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Electrospray Mass Spectrometry Study of 1:1 Ferric Dihydroxamates

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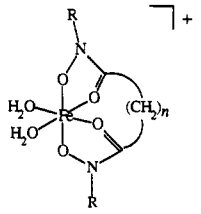
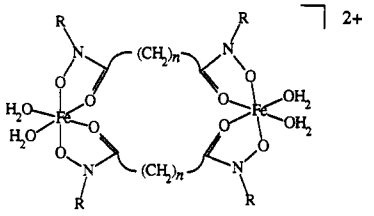
In this communication, we report an early example[1-8] of the use of electrospray mass spectrometry (ESMS) to probe the solution structure of transition metal complexes. Iron hydroxamic acid complexes are important due to their relationship to siderophore-mediated iron transport.[9] Dihydroxamic acids, such as the siderophore rhodotorulic acid and its synthetic models,[10-14] form 1:1 complexes with iron(III) at low pH. Since this species is an intermediate in the ligand exchange pathway of natural and model ferric dihydroxamates, the elucidation of the 1:1 complex as monomer **a** or dimer **b** is an important mechanistic consideration.



a: monomer



b: dimer

Acid-dependent kinetic studies on the dissociation of the 1:1 complexes (R = methyl, n = 2–8) gave strong, though not conclusive, evidence that for $n \leq 6$, the complex was better formulated as the dimer, **b**, while the monomer, **a**, predominates for $n > 6$.[15] In Raymond's series of methylene-bridge dihydroxamic acid (R = isopropyl) complexes,[11] the monomer structure **a** was proposed for connecting chains from 3–10 methylene units. Martell's series of dihydroxamic acid complexes (R = H)[16] show a large discontinuity in the solution stability constant at $n = 6$, suggesting some structural change in the nature of the iron complex. We have used ESMS to show conclusively that for dihydroxamate ligands (R = methyl), the $n = 8$ species forms monomer **a** whereas the $n = 2$ species forms a dimer, most likely with structure **b**.

The dihydroxamic acid ligands $CH_3N(OH)C(O)(CH_2)_nC(O)N(OH)CH_3$ were prepared as described in the literature[11,17] and characterized.[18] Solutions for ESMS analysis were prepared by combining equimolar amounts of Fisher analytical grade ferric chloride, $FeCl_3 \cdot 6H_2O$, and the desired ligand in twice distilled water to give $[Fe]_{tot} = [L]_{tot} = 0.50$ mM. The pH of the solution was adjusted to about 2.3 by dropwise addition of concentrated HCl while stirring vigorously. The UV–vis spectrum of each solution was measured prior to ESMS analysis. The spectra were essentially identical for $n = 2$ (λ_{max} = 472) and $n = 8$ (λ_{max} = 466) complexes. ESMS measurements were performed on mixtures of the iron complex solutions with acetonitrile in varying proportions,[19] using a Fisons Instruments VG BIO-Q quadrupole mass spectrometer equipped with a pneumatically assisted electrostatic ion source operating at atmospheric pressure. A mobile phase of 1:1 (v/v) CH_3CN/water and a sample flow rate of 6 μL/min was used for LC injection. The instrument was operated at unit mass resolution, and the mass scale was calibrated using polyethylene glycol.

The mass spectrum of the iron complex when $n = 8$, Figures 1 and 2,[18] from m/z = 100 to 900 shows major features at m/z = 214, 314, 355, and 663, depending on cone voltage. Upon expansion, the signal at m/z 314 shows an isotope pattern characteristic of the singly charged 1:1 complex FeL^+, Figure 3a. The peak two units down from the main peak is due to the presence of the less abundant ^{54}Fe isotope and is characteristic of an iron complex. Interestingly, the complex has been completely dehydrated, even to removal of the waters of coordination. Certain other iron(III) cations bound to negative oxygen donors have shown a propensity for loss of coordinated water in ESMS

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(19) CH_3CN is added to the sample to facilitate desolvation.

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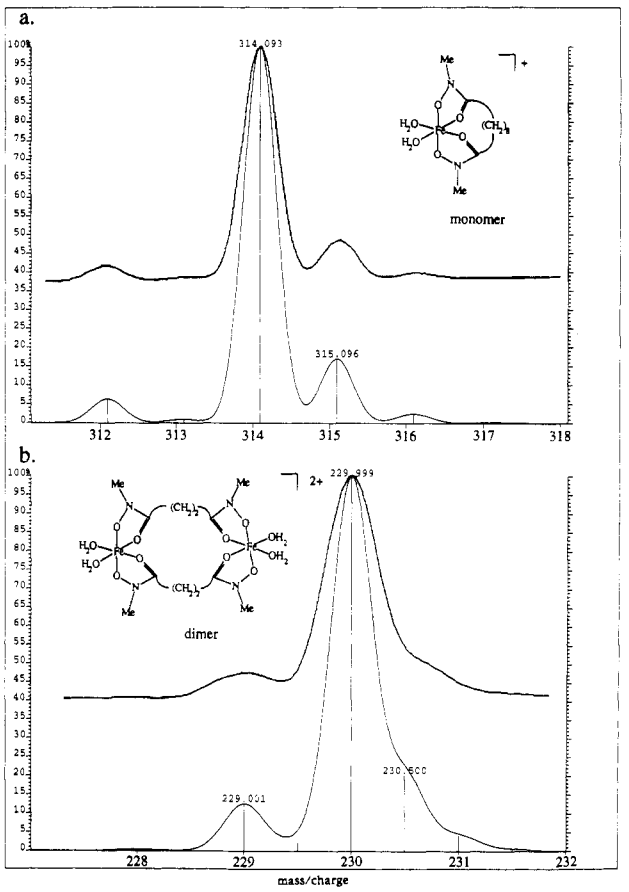

