu(N-O)$ in the Fe(aHa)$_3$ model ($\sim$6 cm$^{-1}$). The large difference between the experimental and the calculated wavenumbers for the amide I band may be caused by the lack of solvated water molecules around the amide and oxime groups in desB. It should also be noted that it was difficult to simulate water solvation and a hydrogen bonding environment using 3 water molecules in these calculations. This can result in the noted differences in the calculated and experimental vibrations of aHa models. However, the Fe(aHa)$_3$ calculations agree closely with the experimental vibrational band energies because all functional groups in Fe(aHa)$_3$ complex are bound to Fe(III) and H-bonding is not an issue.

![Fig. 3. UV-vis spectra for aHa (top) and desB (bottom). (top) aHa (solid line; 0.4 mM, pH = 3.68), aHa$^-$ (dashed; 0.4 mM, pH = 10.33), and Fe(aHa)$_3$ complex (thick solid line; 0.4 mM aHa, 0.13 mM FeCl$_3$, pH = 4.0). (bottom) Neutral desB (solid line; 0.045 mM, pH = 4.07), desB$^+$ (dashed; 0.045 mM, pH = 10.25), and Fe(III) complexed desB (thick solid line; 1:1 complex, pH = 4.29). Inset is enlarged view of ligand-to-metal charge transfer band of desB.](image-url)
Bending the amide I band, the identification of amide I band is not a problem in D$_2$O solutions and in the Raman spectra of aHa.

The amide I band of aHa in the neutral state (below the pKa) is at 1658 cm$^{-1}$ and shifts to 1618 cm$^{-1}$ upon deprotonation (Table 2; Figs. 4 and 5), consistent with the calculated shifts (Table 1, 2). The amide II band overlaps with the δ$_{NOH}$ band and occurs at 1548 cm$^{-1}$ for neutral aHa. Upon deprotonation, this band becomes more discrete, indicating that the δ$_{NOH}$ character is lost and becomes a relatively pure amide II mode. The $v_{(N-O)}$ band shifts from 1091 to 1096 cm$^{-1}$ upon aHa deprotonation, implying a shorter N-O bond length, which is also in agreement with the calculations (Table 1). Although bond length estimates indicate that the change in N-O bond length is greater than the change in the C=O bond length (Table 1), the magnitude of band shifts are small for the $v_{(N-O)}$ (5 cm$^{-1}$) when compared to that of the amide I band (~35 cm$^{-1}$). This small shift in $v_{(N-O)}$ may be caused by coupling of N-O vibrations with the methyl bending modes.

Infrared spectroscopy studies were also conducted in D$_2$O to complement the studies in water. The bending mode of D$_2$O occurs at 1200 cm$^{-1}$ and does not interfere with the amide I band, which occurs at 1648 cm$^{-1}$ for neutral aHa and shifts to 1618 cm$^{-1}$ upon deprotonation. The $v_{(N-O)}$ stretch is coupled to δ$_{ND}$ and exhibits split peaks at 1112 and 989 cm$^{-1}$ for neutral form, which shift to 1120 and 986 cm$^{-1}$ upon aHa deprotonation, respectively. These bands are more discrete at higher pD, indicating stronger coupling with δ$_{ND}$. Calculated vibrational bands also show similar shifts (Table 3, Figs. 4 and 5).

#### Table 2. Theoretical and experimental vibrational modes and their assignments for aHa in H$_2$O.

<table>
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<tr>
<th>Neutral aHa (cm$^{-1}$)</th>
<th>aHa anion (cm$^{-1}$)</th>
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<tr>
<td><strong>Exptl.</strong></td>
<td><strong>Theory</strong></td>
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<td>pH = 4.89</td>
<td>aHa(H$_2$O)$_3$</td>
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*a* sdef = symmetric deformation, adef = asymmetric deformation, rock = denotes a rocking motion, bend = bending mode, out = out of plane bend.

*b* = cis-aHa(H$_2$O)$_3$.

*c* The compositional assignments were determined from the Gaussian calculations. The number in parentheses refers to the percent contribution each mode (stretching, bending, or rock) has on the frequency.

*d* = Oxygen deprotonated cis-aHa(H$_2$O)$_3$.

*e* = Nitrogen deprotonated cis-aHa(H$_2$O)$_3$.

