Mechanistic Insight into the Symmetric Fission of [4Fe–4S] Analogue Complexes and Implications for Cluster Conversions in Iron–Sulfur Proteins

Shuqiang Niu,‡¹ Xue-Bin Wang,‡§ Xin Yang,‡§ Lai-Sheng Wang,*‡§ and Toshiko Ichiye*†,‡

School of Molecular Biosciences, Washington State University, Pullman, Washington 99164-4660, Department of Chemistry, Georgetown University, Washington, DC, 20057-1227, Department of Physics, Washington State University, 2710 University Drive, Richland, Washington 99352, and W. R. Wiley Environmental Molecular Science Laboratory, Pacific Northwest National Laboratory, P.O. Box 999, Richland, Washington 99352

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Assembly and disassembly of protein-bound iron–sulfur clusters are involved in a wide variety of vital biological processes, ranging from biosynthesis to regulation of biological function. The study of the fission of analogue clusters can provide valuable insight into the various reaction mechanisms that can occur in proteins as well as tests of the ability of theoretical studies to interpret experiment. Previously, we observed symmetric fission of doubly charged Fe–S cluster anions, \( [2\text{Fe}S_2X_2]^{2-} \rightarrow [2\text{Fe}S_2X]^- \) (\( X = \text{SC}_2\text{H}_2\text{O}, \text{Cl}, \text{Br} \)) in the gas phase, which is surprising because four strong Fe–S bonds are being broken. Here, we report a study of the detailed fission mechanism using density functional theory in conjunction with photoelectron spectroscopic results for \( X = \text{Cl} \). Both the experimental and theoretical results suggest that the fission daughter products are low-spin \( [2\text{Fe}S_2\text{Cl}]^- \) (\( S = \frac{1}{2} \)) species. However, the layered structure of the cubane would seem to indicate a mechanism that would give high-spin daughter products. Thus, we investigate the symmetric fission of \( [2\text{Fe}S_2\text{Cl}]^{2-} \) along two possible reaction pathways, involving high-spin and low-spin \( [2\text{Fe}S_2\text{Cl}]^- \) fragments, respectively. Though the high-spin channel is endothermic by 1.34 eV with a high barrier of 2.65 eV, the reaction along the low-spin fission channel is more favorable with an exothermicity of 0.53 eV and a lower barrier of 1.51 eV. Two intermediates are observed along the low-spin fission channel, a spin-localized cubane \( [2\text{Fe}S_2\text{Cl}]^{2-} \) cluster, which contains two valence-localized Fe\( ^{2+} \) centers and two valence-localized Fe\( ^{2+} \) centers, and a half-opened \( [2\text{Fe}S_2\text{Cl}]^{2-} \) cluster. The spin-localized cluster is crucial to breaking the four strong Fe–S bonds in the symmetric fission.

Introduction

Iron–sulfur clusters are well-known to be an important class of electron carriers in electron-transfer proteins, which are involved in a wide variety of processes in all organisms, including such essential life-sustaining processes as photosynthesis, respiration, and nitrogen fixation.1 Including such essential life-sustaining processes as photosynthesis, respiration, and nitrogen fixation.1 Including such essential life-sustaining processes as photosynthesis, respiration, and nitrogen fixation.1 Including such essential life-sustaining processes as photosynthesis, respiration, and nitrogen fixation.1

