

Investigating the Structural and Electronic Properties of Nitrile Hydratase Model Iron(III) Complexes Using Projected Unrestricted Hartree–Fock (PUHF) Calculations

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Important structural and mechanistic details concerning the non-heme, low-spin Fe(III) center in nitrile hydratase (NHase) remain poorly understood. We now report projection unrestricted Hartree–Fock (PUHF) calculations on the spin preferences of a series of inorganic complexes in which Fe(III) is coordinated by a mixed set of N/S ligands. Given that many of these compounds have been prepared as models of the NHase metal center, this study has allowed us to evaluate this computational approach as a tool for future calculations on the electronic structure of the NHase Fe(III) center itself. When used in combination with the INDO/S semiempirical model, the PUHF method correctly predicts the experimentally observed spin state for 12 of the 13 Fe(III)-containing complexes studied here. The one compound for which there is disagreement between our theoretical calculations and experimental observation exhibits temperature-dependent spin behavior. In this case, the failure of the PUHF–INDO/S approach may be associated with differences between the structure of the Fe(III) complex present under the conditions used to measure the spin preference and that observed by X-ray crystallography. A preliminary analysis of the role of the N/S ligands and coordination geometry in defining the Fe(III) spin preferences in these complexes has also been undertaken by computing the electronic properties of the lowest energy Fe(III) spin states. While any detailed interpretation of our results is constrained both by the limited set of well-characterized Fe(III) complexes used in this study and by the complicated dependence of Fe(III) spin preference upon metal–ligand interactions and coordination geometry, these PUHF–INDO/S calculations support the hypothesis that the deprotonated amide nitrogens coordinating the metal stabilize the low-spin Fe(III) ground state seen in NHase. Strong evidence that the sulfur ligands exclusively define the Fe(III) spin state preference by forming metal–ligand bonds with significant covalent character is not provided by these computational studies. This might, however, reflect limitations in modeling these systems at the INDO/S level of theory.

Introduction

The hydration of nitriles to amides is an important catabolic step in bacterial and fungal cellular metabolism (eq 1).^{1,2} This



biological transformation is catalyzed by nitrile hydratase (NHase),³ a non-heme metalloenzyme that contains either Fe(III) or Co(III) in the active site.^{4–6} NHase is of considerable biotechnological interest given its role in the industrial production of acrylamide,⁷ its potential for use as an environmental

remediation agent,⁸ and its application to the enantioselective synthesis of primary amides.⁹ The structure of the Fe(III) center

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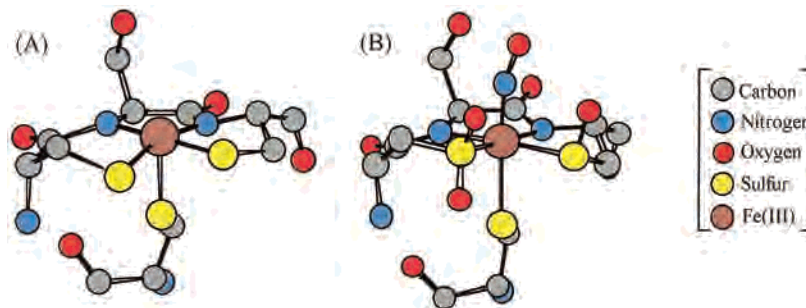


Figure 1. (A) Fe(III) metal center observed in the crystal structure of the nitrile hydratase obtained from *Rhodococcus* sp. R312.¹⁰ (B) Fe(III) metal center observed in the crystal structure of the inactive NO complex of the nitrile hydratase obtained from *Rhodococcus* sp. N-771.¹¹ Atomic coordinates were obtained from entries 1AHJ and 2AHJ, respectively, in the Brookhaven Protein Database.⁴⁴

in the enzyme has been determined in independent X-ray crystallographic studies of (i) the native NHase isolated from *Rhodococcus* sp. R312¹⁰ and (ii) an inactive, nitrosylated form of NHase present in *Rhodococcus* sp. N-771.¹¹ Both structures reveal an octahedral coordination of Fe(III) by the side chains of three conserved cysteine residues, two amide nitrogens from the protein backbone, and a sixth ligand (Figure 1). In the inactive form of NHase, the sixth site is occupied by nitric oxide (NO),^{11,12} whereas spectroscopic evidence suggests that the sixth ligand in the free enzyme is either water^{7,13} or hydroxide ion.¹⁴ X-ray studies have not revealed whether the sixth ligand to the Fe(III) center is displaced by the nitrile substrate during catalytic turnover.¹⁰ This N₂S₃X coordination was unanticipated on the basis of prior EXAFS,¹⁵ resonance Raman,¹⁶ and ENDOR studies on native Fe(III)-dependent NHase.^{13,14} Nitrile hydratases are also unique among known non-heme metalloenzymes in possessing mononuclear, low-spin ($S = 1/2$) Fe(III)^{4,17} or ($S = 0$) Co(III) centers.^{3b,5a} We were intrigued by this observation given that the gas phase, free ion electron pairing energy for d⁵ Fe(III) is approximately 30000 cm⁻¹ in an octahedral environment. A low-spin configuration is therefore favored only when there is a large disparity in the relative energies of the t_{2g} and e_g orbitals in the metal complex. As a consequence, the vast majority of six-coordinate Fe(III) complexes either are high spin ($S = 5/2$) or exhibit spin equilibrium between high and low ($S = 1/2$) states.¹⁸