3.2.2. Fe(III) complexation of aHa

Solution speciation studies indicate that Fe(aHa)$_3$ is the dominant species in the pH range of 4–10 and for Fe(III)/aHa ratios used in this study (Holmen et al., 1997; Farkas et al., 1998). The UV-vis and vibrational spectra show interesting changes when aHa is complexed to Fe(III). While the UV-vis absorption spectrum of the Fe(aHa)$_3$ complex resembles the spectrum of neutral aHa for the p-p* transition of the carbonyl, the vibrational spectrum resembles the anionic form of aHa. The π-π* transition of the carbonyl at 198 nm in the UV-vis spectrum of neutral aHa is unchanged when aHa is complexed with Fe(III) (Fig. 3). In addition, a rela-
Fe(aHa)₃ solutions were also examined at higher pH values, indicating formation of an Fe(III)-hydroxamido complex. The noticeable difference between the low and high pH samples was the increase in the number of Raman active peaks in the latter. This may suggest a change in the coordination, structure, or symmetry of the Fe(aHa)₃, which should result in the appearance of more fundamental vibrational bands in the spectra. In addition, a new band at 1560 cm⁻¹ appears in both H₂O and D₂O solutions, and this may be due to N-deprotonation (data not shown) or to the formation of an Fe(III)-hydroxamido complex.

3.3. Spectroscopy Studies of desB in Aqueous Solution

3.3.1. Protonation and Deprotonation of desB

The spectral variations of desB are similar to those reported for aHa. The π-π* transition of the carbonyl in desB is at 196 nm in acid solutions and shifts to 230 nm as the solution pH is increased. The greater redshift (by ~10 nm more) in desB, when compared to aHa, may be caused by more conjugation in the carbonyl oxygen that stabilizes the negative charge on the oxime group (conjugation through the carbonyl oxygen, the carbonyl carbon, and the nitrogen). In addition, the electron donating ability of the long chain carbon group attached to the nitrogen atom in desB, when compared to aHa, may be caused by more conjugation in the carbonyl group (conjugation through the carbonyl carbon) (Figs. 7 and 8, Table 5), and ii) amide related band around 1620 cm⁻¹, which is not affected by deprotonation significantly. Overlay of the νₐ(C=O) stretch of the amide and oxime groups complicates the identification of...
the $\nu_{C=O}$ of amide group of neutral desB in aqueous solutions (Fig. 7). However, the anionic form of desB exhibits two distinct peaks at 1622 cm$^{-1}$ and 1582 cm$^{-1}$ in D$_2$O solutions, which are assigned to the amide and oxime $\nu_{C=O}$ stretching modes, respectively. The infrared spectra calculated using the desB model verifies that the amide carbonyl is at a higher wave number than the oxime carbonyl in the neutral form (Fig. 7).

### 3.3.2. Fe(III) Complexation of desB

Like the Fe-aHa complex discussed above, the UV-vis absorption spectrum of carboxyls ($\pi$-$\pi^*$ transitions) in Fe-desB complex is similar to that of neutral desB (Fig. 3), and the vibrational band of the oxime carboxyl of Fe-desB is similar to that of the anionic form of desB (Fig. 8). The charge-transfer band of Fe-desB complex occurs at 420 nm, which is blue-shifted by 10 nm when compared to that of the Fe(aHa)$_3$ complex. This suggests that the ligand field stabilization is greater in Fe-desB than the Fe(aHa)$_3$ complex. In the infrared spectrum, the $\nu_{C=O}$ stretch of the Fe(III)-desB complex exhibits two bands at 1572 cm$^{-1}$ and 1587 cm$^{-1}$, which correspond to the amide and oxime carboxyls, respectively (Fig. 8), and these are similar to those reported for the anionic desB.

Ab initio calculations and electronic and vibrational spectroscopy results were useful in the identification of the most likely conformations of aHa, and variations in the functional group chemistry of aHa and desB as a function of pH. This spectral information was used in interpreting Fe(III) complexation by aHa and desB. Since the spectral variations are similar for aHa and desB, they are discussed together in the following section.