Many of the iron–sulfur clusters found in proteins assemble spontaneously and interconvert nearly quantitatively under appropriate conditions.4 The most common transformation is the interconversion between the cubane [4Fe–4S]\( ^{2+} \) and the cuboidal [3Fe–4S]1+ observed in the Desulfovibrio gigas ferredoxin II and the enzyme aconitase.10,11 On the other hand, since the first example of a direct [4Fe–4S]\( ^{2+} \) to [2Fe–2S]\( ^{2+} \) conversion in the iron–sulfur protein of Azotobacter vinelandii nitrogenase was reported in 1984,12 this type of conversion has been also found to function as a biosensor in living cells13 and as the radical and sulfur sources in many biosynthetic processes.14,15 For instance, it appears to be important for the function of fumarate nitrate reductase (FNR), which in Escherichia coli is a global transcription regulator that controls numerous genes required for the synthesis of components of its anaerobic respiratory pathways.9,16 The anaerobically purified form of FNR is a dimer containing a highly oxygen-sensitive [4Fe–4S]\( ^{2+} \) cluster per subunit.17 On exposure to dioxygen, these [4Fe–4S]\( ^{2+} \) clusters are converted to [2Fe–2S]\( ^{2+} \) either in vitro13 or in vivo,18 which decreases DNA binding and results in the dissociation to the monomeric form. The [2Fe–2S]\( ^{2+} \) cluster can be largely converted to the [4Fe–4S]\( ^{2+} \) form under anaerobic conditions in vitro or in vivo, and the corresponding enzymatic activity is regained. Similar conversion processes have been also observed in biotin14 and lipoate synthases.14b Biotin synthase contains only [2Fe–2S]\( ^{2+} \) clusters when isolated under aerobic conditions, which catalyzes the last step of the biosynthesis of biotin, an essential vitamin. Although the [2Fe–2S]\( ^{2+} \) cluster is proposed to be the sulfur source for biotin synthesis, absorption and resonance Raman studies show that the two reduced [2Fe–2S]\( ^{2+} \) clusters may dissociate from the protein and reassemble as a [4Fe–4S]\( ^{2+} \) cluster in the absence of additional iron and sulfide.14a Although these show that the conversion between [4Fe–4S]\( ^{2+} \) and [2Fe–2S]\( ^{2+} \) is a reversible process, the reaction mechanisms still remain unknown.
Determination of the electronic structure, energetics, and interconversion mechanisms of the iron–sulfur clusters is crucial to understand the properties and function of the iron–sulfur proteins. The intrinsic thermodynamic and kinetic studies of iron–sulfur cluster assembly/disassembly in proteins are relatively challenging using quantum mechanical (QM) approaches. Given the complexity of the reaction of the clusters in the protein environment, it is essential to prove that our computational methods can predict the physicochemical properties of simple clusters in the gas phase for which we have accurate experimental values. Although recent progress in combined quantum mechanical/molecular mechanical (QM/MM) methods may lead to information about protein environmental effects on the clusters, we have carried out a series of investigations using a combination of techniques including electrospray ionization (ESI), photoelectron spectroscopy (PES), and density functional theory (DFT) calculations. Although the reactions in the proteins may be influenced by the protein environment of the clusters, careful experimental and theoretical studies of analogue complexes can provide insight into possible reaction mechanisms as well as tests of the theoretical studies to interpret experiment.

A key question in these studies is the formation and the dissociation of the strong Fe–S bonds. In previous studies of the electronic structure and reduction potentials of the rubredoxin redox site analogues, [Fe(SCH3)3]n− (n = 0, 1, 2) and [Fe(SCH3)4]n− (n = 0, 1), we have shown a thermodynamic cycle that relates the [Fe(SCH3)4]1− couple and the ligand association reaction, [Fe(SCH3)2]2− + 2S− → [Fe(SCH3)3]1− + S2−. Our studies showed that the [FeX2]− and [FeX4]n− (X = Cl, Br, SCH3) are the most stable states, because the high-spin FeII site favors a planar three-coordinated structure, whereas the high-spin FeIII site favors a tetrahedral four-coordinated one. Thus, it appears that the FeII ↔ FeIII conversion through an electron transfer is a crucial factor in determining the formation and breaking of Fe–S bonds in the iron–sulfur clusters in the gas phase.