Theoretical methods offer an approach to defining the structural basis for the low-spin preference of Fe(III) in the enzyme but need careful calibration using model inorganic complexes before their application to systems as complicated as NHase itself.¹⁹ Such calculations can complement experimental studies of questions pertaining to the relationship between structure and spin preference in a number of ways. For example, spin-state-dependent information on the electronic structure and spectroscopy of relevant Fe(III) complexes might allow insight into the role of the protein ligands in modulating the spin properties of the NHase metal center. Computational studies of the electronic structure in either the unique NHase Fe(III) center or related inorganic model Fe(III) complexes have not, however, been reported. The lack of previous work in this area probably reflects the difficulty of applying quantum mechanical (QM) methods to determining the electronic structure of transition metal complexes, especially given that such systems possess a manifold of electronic states that lie close in energy and exhibit different chemical behavior.²⁰ Unfortunately the problem is further complicated by the need to include the effects of electronic correlation. Methods to address such problems, such as MCSCF²¹ and CASPT2,²² are limited to an active space of relatively few orbitals in order to ensure their computational tractability. As a result, only the spin properties of small metal-containing complexes can be studied using these approaches without significant effort. Density functional theoretical (DFT) methods represent an alternate technique for computing the ground state properties of Fe(III) complexes²³ and are finding increasing utility in modeling enzymes that contain transition metals.²⁴ The treatment of open-shell systems using DFT calculations, however, can often be complicated by the fact that the Kohn–Sham wave function may be heavily spin-contami-

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nated.²⁵ We now report that projection unrestricted Hartree–Fock (PUHF) calculations,²⁶ in combination with the INDO/S semiempirical model,²⁷ offer an intriguing alternative to CASPT2 and DFT methods in modeling the spin properties and electronic structure of inorganic Fe(III) complexes. We have used the PUHF method to study a number of complexes in which Fe(III) is coordinated by a mixed set of N/S ligands (Figure 2),²⁸ a subset of which have spectroscopic and ligand-binding properties similar to those observed for NHase.²⁹ For comparison, we have also examined Fe(III) complexes in which the metal is octahedrally coordinated by a set of N/O ligands.³⁰ Our results suggest that the PUHF methodology offers a new theoretical approach for investigating spin-dependent structural and chemical reactivity of Fe(III)-containing metalloenzymes including NHase.

Methods

Crystallographic coordinates for Fe(III) complexes **1–6** and **13** were obtained from the Cambridge Crystallographic Database, release 5.14,³¹ while those for compounds **7–9** were obtained from the supplementary material deposited for the relevant papers. Coordinate data were also obtained prior to publication directly from Dr. Alex Nivorozhkin (Harvard) (**10**) or Dr. Pradip Mascharak (Santa Cruz) (**11** and **12**). For complexes **3**, **6–9**, **11**, and **12**, all atoms were fixed at the coordinates reported in the crystal structures. Coordinates for hydrogens were

calculated with Chem3D Pro version 3.5.2 (CambridgeSoft Corp., Cambridge, MA), when necessary, to the non-hydrogen atoms observed in the crystal for complexes **1**, **2**, and **13** and the *tert*-butyl groups in **4**, using standard bond lengths and bond angles. In the case of the remaining structure, **5**, hydrogen atoms were attached to the non-hydrogen atoms in standard geometries using the CAChe Editor version 3.8 (Oxford Molecular Inc., Beaverton, OR).

All calculations were carried out using the ZINDO software package (98.1 release) running on Enterprise 5000 server running the Solaris 2.6 operating system. Fully projected UHF calculations,²⁶ using the INDO/S semiempirical model,²⁷ were used to obtain the energies associated with the $S = 1/2$, $S = 3/2$, and $S = 5/2$ states of each complex at the experimental geometry observed in the crystal. Each calculation was complete in 10–20 CPU minutes. The default INDO/S β -values in ZINDO describing Fe, H, C, N, and S were employed in these computations,²⁷ with the exception of that for O ($\beta = -54.0$ eV). The interaction factors for $s-\sigma$, $p-\sigma$, $p-\pi$, $d-\sigma$, $d-\pi$, and $d-\delta$ were 1.0, 1.267, 0.585, 1.0, 1.0, and 1.0, respectively. In the case of charged complexes, counterions were not included in the calculation. Solvent molecules observed in the crystal structure were also omitted from the structures used to compute spin-state energies. Mulliken partial charges,³² spin densities, and Wiberg bond indices³³ were determined for the pure spin-state component of each complex by projection, assuming a basis of symmetrically orthogonal Slater type orbitals (STOs).

Results and Discussion

Overview of Projection UHF Calculations. Although the detailed theoretical basis of the PUHF approach has been presented elsewhere,²⁶ the principles of the method are briefly discussed here. The simplest treatment of open-shell systems at the Hartree–Fock level of theory is the UHF approach. Unfortunately UHF descriptions of electronic structure are generally useful only for systems that are well described by a single determinant wave function. This requires that there are spatially nondegenerate states of the highest multiplicity associated with the open-shell system. For most structures of biological interest, the UHF wave function is consequently an admixture of several pure spin states of differing multiplicities. Using a basis of corresponding orbitals the UHF wave function can be written as a linear combination of restricted pure-spin configuration state functions:

$$\Psi_{S_z}^{\text{UHF}} = \sum_{S=S_z}^{S_z+N_\beta} \omega_s \sum_{k=0}^{N_\beta} C_k(S, S_z, n)^{2S+1} \Psi_{S_z}^k$$

where N_β and N_α are the numbers of β - and α -spin electrons, respectively. By convention, $N_\alpha > N_\beta$ for open-shell systems. S is the total spin angular momentum, $n = (N_\beta + N_\alpha)/2$, and $S_z = (N_\beta - N_\alpha)/2$ in atomic units. The squares of the ω_s are weighting coefficients and sum to unity for a normalized wave function. Determining the electronic properties associated with a pure spin state therefore requires treatment of the UHF wave function with an operator that projects out the contribution from the appropriate Slater determinant. From the definition of ω_s , the following expression can be derived for the PUHF energy, E , of a given spin state in the system:

$$E(\Psi_{S_z}^{\text{UHF}}) = \sum_{k=0}^{N_\beta} \omega_{S_z+k}^2 E^{(2(S_z+k)+1)} \Psi_{S_z} \leq E^{(2S_z+1)} \Psi_{S_z}^{\text{ROHF}}$$