### 4. Discussion

The dominant form of the neutral keto aHa in aqueous solution is identified as cis-aHa, since the calculated energy for the cis form is $\sim$3.5 kJ/mol less than the trans-aHa form. The ab initio vibrational spectrum of trans-aHa (data not shown) indicates that the amide II and III bands (strong contributions

### Table 4. Experimental and theoretical vibrational modes for the Fe(aHa) complex in H$_2$O and D$_2$O.

<table>
<thead>
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<th>Raman</th>
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<th>Intensity$^b$</th>
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<td>$^\text{N-D}$</td>
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<td>H$_2$O</td>
<td>D$_2$O</td>
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$^a$ sdef = symmetric deformation; adef = asymmetric deformation; rock = rocking motion.

$^b$ Intensity of the peak in off-resonance Raman and IR.

$^c$ Symmetry of the frequency in the Fe(aHa)$_3$ model.

$^d$ Calculated frequency of the symmetry mode.
from N-H vibrations) shift to lower energies than those of the cis form because there is stronger H-bonding between N-H and C=O in trans-aHa. Absence of such strong redshifts in the experimental spectrum of aHa (Figs. 4a and 5a) rules out the presence of detectable trans-aHa and suggests that the cis-aHa is the dominant species in aqueous solutions at pH values below the pKₐ.

As aHa deprotonates in aqueous solutions, the ν(C=O) stretch and the π-π* transition of the carbonyl are redshifted, indicating that the C=O is weakening and the C-O bond distance increases. Deprotonation at the OH or NH sites can cause these variations in C=O. The question is, where does deprotonation occur, at the N? or at the oxime O?

The ν(N=O) stretch, together with the ab initio analysis, provide clues to the site of deprotonation. However, it is important to correctly identify the location of the ν(N=O) band in aHa. In this study, the ν(N=O) stretch is assigned to a band at 1091 cm⁻¹ for aqueous aHa based on the calculations, IR and resonance Raman analysis. This contradicts the previous report of Holmen et al. (1997), who assigned the ν(N=O) stretch to a band at 994 cm⁻¹. All calculations of aHa models in our study indicate that the ν(N=O) stretch is strongly coupled to the δ(CHO) bending and rocking vibrations and occurs in the range of 1075–1097 cm⁻¹. Only N-deprotonated aHa exhibits this band at 866 cm⁻¹ (Tables 2, 3). The unequivocal experimental evidence for this assignment comes from the resonance Raman spectra of the Fe-aHa complexes. Because of the direct involvement of the NO group in the Fe(III) complex, the ν(N=O) stretch is enhanced in the resonance Raman spectra and appears as a strong peak at 1097 cm⁻¹. The ν(N=O) stretch of aHa also strongly couples with the δ(ND₃) bending mode in D₂O solutions, and results in the splitting of the ν(N=O) stretch. Both ab initio and experimental studies show the splitting of the oxime band in neutral aHa and anionic aHa, supporting the above observations (Figs. 4 and 5; Table 3). The ν(N=O) stretch of aqueous aHa shifts to higher energy with deprotonation. Theoretical calculations indicate that N-deprotonation should result in the lengthening of the N-O bond length, while O-deprotonation shows a decrease in the N-O bond length (Table 1), further confirming O-deprotonation of aHa in aqueous solutions.

The structure of O-deprotonated aHa can be identified using the vibrational band position of the amide II band. According to the calculated spectra, the H atom on the N atom interacts with the carbonyl oxygen in the trans configuration (partial intramolecular hydrogen bonding), and leads to a weaker coupling between the C-N and H-N bonds when compared to the cis configuration of aHa⁻. Since the experimental spectra of aHa solutions do not indicate that this is occurring, the O-deprotonated form of cis-aHa⁻ is considered as the dominant conformation in aqueous solution.

Infrared and resonance Raman spectra of Fe-aHa solutions suggest that the spectra of aHa⁻ and Fe(aHa)₃ are similar, which indicate that the electronic structural changes in the...
oxime and carbonyl groups of anionic aHa are small upon Fe(III) complexation. These spectral changes are also in agreement with the ab initio calculations. However, the transition (from the UV-vis spectra) of the carbonyl in aHa shifted to high energy upon Fe(III) complexation, and this energy is similar to that of neutral aHa. This discrepancy in the vibrational and electronic spectra may be caused by the contributions from the C-N bond and π-back-bonding within the Fe-complex.