More recently, we observed symmetric fission in a series of doubly charged [4Fe–4S] complexes, [Fe2S4X4]2− (X = Cl, Br, SC2H5), through collision-induced dissociation (CID) and PES experiments.20,21 The observation of the symmetric fission was unexpected because it involves breaking four strong Fe–S bonds. Our prior PES results and DFT calculations of the cubelike [Fe4S4X4]2− complexes and the fission products [Fe2S2X2]− demonstrated: (i) the parent and the fission species had similar electronic structures, owing to the unique layered structure of the [Fe4S4X4]2− cluster and its antiferromagnetic coupling, (ii) the fission may proceed either along a high-spin channel or a low-spin channel according to Scheme 2, and (iii) the low-spin fission channel was almost thermoneutral with an endothermicity of only 0.09 eV, whereas the high-spin fission channel had a much higher endothermicity of 1.26 eV.22 The stability of the low-spin product is not surprising because previous experimental26 and theoretical27,28 studies of the reduced [Fe2S2R4]3− species showed that the [Fe2S2]3+ core contains a valence-localized FeII site and a valence-localized Fe2+ site with a net spin S = 5/2. On the other hand, the first example of a synthetic or biological [Fe2S2]1+ cluster with an S = 7/2 ground state has been observed in spectroscopic studies of the Cys56Ser mutant [2Fe] ferredoxin from Clostridium pasteurianum.29 Despite the thermodynamic favorability of the low-spin species, the layered structure of [Fe4S4X4]2− seemed to suggest that the high-spin channel may be more favored kinetically because of magnetic repulsion and strong electron delocalization, in addition to the electrostatic repulsion. However, the high-spin [Fe2S2X2]− daughter anions were not consistent with the PES data, which suggested that the observed [Fe2S2X2]− fission products had low-spin states. Consequently, a study of the fission mechanism is necessary to resolve the barrier involved.

The DFT method is now a widely used computational technique to study real reaction systems containing transition metals.30 In the current paper, we report a detailed DFT study, verified by the PES data, on the fission mechanisms of [Fe4S4Cl4]2−. This analogue has proved to be a sufficient model to obtain geometries and electronic structures of the cubane core found in the active site of the proteins because of the similarity in geometrical and electronic structure to the [Fe4S4Cl4]2−, [Fe(SC2H5)4]−, and [Fe3S4(SC2H5)4]2−/− protein redox site analogues.31 The electronic structures, geometries, and energetics of the parent and fission complexes, the intermediate complexes, and the transition states along the possible fission channels are calculated using the broken-symmetry DFT method. A major effort was made to search for transition states and intermediates along both the high-spin and low-spin fission channels. Although
no fission intermediates were found for the high-spin channel, two interesting intermediates were located for the low-spin channel, which was shown to be the favored fission channel with a significantly lower barrier. Combined with available experimental data in biological and synthetic systems, the current study provides valuable insight into iron–sulfur cluster assembly/disassembly mechanisms, as well as other enzymatic processes.

**Methods**

In our work on the electronic structure of [Fe₄S₄X₄]²− (X = SC₂H₅, SH, Cl, Br, I), we have shown that although the weaker electron donor Cl ligands lead to a larger electron binding energy of [Fe₄S₄X₄]²− by ~0.5 eV than the SC₂H₅ ligands, both terminal ligands lead to similar [4Fe–4S]²⁺ core geometries and similar electronic structures. Therefore, in the current study, we focus on the cubane core structure for the detailed exploration of the conversion mechanism of [4Fe–4S] to [2Fe–2S] using the [Fe₄S₄Cl₄]²− complex as models. This avoids the necessity of exploring the conformational changes in the terminal ligands that could occur in the transition states or intermediates of [Fe₄S₄(SC₂H₅)₄]²−.

The broken-symmetry DFT method, specifically with the Becke’s three-parameter hybrid exchange functional and the Lee–Yang–Parr correlation functional (B3LYP) using the 6-31G** basis sets, was utilized for the geometry optimizations and electronic structure calculations of the iron–sulfur clusters and transition structures. An approximate spin projection procedure based on the developed method by Noodleman was carried out on the broken-symmetry DFT energies of the fission reactant and products because the broken-symmetry energy of a spin polarized low-spin state is not a pure spin-state energy and is a weighted average of pure-spin states. Transition states (TS) were optimized by an eigenvalue-following optimization method, in which the final updated Hessian shows only one negative eigenvalue, and were verified by several separate calculations along the reaction coordinate (RC). No symmetry restraints were imposed during geometry optimizations, and each structure was confirmed to be a ground-state structure by several separate calculations on different possible configuration states. This procedure is necessary because the electronic structure is very sensitive to the iron–sulfur cluster bonding structure. The calculated energies were refined at the B3LYP/6-31G**/(B3LYP/6-31G**) level, where sp-type diffuse functions were added to the 31G** basis set of the sulfur atoms, which significantly improved the accuracy of the calculated oxidation potentials of the iron–sulfur redox couples.