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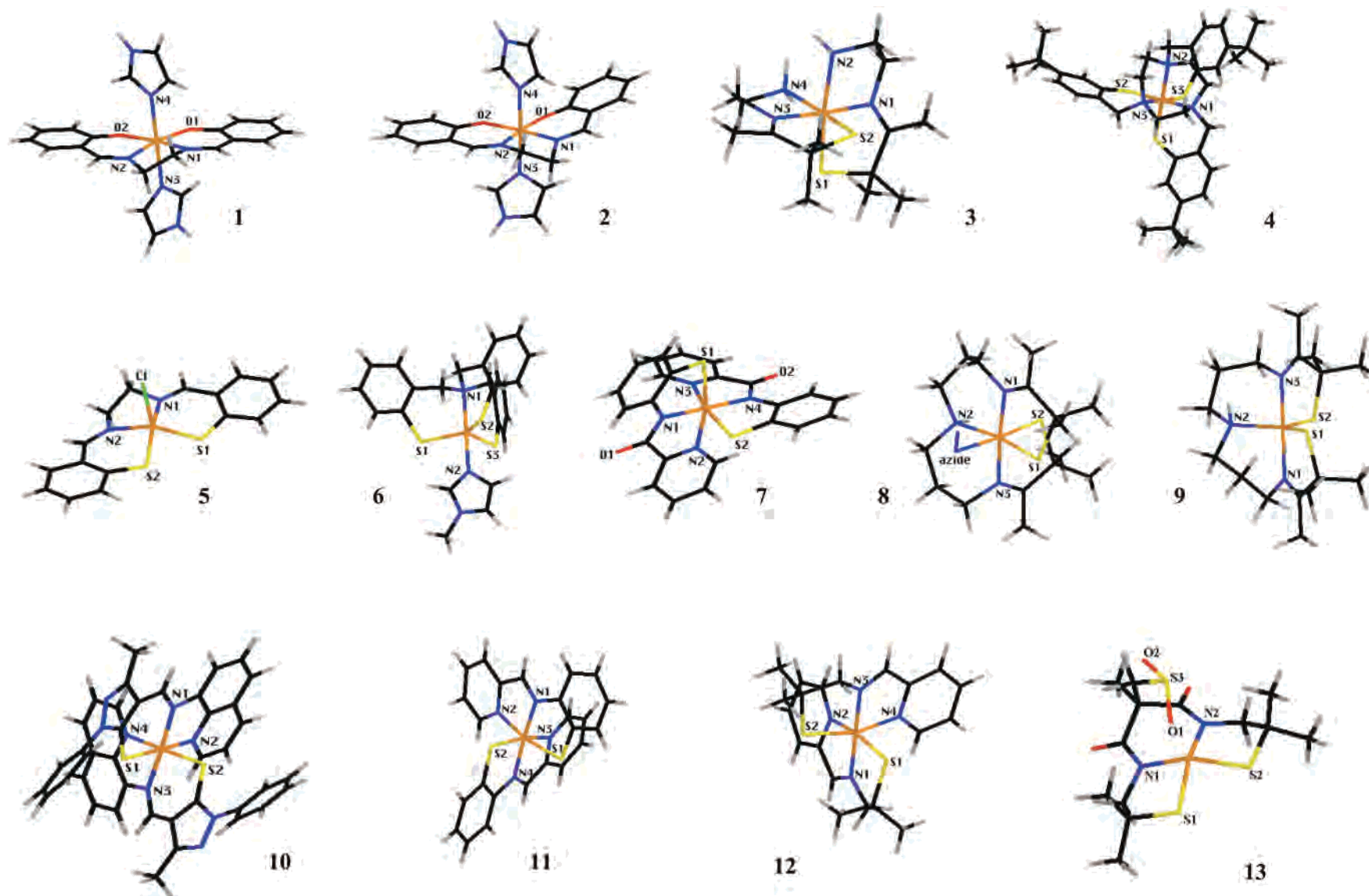


Figure 2. Fe(III) complexes 1–13 used in PUHF study of preferred spin states and electronic structure. Numbered atoms correspond to those shown in subsequent figures. X-ray crystal structures were visualized using RasMol V2.6.⁴⁵ Atoms are colored using the following scheme: C, black; H, white; N, blue; O, red; S, yellow; Fe, orange.