Figure 3. ESMS of ferric dihydroxamate complexes, $n = 2$, 8. $[Fe]_{tot} = [L]_{tot} = 0.52$ mM, pH = 2.3 (HCl); solvent = 1/1 (v/v) CH_3CN/H_2O; mobile phase = 1/1 (v/v) CH_3CN/H_2O. Key: (a) FeL, molecular formula $FeC_{12}H_{22}N_2O_4$, cone voltage (B1) = 42 V, top = experimental, bottom = calculated on the basis of isotope ratios; (b) $Fe_2L_2^{2+}$, molecular formula $Fe_2C_{12}H_{20}N_4O_8$, cone voltage (B1) = 42 V, top = experimental; bottom = calculated on the basis of isotope ratios.

experiments.[3] This is presumably due to the lability imparted to the coordinated water upon addition of a hard negative donor to the inner coordination sphere.

The signal at m/z 355 is 41 mass units higher than m/z 314 and is most likely an acetonitrile adduct, $FeL{\cdot}MeCN^+$. Acetonitrile is a slightly coordinating solvent, and we do not expect this adduct to be extremely stable. This is consistent with the observation that the signal at m/z 355 disappears as the cone voltage B1 is increased from 29 to 46 V, Figures 1 and 2,[18] where conversion of increased translational energy to internal energy results in disruption of weak adducts.

The signal at m/z 633, Figure 4,[18] is assigned to a chloride-containing dimer, $Fe_2L_2Cl^+$, as determined by isotopic distribution. It is not clear from this experiment just how the chloride is associated with the complex. Since chloride is a coordinating ligand for iron, it is possible that the chloride anion is directly bound to iron, either in a terminal or bridging mode. On the other hand, given the nature of the proposed desolvation mechanism, it is possible that this is only an ion pair or cluster formed in the ion source.[20,21] This experiment gives no information about whether this species is bridged by a dihydroxamate ligand or consists of two monomers, **a**, bridged by the chloride ion. The observation that a dehydrated monomer can form and that there is no evidence for a dehydrated dimer $Fe_2L_2^{2+}$ suggests that the species responsible for the signal at 663 may contain a chloride bridging two monomer units.

The signal at m/z 214, which is 46 mass units less than the ligand mass of m/z 260, can be formed by loss of *N*-methylhydroxylamine from the free protonated ligand. However, there is also a significant background ion peak at m/z 214, Figure 5.[18] ESMS measurements on the free ligand show a major $(MH)^+$ peak at m/z 261, but also a significant peak at m/z 214 as well, Figure 6.[18] Selecting the peak at m/z 261 for ESMS-MS analysis using collisionally-activated decomposition (argon gas) to fragment the species confirms that the major fragmentation product of the free ligand is at m/z 214, Figure 7.[18] This is not surprising since in acid solution, acid catalyzed hydrolysis of the hydroxamate group occurs at the carbonyl carbon, with loss of the hydroxylamine linkage.[22-24]

The mass spectrum of the iron complex when $n = 2$ has some of the same features that are observed for $n = 8$. At a cone voltage of 42 V, the parent peak is observed at m/z 230, and two other major peaks are observed at m/z 130 and 495, Figure 8.[18] The signal at m/z 230 is consistent with a species empirically having a 1:1 metal-to-ligand ratio, Figure 3b. However, the isotopic distribution pattern cannot be explained on the basis of a singly charged species. If the species here were FeL^+, then we would expect to observe an isotope distribution pattern similar to that in Figure 3a. The observed compression of the isotope distribution pattern in Figure 3b is consistent with an iron complex with m/z 230 and $z = 2$: i.e., $Fe_2L_2^{2+}$. This is very strong evidence that when only two methylene units separate the hydroxamate groups, ring strain precludes formation of the monomer and that, in fact, a 2:2 dimer is the species existing in solution. There is no evidence, in this case, for the formation of a monomer at m/z 230 or for formation of higher oligomers.

For the $n = 2$ complex, there is also a signal at m/z 495, Figure 9,[18] whose isotopic distribution is consistent with a chloride-containing dimer, $Fe_2L_2Cl^+$. In this case it is likely that the dihydroxamate ligand is bridging, though the nature of the interaction with the chloride is still uncertain. The isotopic distribution is, once again, consistent with this stoichiometry. This sample also shows a signal at m/z 130, 46 mass units less than the free ligand mass of m/z 176, and can be explained by loss of *N*-methylhydroxylamine from the free protonated ligand. ESMS and ESMS-MS experiments, Figures 10 and 11,[18] performed on the free ligand show an $(MH)^+$ species at m/z 177 which can be collisionally fragmented to give a peak at m/z 130.

The data reported here constitute evidence that very short chain *N*-methyl-substituted dihydroxamate ligands do not form a 1:1 metal-to-ligand monomer in which a single ligand spans a single iron to coordinate in a tetradentate fashion. This would entail formation of a 7-membered ring in the case where $n = 2$, which is not itself a highly strained situation. However, the geometric constraints of the two hydroxamate groups upon binding to a single iron forces the carbonyl carbons into a position in which they cannot be easily spanned by anything less than a six-atom bridge, e.g. $n = 6$.

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Supplementary Material Available: Figures 1, 2, and 4–11 showing ESMS spectra, and a textural listing of ligand characterization data (11 pages). Ordering information is given on any current masthead page.

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