Furthermore, our recent PES and DFT studies on the tetrahedral ferric complexes Fe₃III⁻X⁻ (X = Cl, Br) and the three-coordinate complexes M²⁺X⁻ (M = Mn, Fe, Co, Ni; X = Cl, Br) have shown that increasing the basis set size for all atoms, for example, using triple-ζ basis sets, does not significantly change the calculated geometric parameters and redox energies with respect to the experimental data or the calculated values using double-ζ basis sets. Self-interaction error (SIE), which arises owing to the difficulty in describing the dissociation behavior of molecules with a noninteger number of electrons or spins using the DFT method, is roughly estimated for the transition states by comparing the total energy of the dissociating fragments at 40 Å with that of two separated fragments. Although the error introduced by the SIE most likely exceeds the improvement by recalculating energies at the B3LYP/6-31G**(B3LYP/6-31G**) level, especially for the later stages of the fission, this level is used to maintain consistency with our previous work. In addition, because our mechanistic calculations involve the comparisons of different reaction pathways with similar dissociated fragments, the SIE tends to cancel and should not affect our conclusions.

All calculations were performed using the NWChem program package. The molecular orbital visualizations were performed using the extensible computational chemistry environment (Ecce) application software.

**Results and Discussion**

**Geometries and Electronic Structures of [Fe₄S₄Cl₄]²− and [Fe₄S₄X₄]²−.** It is well-known that the [4Fe–4S]²⁺ core can be viewed as two ferromagnetic [2Fe–2S] sublayers, which are coupled antiferromagnetically to give a low-spin state. The [Fe₄S₄Cl₄]²− (R) fission (Scheme 2) can proceed by breaking four Fe–S bonds between the two ferromagnetic sublayers to generate two high-spin [Fe₂S₂Cl₂]− daughter anions [P(H)] or within the ferromagnetic sublayers to give two low-spin [Fe₄S₄Cl₄]²− daughter anions [P(L)]. The latter, however, also requires breaking the additional Fe–Fe bonding arising from the delocalization of the minority spin electrons within each sublayer. To elucidate the influence of spin states on the properties of [Fe₄S₄Cl₄]²− and [Fe₄S₄X₄]²−, we investigate their geometries and electronic structures by combining the DFT calculations with the PES measurements.

(a) Parent [Fe₄S₄Cl₄]²−: Dianion (R). The electronic structure of the cubane [4Fe–4S]²⁺ have been studied theoretically by Noodleman and co-workers extensively using the broken symmetry DFT methods. The spin-couplings within the [4Fe–4S]²⁺ core can be described by the following Heisenberg–Dirac–van Vleck (HVV) spin Hamiltonian:

$$H_{\text{spin}} = J \sum_{i<j=1,4} S_i S_j + B(S_{AB} + \frac{1}{2}) \pm B(S_{CD} + \frac{1}{2})$$

and eigenvalues

$$E(S) = (J/2) S(S + 1) \pm B(S_{AB} + \frac{1}{2}) \pm B(S_{CD} + \frac{1}{2})$$

where A, B, C, and D represent the four high-spin sites, J is the exchange coupling constant, B is a resonance parameter describing the delocalization in the Fe–Fe pair, S is the spin operator of the ith iron in [4Fe–4S]²⁺, S₁ and S₂ are the Fe–Fe pair spin operator ranging from [S₁ − S₂] to S₁ + S₂ (i.e., 1/2 to 9/2), and S is the total spin ranging from [S_{AB} − S_{CD}] to S_{AB} + S_{CD} (i.e., 0–9). The spin-state energy usually represents a compromise of the competing forces between Heisenberg exchange and resonance delocalization (double exchange) in a given system. Furthermore, it was found that the [4Fe–4S]²⁺ core exhibits the mixed valence state, where two high-spin Fe centers in each [2Fe–2S] sublayer are coupled ferromagnetically and one minority spin electron is delocalized between the two Fe on each sublayer, consistent with experimental observations from Mössbauer and EPR spectroscopy.