Table 1. Spin-State Energies for Fe(III) Complexes with S/N/O Coordination Computed Using the Fully Projected UHF Method

| entry | coordination geometry | charge ^b | Fe(III) spin density (%) ^c | rel PUHF-INDO/S spin-state energies (cm ⁻¹) ^a | | | obsd | ref |
|-------|-----------------------|---------------------|---------------------------------------|--|----------------------|----------------------|------------------|-----|
| | | | | S _z = 1/2 | S _z = 3/2 | S _z = 5/2 | | |
| 1 | octahedral | +1 | 81 | 1670 | 4034 | 0 | 5/2 ^d | 30a |
| 2 | octahedral | +1 | 74 | 0 | 16710 | 16348 | 1/2 ^e | 30a |
| 3 | octahedral | +1 | 100 | 0 | 4829 | 8114 | 1/2 | 28a |
| 4 | octahedral | 0 | 86 | 0 | 4197 | 6091 | 1/2 | 28b |
| 5 | square pyramidal | 0 | 97 | 9636 | 0 | 6735 | 3/2 | 28c |
| 6 | trigonal bipyramidal | 0 | 81 | 9265 | 1779 | 0 | 5/2 | 28d |
| 7 | octahedral | -1 | 72 | 0 | 9013 | 11571 | 1/2 | 28e |
| 8 | octahedral | 0 | 92 | 0 | 3228 | 4673 | 1/2 | 28f |
| 9 | trigonal bipyramidal | +1 | 93 ^f | 6695 | 0 | 4608 | 1/2 ^g | 28f |
| 10 | octahedral | +1 | 66 | 0 | 8786 | 12857 | 1/2 | 28g |
| 11 | octahedral | +1 | 69 | 0 | 12385 | 26547 | 1/2 | 28h |
| 12 | octahedral | +1 | 88 | 0 | 7666 | 12073 | 1/2 | 28h |
| 13 | square pyramidal | -2 | 95 | 10708 | 0 | 4341 | 3/2 | 28i |

^a 83.6 cm⁻¹ = 1 kJ/mol. ^b Counterions are not included in PUHF calculations. ^c Calculated percentage of spin density associated with the Fe(III) center in the spin state of lowest energy. ^d Structure of complex corresponds to that observed in the crystal when hexafluorophosphate (PF₆⁻) is the counterion. ^e Structure of complex corresponds to that observed in the crystal obtained at 120 K when perchlorate (ClO₄⁻) is the counterion. ^f Spin density on Fe(III) calculated for the pure S = 3/2 spin state. ^g This complex exhibits temperature-dependent spin crossover. The experimental spin preference corresponds to that observed for the complex below 125 K.

In general, this projection approach yields spin-state energies that are considerably lower than those obtained using standard ROHF calculations, as different orbitals are obtained for the different electron spins (DODS). This greater degree of variational freedom leads to lower energies.²⁶ Although the UHF energy is bounded, it contains some of the correlation energy by construction. The projection generally lowers the energy, as seen in the above expressions, and the $^{2S+1}\Psi_{S_z}^k$ are linear combinations of the $(N_\beta + N_\alpha)! \binom{N_\beta}{k} \binom{N_\alpha}{k}$ possible spin-restricted determinants constructed from the corresponding orbitals and are thus somewhat akin to configuration interaction (CI) wave functions. In addition, the PUHF procedure allows extraction of the one-particle density matrix for each pure spin state, and from this matrix we can compute all one-electron properties such as bond orders, dipole moments, spin densities, and atomic partial charges.

Computed Fe(III) Spin-State Preferences in Model Complexes. Our initial goal was to calibrate the ability of PUHF calculations to predict Fe(III) spin-state preferences using a series of model inorganic complexes for which structural data was available (Figure 2).²⁸⁻³⁰ The INDO/S model is parametrized to reproduce energy differences between electronic states, which determine the observed spin state of the system, at fixed molecular geometries. In light of our interest in understanding the electronic structure of the NHase Fe(III) center, this test set comprised 10 complexes in which the metal was coordinated by a mixture of nitrogen and sulfur ligands. Systematic variation of the electronic properties of the metal ligands was not possible given the relatively few examples of thiolate-containing Fe(III) complexes (i) that have been synthesized and (ii) for which data has been obtained concerning their structure, spin preferences, and spectroscopic properties. We note that, without careful design of the metal ligands, such Fe(III) complexes are often thermodynamically unstable and undergo electron transfer to generate Fe(II) with concomitant disulfide bond formation.³⁴ Nevertheless, our test series included complexes possessing a variety of coordination geometries and charge states. The relative energies of each of the three possible spin states for the set of inorganic Fe(III) complexes were calculated using the PUHF-

INDO/S method (Table 1). Optimization of the crystal structure geometries observed for the 13 model compounds was not carried out since the primary goal of our studies was to evaluate the utility of the PUHF-INDO/S method for calculating the spin-state preferences of structurally characterized Fe(III) complexes. Counterions and solvent molecules present in the crystal were also absent in the systems used in these UHF calculations. With the exception of complex **9**, the agreement between the calculated and observed spin states is remarkable in light of the variation in coordination geometry and total charge of the complexes used in these studies. The energetic separations between the preferred spin state and that of next highest energy were greater than 1670 cm⁻¹ (20 kJ/mol). Although it is difficult to estimate the size of the errors in the calculated energies, due to the limited number of prior studies using the PUHF-INDO/S method,²⁶ it is likely that the observed differences in spin-state energies are significant for this series of complexes.

With this apparent success in calculating the spin preferences of 12-model Fe(III) compounds, we were intrigued by the failure of PUHF-INDO/S calculations to predict the observed spin state of **9**. This complex is of special interest because (i) it was synthesized specifically as a model of the Fe(III) center in NHase^{28f} and (ii) it reacts with both azide and nitric oxide to yield 6-coordinate complexes with spectroscopic properties similar to those observed for the products of the cognate reactions with the enzyme.^{28f,29} On the other hand, the N₃S₂ coordination in complex **9** differs from that observed in NHase (N₂S₃O), primarily because it was designed on the basis of spectroscopic characterizations¹³⁻¹⁶ obtained prior to the X-ray crystallographic determinations of the enzyme structure.^{10,11} Experimental measurements of the magnetic properties of **9** in the solid state show that the complex exhibits a magnetic moment (μ) of 2.3 μ_B , consistent with a preference for the S = 1/2 spin state, at temperatures below 100 K.³⁵ The low-spin preference is highly unusual for a five-coordinate Fe(III) complex on the basis of ligand field theory.³⁶ Between 100 and 300 K, μ for the complex **9** gradually increases to 4 μ_B .^{28f} This spin crossover^{37,38} can be attributed to a thermal equilibrium