4.2. Speciation of desB and Its Fe(III) Complexes in Aqueous Solutions

The strong coupling of the methylene groups in desB when compared to aHa made it difficult to assess all of the vibrational bands in desB. Hence, this discussion focuses on the carbonyls in desB (the amide and oxime carbonyls). The infrared spectra of aqueous desB show that the vibrational bands of the amide and oxime carbonyls overlap significantly, although the ab initio calculations indicate that the νC=O stretch of the amide carbonyl is at a higher wave number than the oxime carbonyl (Figs. 7 and 8; Table 5). Poor separation of these bands in aqueous solutions may be caused by the problems associated with background subtraction for water in the H2O bending region. Water normalization is not an issue in alkaline D2O solutions, where these carbonyl bands are well separated (Fig. 8).
The $v_{(C=O)}$ stretch of the oxime group is 50 cm$^{-1}$ lower than the $v_{(C=O)}$ band in aHa, and this is attributed to the differences in the structure of the hydroxamate group in aHa and desB. According to the calculated bond distances in the desB model, the C=O (and N-O) bonds are longer in desB (Table 1). The longer bond lengths correspond to lower wavenumbers in the IR spectra and this factor may be attributed to the differences in the substituents on the nitrogen atom. With an increase in pH, the oxime carbonyl shifts to lower wavenumber because of the conjugation as well as the substituent attached to the nitrogen atom. When compared to the $v_{(C=O)}$ band of these compounds (aHa and desB), N-methyl aHa shows this band at 1167 cm$^{-1}$ (Brown et al., 1979). In aHa, there is a hydrogen atom attached to the N, while in N-methyl acetoxyhydroxamic acid and desB a methyl group and a long hydrocarbon chain are attached, respectively. The carbon groups in the latter two compounds are electron donating, adding more electron density to the conjugation site. This adds more conjugation in the CONO core of desB than in aHa, and accounts for the decrease in energy for the amide I and increase in the energy of the $v_{(N-O)}$ modes. This substitution also influences their binding constants. The complexation constants (log K, where K = stability constant) of Fe(III) with aHa, N-methyl aHa and desB are 28.8, 29.4, and 32.6, respectively (Schwarzenbach and Schwarzenbach, 1963; Schwarzenbach and Schwarzenbach, 1963; Farkas et al., 1998). The spectral variations and differences in conjugation within the oxime group of these compounds explain these observed differences in complexation.

5. SUMMARY AND CONCLUSIONS

The functional group chemistry of aHa and desB, and their Fe(III) complexes were characterized using vibrational (infrared and resonance Raman) and electron (UV-vis) spectroscopy methods. The proposed structures and the deprotonation sites in aHa, and metal bonding environments of aHa and desB were also corroborated with the ab initio calculations. The summary of our observations is as follows,

1) The most stable form of aHa in aqueous solution is the cis-keto configuration, and deprotonation occurs at the O-...
site. However, deprotonation may occur at the N-site in highly alkaline solutions of pH >11.0. A combination of infrared and Resonance Raman spectra, and ab initio calculations were helpful in making band assignments unambiguously and in arriving at these conclusions.

2) Changes associated with the deprotonation of oxime group are similar for aHa and desB. However, the vibrational and electronic spectra indicate that electron delocalization is stronger in desB, and this may be caused by the differences in substitution at the N-site (i.e., a proton in aHa, and long aliphatic chain in desB).

3) Differences in electron delocalization in the CONO core of the reactive oxime group in aHa and desB may lead to the significant differences in their binding constants. Stronger electron delocalization and increased conjugation in the CONO core of desB is responsible for very high Fe(III) binding constants.

The molecular information presented here is important in assessing the metal binding affinities of different hydroxamate siderophores and the chemical states of their metal complexes. This information is also useful in synthesizing different siderophore analogues, which have high affinity for specific metals, such as actinides, and in selective sequestration of these metals from waste repositories. Recently, researchers have focused on the specific interactions of siderophores with different mineral substrates (Kraemer et al., 1999). Although siderophores have high affinity for Fe(III) in aqueous solutions, these studies indicate that siderophores alone do not increase the dissolution kinetics of Fe(III)-hydr(oxydes) because of steric limitations of large ligands at mineral-water interfaces. Information presented here for solution complexes of Fe(III) is useful in interpreting the nature of siderophore interactions on Fe(III)-hydr(oxyde) surfaces.

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