The PES data and DFT calculations indicate that [Fe₄S₄Cl₄]²− is relatively stable with an ADT of about 0.8 eV (Table 1). Our previous studies showed that the ADEs of [Fe₄S₄L₄]²− (L = SH, SC₂H₅, Cl, Br, I) depend on the terminal ligands, because the HOMO’s of [Fe₄S₄L₄]²− exhibit terminal Fe–L antibonding and strong Fe–Fe bonding character in each sublayer. Similar to other ligands, in [Fe₄S₄Cl₄]²−, the two minority electrons delocalize on the two ferromagnetic sublayers, respectively, resulting in significant intralayer Fe–Fe bonding interactions, in addition to the HDV antiferromagnetic coupling interaction between the two ferromagnetic sublayers. As shown in Figure
1. the B3LYP/6-31G** optimized Fe–Fe distance within the two sublayers of [Fe₅S₄Cl₂]⁻ is shorter by 0.087 Å. In addition, the four Fe–S bonds within each ferromagnetic layers become equal because of the effective conversion from an Fe²⁺–Fe³⁺ pair to an Fe⁴⁺–Fe⁵⁺ delocalized pair. The average Fe–S bond energy in the [4Fe–4S]ₚ cubane core was estimated to be ~2 eV, close to the calculated Fe–S bond energy of the tetrahedral mono-ferric thiolate cluster.²⁵

(b) High-Spin and Low-Spin [Fe₅S₄Cl₂]⁻ Anions. Upon the fission of [Fe₅S₄Cl₂]⁻ (R), two possible [Fe₄S₄Cl₂]⁻ daughter anions may be produced: either a high-spin product P(H) (S = ⁹/₂) or a low-spin product P(L) (S = ⁷/₂). As shown in Figure 2, the optimized geometry of P(L) with lowest energy has a planar structure with a valence-localized Fe²⁺–Fe³⁺ pair, whereas the optimized geometry of P(H) has the lowest energy has a folded structure of ⁵¹° with a valence-delocalized Fe²⁺–Fe³⁺ pair.

The Fe–Cl and Fe–S bond distances increase in the order of Fe³⁺ < Fe²⁺⁺ < Fe²⁺. The folded P(H) is a direct consequence of the delocalized minority spin, which has strong Fe–Fe σ bonding character. Analyses of the molecular orbitals and the spin densities show that the majority and minority spin Fe 3d orbitals of P(H) are entirely delocalized over both Fe centers, whereas those of P(L) are localized on the individual Fe sites with antiferromagnetic coupling (Table 2). In particular, the minority spin of P(H) is delocalized on the Fe–Cl antibonding orbital with very strong Fe dₓz character. Because the energy difference between P(H) and P(L) can be attributed to a competition between HDV spin coupling and resonance delocalization, the bonding interactions of the antiferromagnetically coupled [2Fe–2S] binuclear site in P(L) are larger than those of the ferromagnetically coupled [2Fe–2S] site with spin delocalization in P(H), contrary to the [4Fe–4S]₂⁺ cubane core. Overall, our DFT calculations show that P(L) is more stable than P(H) by 0.58 eV at the B3LYP/6-31+(++g)° level and by 0.89 eV when the spin-projection correction is taken into account.

Upon oxidation from [Fe₅S₄Cl₂]⁻ to [Fe₅S₄Cl₂]⁰, the Fe–Fe spin coupling interaction and Coulomb repulsion of the oxidized species P₉/₁(L) and P₇/₁(H) increase. In comparison to P(L), the Fe–Fe distance of P₉/₁(L) only shortens by 0.01 Å, indicating that the Fe–Fe antiferromagnetic coupling interaction partially counteracts the strong Coulomb repulsion of the ferric ferric centers. With the increase in charge of the former ferrous center (+0.31 e) and the Cl atom (−0.18 e) upon the oxidation, average Fe–S bond distances and the Fe–Cl bond distances of P₉/₁(L) shorten relative to those of P(L) by 0.03 and 0.08 Å, respectively. On the other hand, the Fe–Fe distance of P₇/₁(H) significantly increases by 0.48 Å with respect to P(H) and P₉/₁(L) becomes planar because there is no longer strong Fe–Fe bonding from a delocalized minority spin and the ferromagnetic coupling interaction of Poxd(H) decreases the bonding interactions between the iron centers and the ligands. Consequently, P₉/₁(H) is less stable by 0.85 eV than P₇/₁(L). Therefore, the

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TABLE 1: Experimental and Calculated Adiabatic (ADE) and Vertical (VDE) Detachment Energies (eV) and the Oxidant Relaxation Energies (eV) and the 