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involving the $S = 1/2$ and $S = 5/2$ states of **9**. Further evidence for the low-spin preference of **9** at 125 K was provided by EPR measurements of the complex in a 9:1 MeOH/EtOH glass.^{28f} For comparison, octahedral complex **8** which was obtained by reaction of **9** with azide anion has a magnetic moment of 1.73 μ_B independent of temperature.³⁵

Two explanations may underlie the inability of the PUHF method to predict the spin preference of **9** at low temperatures. First, this theoretical approach may not be applicable to Fe(III) complexes that possess a trigonal bipyramidal coordination. The agreement between the calculated and observed spin preference for **6**, however, appears inconsistent with this hypothesis. Our ability to investigate this possible limitation of the PUHF method in a systematic fashion is hampered by other examples of trigonal bipyramidal Fe(III) complexes in which the metal is coordinated only by nitrogen and sulfur-containing ligands. A second explanation is that the structure of **9** employed in our calculations is not that present in the solid at temperatures below 100 K. Evidence supporting the intrinsic flexibility of the coordinating ligands is provided by the ability of **9** to undergo structural rearrangement yielding an octahedrally coordinated complex on treatment with either azide or nitric oxide.^{28f,29} It is also possible that the conformational preferences of the six-membered rings in the complex may change at low temperature in response to the decrease in ionic radius of the metal in the low-spin ground state. This would result in shortening of the metal–ligand bond lengths. We note that for Fe(S₂CNEt₃)₃, which has $\mu = 2.3 \mu_B$ at 79 K and 4.3 μ_B at 297 K, it has been found that the crystal packing and molecular dimensions are significantly different at the two temperatures.^{19,38h}

Although possessing an N/O coordination sphere, our results for structures **1** and **2** yield some insight into the magnitude of the structural changes needed to modify the spin preference of the Fe(III) center as calculated using the PUHF–INDO/S method. Complexes **1** and **2** are different forms of the same complex that differ in molecular geometry due to the nature of the counterion with which they were crystallized. Thus, **1** is observed in a crystal obtained at 295 K when hexafluorophosphate is the counterion, while **2** is that measured at 120 K for the perchlorate salt. Interestingly, the $S = 5/2$ spin state is observed for the hexafluorophosphate salt independent of temperature. The perchlorate salt, however, exhibits temperature-dependent spin crossover,^{30a,37} and so the structure of this derivative was determined at temperatures on each side of that at which the spin crossover occurred. Structure **2** represents the form of the complex with a low-spin ground state and was determined at 120 K. Superimposition of **1** and **2** clearly shows the extent to which the counterion and temperature modify the structure of the complex (Figure 3). The observed change in spin-state preference for these two variants of the same complex is correctly reproduced by the PUHF–INDO/S method (Table 1). On the other hand, our approach does not calculate the observed spin properties of the perchlorate salt complex using

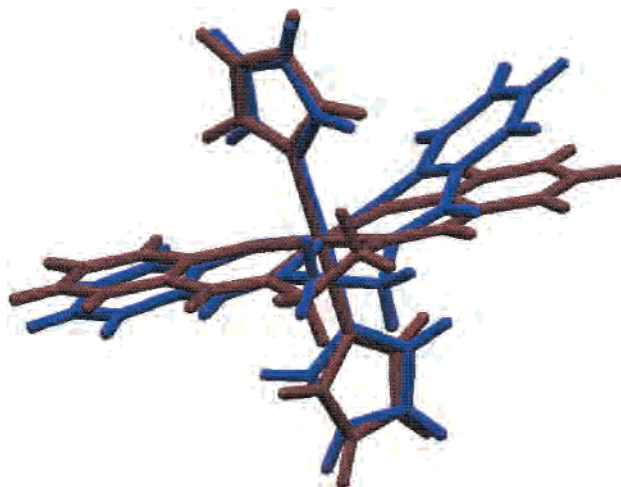


Figure 3. Superimposed structures for **1** (red) and **2** (blue).

the crystal structure measured at 295 K. Presumably this failure of the method reflects the constraint of employing a single structure to describe a system in which the observed spin properties reflect a thermal equilibrium between multiple forms of this Fe(III) complex. The calculated spin behavior of structures **1** and **2**, however, supports the notion that the disagreement between the theoretical and observed spin preference for **9** is due to structural reorganization at temperatures below 150 K.

Electronic Structure of Fe(III) Model Complexes. The spin preference of the NHase metal center is unique among non-heme Fe(III)-containing metalloenzymes, and indeed the π -donor properties of the cysteine ligands might be expected to favor a high-spin electronic configuration. In addition, experimental measurements suggest that no changes in metal oxidation state take place during the enzyme-catalyzed reaction. This lack of redox activity is interesting given that three cysteine residues are coordinated to Fe(III) in NHase.³⁴ The role of the protein ligands in conferring these unusual properties on the NHase metal center has been the subject of considerable discussion in the literature.^{10,11,28} Recent studies employing complex **7** have suggested that amide nitrogen coordination might be an important element in controlling the redox potential of the NHase Fe(III) center,^{28c} and it is noteworthy that nitrogenase is presently the only other protein in which a metal is coordinated by deprotonated, main chain amide bonds.³⁹ On the other hand, the sulfur ligands in the NHase center might be critical in determining the unusual Fe(III) spin preference due to the metal–thiolate bonds possessing significant covalent character. As the radial distribution of the d orbitals would be extended, this nephelauxetic effect would lower the energy of the ³P state, thereby increasing the t_{2g} and e_g orbital splitting in the octahedral complex.⁴⁰ As the PUHF–INDO/S method appeared sufficiently robust in calculating the spin preferences of Fe(III) centers in cationic, anionic, and neutral complexes of varied coordination geometry, we examined whether information on the role of N/S coordination in controlling the observed electronic properties of Fe(III) in complexes **1–13** could be obtained from spin-dependent electronic properties.

The Mulliken partial charges (Figure 4) and Wiberg bond indices (Figure 5) were calculated for the lowest energy, pure

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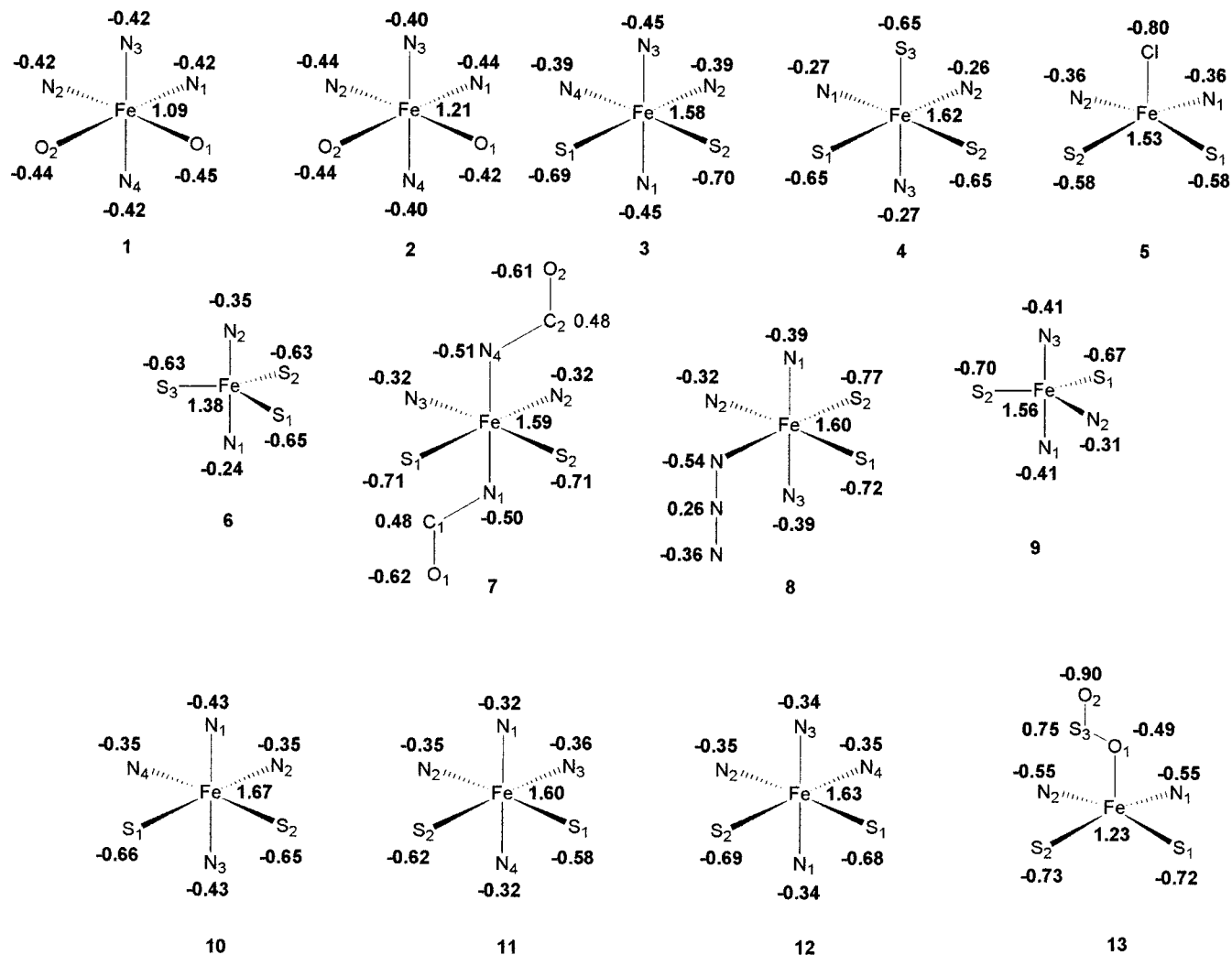


Figure 4. Partial atomic charges calculated for the pure spin state of lowest energy of Fe(III) complexes **1**–**13** using the PUHF–INDO/S method.