TABLE 2: Calculated Charges and Net Spin Densities at the B3LYP/6-31G** Level for the Parent [Fe₅S₄Cl₂]⁻ Dianion (R), the Daughter [Fe₅S₄Cl₂]⁻ Anions [P(H) and P(L)], the Fission Intermediates [I₁(L) and I₂(L)], and the Fission Transition States [TS₁(H), TS₁(L), and TS₂(L)]

R | P(H) | P(L) | TS₁(H) | TS₁(L) | TS₂(L) | TS₂(L) | (S = 0) | (S = ⁹/₂) | (S = ⁷/₂) | (S = 0) | (S = 0) | (S = 0) | (S = 0) |
---|---|---|---|---|---|---|---|---|---|---|---|---|---|
 unspecified | +0.57 | +0.64 | +0.80 | +0.67 | +0.70 | +0.75 | +0.84 |
 Fe₉/₁ | +0.57 | +0.64 | +0.45 | +0.67 | +0.55 | +0.54 | +0.51 | +0.44 |
 S₁ | −0.56 | −0.64 | −0.40 | −0.64 | −0.62 | −0.64 | −0.60 |
 S₁ | −0.56 | −0.64 | −0.40 | −0.64 | −0.62 | −0.59 | −0.58 | −0.62 |
 C₁ | −0.51 | −0.51 | −0.51 | −0.53 | −0.49 | −0.49 | −0.48 | −0.50 |
 C₁ | −0.51 | −0.51 | −0.54 | −0.53 | −0.52 | −0.55 | −0.55 | −0.56 |
 a. Mulliken Charges | | | | | | | | | | | | | |
 Fe₉/₁ | +3.86 | +3.96 | +3.99 | +3.96 | +4.02 | +3.99 | +3.97 | +3.99 |
 Fe₇/₁ | −3.86 | +3.96 | −3.57 | −3.96 | −3.82 | −3.68 | −3.62 | −3.57 |
 S₁ | +0.14 | +0.43 | +0.26 | +0.43 | +0.00 | −0.03 | +0.01 | +0.26 |
 S₁ | −0.14 | +0.43 | +0.26 | +0.43 | +0.15 | +0.25 | +0.25 | +0.26 |
 C₁ | +0.11 | +0.11 | +0.12 | +0.10 | +0.14 | +0.14 | +0.15 | +0.13 |
 C₁ | −0.11 | +0.11 | −0.07 | −0.10 | −0.09 | −0.07 | −0.06 | −0.06 |
 b. Net Spin Densities | | | | | | | | | | | | | |
The major factor contributing to stabilization energy of the redox sites is the spin-coupling character of the system.

Overall, our DFT calculations support that the reduced $[\text{Fe}_2\text{S}_2\text{Cl}_2^-]$ species is a low-spin ground state, in which the minority spin has Fe(d$^2$)$_{z^2}$-Cl antibonding character. Further confirmation of the DFT results is made by the PES measurements. Our PES data revealed that the $[\text{Fe}_2\text{S}_2\text{Cl}_2^-]$ daughter anions have similar electronic structure to the $[\text{Fe}_4\text{S}_4\text{Cl}_4^-]$ dianion, giving rise to an inverted level scheme. The B3LYP/6-31G** calculations show that the calculated ADE and relaxation energy ($\lambda_{\text{relax}}$) of the low-spin $[\text{Fe}_2\text{S}_2\text{Cl}_2^-]$ are 3.97 and 0.15 eV, respectively, in very good agreement with the experimental values of 3.80 and 0.13 eV (Table 1). On the other hand, a high-spin $[\text{Fe}_2\text{S}_2\text{Cl}_2^-]$ would have significant geometry distortions upon photodetachment of the delocalized electron, resulting in a larger ADE of 4.09 and a much larger $\lambda_{\text{relax}}$ of 0.64 eV, obviously inconsistent with the PES measurements. Although a more stable high-spin species of $[\text{Fe}_2\text{S}_2\text{Cl}_2^-]$ with $S = \frac{7}{2}$ is lower by 0.23 eV in energy than that of the $S = \frac{3}{2}$ state (through an $\alpha$ spin flip), the larger ADE of 4.22 eV and the larger $\lambda_{\text{relax}}$ of 0.27 eV of the $S = \frac{7}{2}$ state also disagree with the PES values.