spin states of complexes **1**–**13** using the PUHF–INDO/S methodology. Although any conclusions are therefore necessarily qualified, especially in light of the relatively small number of model Fe(III) inorganic complexes, a number of points arise from this computational analysis. First, for complexes in which Fe(III) was coordinated by an N/S ligation sphere, there was a strong correlation of the calculated partial charge on the metal and low-spin preference. Comparison of the low-spin Fe(III) complexes **7** and **11**, in which the Fe(III) is coordinated by deprotonated amide bonds and imines, respectively, did not reveal any significant differences in the calculated metal–sulfur bond orders (Figure 6). Analysis of the C–N bond orders and partial charge distributions in the coordinating amides of **7** and **13** did indicate, however, that the deprotonated amides might be better regarded as imidates when complexed to Fe(III). Thus, our PUHF–INDO/S calculations suggest that the C–N bonds of the amide anions in **7** have substantial double-bond character when compared with that computed for the cognate bonds of the imino groups in complexes such as **11** and **12**. Although the negative charge is delocalized onto the oxygen atom, the imidate ligands can still act as strong σ donors to the metal, increasing the t_{2g} and e_g orbital splitting in the octahedral complex. An examination of experimental data that may support or refute these theoretical observations, however, has revealed conflicting interpretations of the bonding between metals in high oxidation states and deprotonated amides.^{28e,41} Support for the hypothesis that the deprotonated amides are best described as

imidates is provided by the red shift in carbonyl stretching frequency that is observed on metal coordination, from 1688 cm^{-1} (in the free ligand) to 1612 cm^{-1} in complex **7**.^{28e} A similar $\nu_{\text{C-O}}$ red shift in carboxamide complexes of Ni(IV), together with evidence from hydrogen-bonding patterns, has also been claimed to demonstrate a significant delocalization of charge from nitrogen to oxygen in the deprotonated amide.^{41a} Comparable shifts in $\nu_{\text{C-O}}$ are observed for complexes in which Fe(III) is coordinated by deprotonated amides.²⁸ⁱ On the other hand, a recent comparison of the $\nu_{\text{C-O}}$ red shifts for a single ligand complexed to Fe(II) and Fe(III) has been interpreted as evidence for higher localization of charge on the nitrogen of the deprotonated amide when coordinated to Fe(III) relative to Fe(II).^{41d} A definitive assessment of our computational results will therefore demand additional theoretical and experimental study. These PUHF–INDO/S calculations do not suggest that the bonds between the metal and the amide nitrogens are unusual. It is therefore interesting that complex **7** is surprisingly stable to reduction. The electronic basis for this behavior is not readily apparent from our analysis, and the relative contributions of the amide and thiolate ligands in modulating the reduction potential of **7** remain to be elucidated. The theoretical analysis

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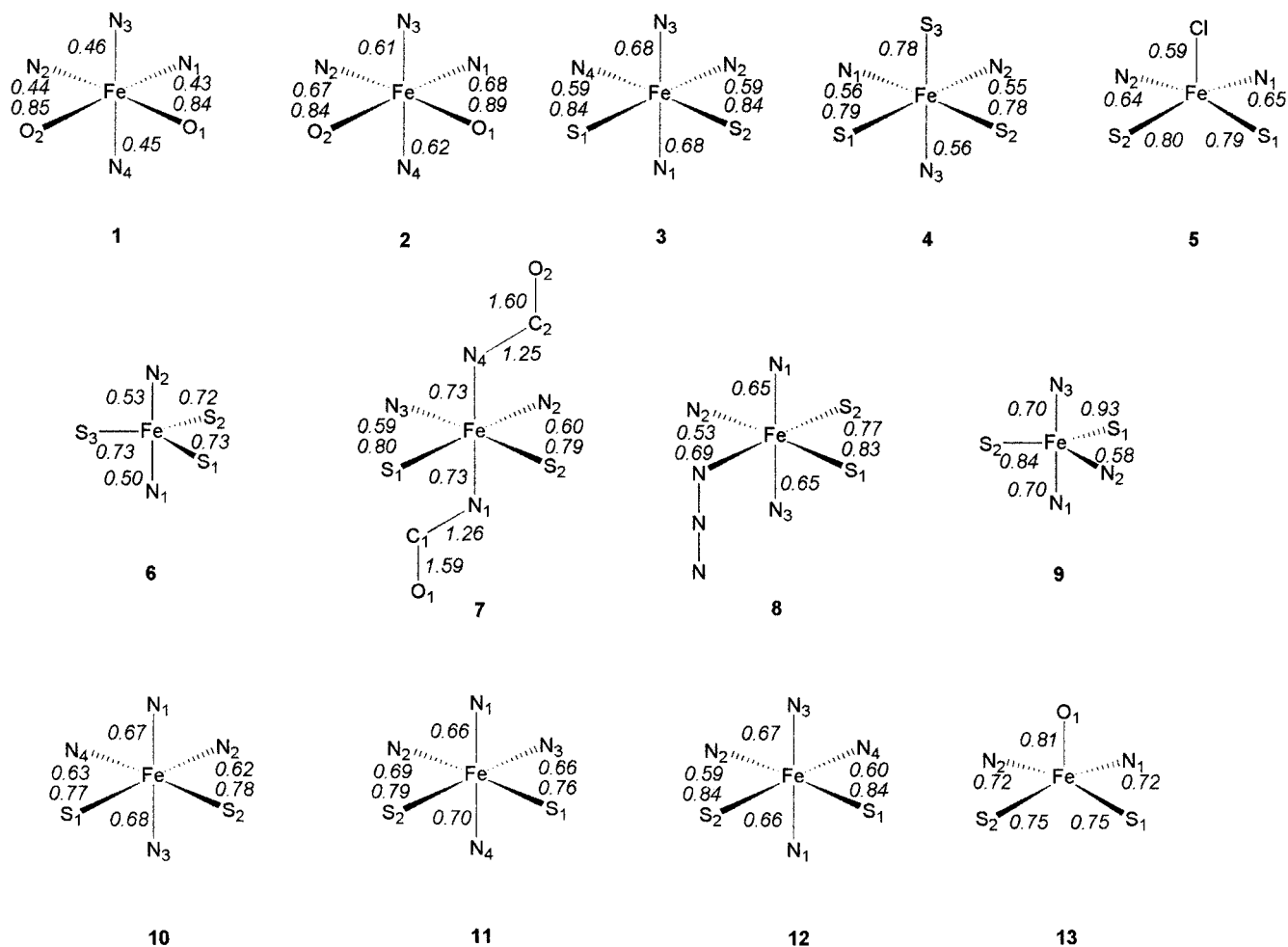


Figure 5. Wiberg bond orders calculated for the pure spin state of lowest energy of Fe(III) complexes **1–13** using the PUHF–INDO/S method.