Disassembly of $[\text{Fe}_4\text{S}_4\text{Cl}_4^-]^-$. After confirming that the fission products are the low-spin $[\text{Fe}_2\text{S}_2\text{Cl}_2^-]$ anions [P(L)] and that the symmetric fission in $[\text{Fe}_4\text{S}_4\text{Cl}_4^-]$ is almost thermo-neutral, the next questions are how the fission reaction overcomes an expected large energy barrier arising from the breaking of four strong Fe-S bonds and whether it is possible that the fission proceeds via a high-spin channel and then the products decay to a low-spin species. To understand the kinetic behavior of the fission reactions, we performed B3LYP geometry optimizations and energy calculations to search for transition states and possible fission intermediates along the two fission channels.

(a) High-Spin Fission Channel of $[\text{Fe}_4\text{S}_4\text{Cl}_4^-]^-$. Because the high-spin fission channel involves the breaking of four Fe-S bonds between the two antiferromagnetically coupled sublayers of $[\text{Fe}_4\text{S}_4\text{Cl}_4^-]^-$, this channel would be expected to have a lower barrier than the low-spin fission channel, which involves the breaking of two additional Fe-Fe bonding interactions within the two sublayers. We carefully searched the stationary points along the high-spin fission pathway and only found a late transition state TS(H) (Figure 3), in which the distance between the two sublayers is 4.774 Å and the structure of each sublayer already resembles that of the daughter anion (H). In comparison to R, the Fe-Fe and Fe-S bond distances of TS(H) are shorter by 0.398 and 0.105 Å, respectively. The intracluster Coulomb repulsion, the increasing bonding interaction between the Fe

![Figure 3](image-url)
centers and ligands within the two sublayers, and the magnetic repulsion derived from the antiferromagnetic coupling between the two ferromagnetically coupled sublayers all play a role in this fission channel. This channel is endothermic by 1.26 eV with a high barrier of 2.95 eV. Taking the SIE correction and spin projection into account, the fission reaction is endothermic by 1.34 eV and the fission barrier is estimated to be ~2.7 eV, as shown in Figure 3. This fission channel is less endothermic per bond broken than the thiolate dissociation from the [1Fe] cluster (2 eV)\textsuperscript{25} because the [2Fe–2S] daughter anions have stronger Fe–S and Fe–Fe bonding interactions than the [4Fe–4S] parent. However, the fission barrier is sufficiently high and the reaction is significantly endothermic so that this fission channel is expected to be unfavorable, indicating that a high-spin channel fission followed by decay to low-spin products is unlikely.

(b) Low-Spin Fission Channel of [Fe\textsubscript{4}S\textsubscript{4}Cl\textsubscript{4}]\textsuperscript{2–}. The low-spin fission channel of [Fe\textsubscript{4}S\textsubscript{4}Cl\textsubscript{4}]\textsuperscript{2–} would appear to be favored over the high-spin channel on the basis of the low-spin state of the antiferromagnetically coupled [Fe\textsubscript{2}S\textsubscript{2}Cl\textsubscript{2}]– daughter anions [P(L)]. Although the low-spin fission process is favored thermodynamically over the high-spin fission process, if the low-spin fission of [Fe\textsubscript{4}S\textsubscript{4}Cl\textsubscript{4}]\textsuperscript{2–} proceeded through a symmetric fission transition state like in the high-spin channel, the reaction might encounter a higher activation energy than the high-spin channel because it involves the breaking of four strong Fe–S bonds and two additional Fe–Fe bonds. Such a high barrier for the low-spin channel might result in some high-spin products as well. However, because only low-spin [Fe\textsubscript{2}S\textsubscript{2}Cl\textsubscript{2}]– products were observed experimentally, it suggests that there might be other low-barrier pathways along this fission channel.