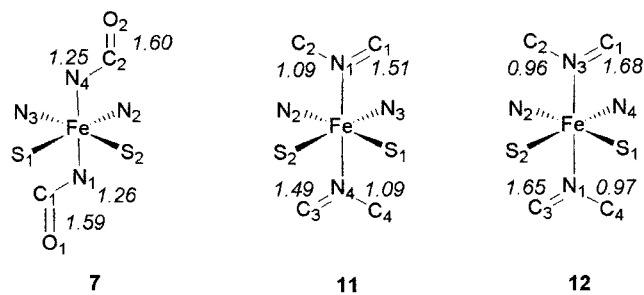


Figure 6. Comparison of selected Wiberg bond orders for the amide and imine ligands in complexes **7**, **11**, and **12**. The values shown are those computed for the pure spin state of lowest energy.

is further complicated by the fact that **7** has an overall negative charge. While it can be argued that charge effects are not important given that the positively charged complex **9** is resistant to reduction (-1.1 V in water),^{28f} direct comparison of these two Fe(III) complexes is complicated due to differences in their coordination geometries.

The contribution of metal–sulfur interactions in stabilizing the ($S = 1/2$) spin state of Fe(III) in the NHase metal center is not readily apparent on the basis of our computational results. For example, while the calculated partial atomic charges on the sulfur ligands span a wide range (-0.58 to -0.77) throughout the low-spin octahedral complexes, there appears to be little variation in the Wiberg bond orders. Thus the range of values computed for the Fe–S bond order seen for the low-spin complexes is not very different from that seen for complexes

that exhibit high, or intermediate, spin preferences. Comparison of complexes **1** ($S = 5/2$) and **3** ($S = 1/2$) suggests that the ability of the metal to donate electron density into the unoccupied 3d orbitals on sulfur could result in increased separation of the t_{2g} and e_g orbital splitting in these octahedral complexes. Even in these cases, however, the importance of Fe–S interactions is hard to evaluate since the covalent character of the Fe–N bonds seems higher in complex **3** than **1**. The effects of coordination geometry in defining preferred spin state further complicate the situation. For example, the Fe(III) center is calculated to be low spin in all of the octahedral complexes possessing an N/S ligand shell. Even complex **4**, which exhibits spin equilibria between high and low states, is predominantly low spin at ambient temperature.^{28b} Our inability to separate out effects associated with the nature of Fe–S bonding from those of coordination geometry is a significant problem in interpreting our spin-dependent, theoretical results. These INDO/S calculations therefore do not provide substantial evidence that the sulfur ligands play an unusual role in controlling the Fe(III) spin state through formation of metal–ligand bonds with significant covalent character. This is consistent with the observation that complex **9** mimics the ability of the enzyme active site in spin preference, electronic spectroscopy, and its ability to form six-coordinate structures on treatment with either azide anion^{28h} or nitric oxide,²⁹ even though (i) one of the three sulfur ligands is replaced by nitrogen and (ii) the coordinating sulfurs are not at the oxidation level of those present in the NHase metal center.^{11,42} We also note that two octahedral Fe(III) complexes in which the metal is coordinated by carboxamide moieties in

the absence of Fe–S bonds also have a low-spin electronic configuration.^{41b}

On the other hand, recent studies on the behavior of complexes **7**, **11**, a Co(III)-containing analogue of **7**, and two other Fe(III) complexes prepared as models of the NHase center when treated with various oxidants suggest that there are significant differences in the chemical reactivity of the Fe–S bonds in these structures.^{28h,i,43} Although these observations might reflect limitations in modeling the Fe–S bonding in these systems at the INDO/S level of theory, more work has to be performed to elucidate the mechanisms underlying these reactions. In addition, the calculations described here only reflect the electronic structure of the molecular ground states. Important effects on the transition state energetics and metal–sulfur bonding during the oxidation reaction are therefore unlikely to be evident in the bond orders computed using the PUHF–INDO/S method.

Conclusions

Fully projected UHF calculations, in combination with the INDO/S semiempirical model, represent a computationally

inexpensive method for computing the spin-state preferences of Fe(III) complexes in which there is an N/O/S ligation shell. Given the crystal structure of a complex that does not exhibit temperature-dependent spin equilibrium, the PUHF method correctly computes the observed spin preferences of the Fe(III) center in this class of compounds. Conversely, the disagreement between the observed and PUHF-derived spin states may indicate some disparity between the structure of the complex under the conditions used for the magnetism measurements and that present in the single crystal. These calculations at the INDO/S level of theory provide no substantial evidence that the sulfur ligands play an unusual role in controlling the Fe(III) spin state through formation of metal–ligand bonds with significant covalent character. Such bonding, however, likely does affect the chemical reactivity of the Fe(III) center. On the other hand, these semiempirical calculations suggest that low-spin character may be stabilized by the σ -donating ability of the deprotonated amide ligands. In light of the observed spin-crossover behavior of **9**, and the lack of a clear pattern between metal–sulfur bonding and metal spin preferences in the INDO/S description of complexes **3–13**, the stability of the low-spin Fe(III) in the NHase center probably reflects a subtle combination of geometric and electronic effects. Further computational studies to delineate the relative importance of these contributions in defining the spin-state preferences of Fe(III) are underway.

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