Our calculations of the [1Fe] complexes suggest that the conversion of the high-spin tetrahedral ferrous site to the trigonal planar ferrous site is exothermic by ~2 eV with a low barrier of 0.5 eV.\textsuperscript{25,44} Because a relatively stable three-coordinate ferrous center exists in the antiferromagnetic daughter anion P(L), we suspected that a low barrier pathway might be possible via an intermediate with ferrous centers. Indeed, we found a half-open and relatively low-energy [Fe\textsubscript{4}S\textsubscript{4}Cl\textsubscript{4}]\textsuperscript{2–} intermediate I\textsubscript{2}(L) with two tetrahedral ferric centers and two trigonal planar ferrous centers (Figure 3). This intermediate I\textsubscript{2}(L) is only 0.33 eV higher in energy than the parent R. Reoptimization of the cubane structure [Fe\textsubscript{4}S\textsubscript{4}Cl\textsubscript{4}]\textsuperscript{2–} along the reaction coordinate from I\textsubscript{2}(L) to R using the electron configuration state of I\textsubscript{2}(L) failed to directly recover the parent R. Instead, a new antiferromagnetically coupled [Fe\textsubscript{4}S\textsubscript{4}Cl\textsubscript{4}]\textsuperscript{2–} I\textsubscript{1}(L) with a localized core spin character was unexpectedly found through searching for stationary points. The vibrational frequency calculation of the optimized [Fe\textsubscript{4}S\textsubscript{4}Cl\textsubscript{4}]\textsuperscript{2–} core of I\textsubscript{1}(L) indicates it is a local minimum.

The intermediate I\textsubscript{1}(L) contains two regular ferric and two ferrous centers, ([Fe\textsuperscript{2+}]\textsubscript{2}([Fe\textsuperscript{3+}]\textsubscript{2}S\textsubscript{4})\textsuperscript{2–}, in which the Fe\textsuperscript{2+}–S bonds are longer by 0.035–0.118 Å and the Fe\textsuperscript{3+}–S bonds are shorter by 0.029–0.041 Å than the Fe\textsuperscript{2.5+}–S bonds in the parent R (Figure 1). It is somewhat surprising that the new intermediate I\textsubscript{1}(L) is only less stable by 0.13 eV than the parent [Fe\textsubscript{4}S\textsubscript{4}Cl\textsubscript{4}]\textsuperscript{2–} (R). Moreover, we found that the intermediate I\textsubscript{1}(L) exhibits distinctly different electronic structure in its spin state (Table 2). In the parent [Fe\textsubscript{4}S\textsubscript{4}Cl\textsubscript{4}]\textsuperscript{2–}, the two minority electrons delocalize on the two ferromagnetic sublayers, resulting in additional stabilizing interactions beyond the HD\textsubscript{V} antiferromagnetic coupling between the two ferromagnetic sublayers. In the intermediate I\textsubscript{1}(L), the resonance delocalization within the two ferromagnetic sublayers vanishes and the spin densities localize at each Fe site, shown as HOMO and LUMO in Figure 4, consequently, the four Fe\textsuperscript{2.5+} sites are converted to two Fe\textsuperscript{3+} and two Fe\textsuperscript{2+} sites without spin flipping.

Furthermore, a transition structure TS\textsubscript{1}(L) was found to connect the intermediates I\textsubscript{1}(L) and I\textsubscript{2}(L) with a low barrier of 0.28 eV. In the transition state TS\textsubscript{1}(L), the first step of fission starts within the Fe\textsuperscript{2+}–Fe\textsuperscript{2+} sublayer. The Fe\textsuperscript{2+}–S and Fe\textsuperscript{3+}–S bonding interactions on the two Fe\textsuperscript{2+}–Fe\textsuperscript{3+} sublayers of TS\textsubscript{1}(L) increase, whereas the Fe\textsuperscript{3+}–S bonding interactions in the Fe\textsuperscript{3+}–Fe\textsuperscript{3+} sublayer slightly decrease and the Fe\textsuperscript{2+}–S bonding interactions in the Fe\textsuperscript{2+}–Fe\textsuperscript{3+} sublayer decrease even more with respect to the intermediate I\textsubscript{1}(L). The breaking of the two Fe\textsuperscript{2+}–S bonds to a half-open intermediate I\textsubscript{2}(L) only leads to an endothermicity of 0.20 eV because of the increasing antiferromagnetic coupling within the two Fe\textsuperscript{2+}–Fe\textsuperscript{3+} sublayers. Upon breaking of the two Fe\textsuperscript{2+}–S bonds from the intermediate I\textsubscript{2}(L) to the final product P(L), we located another late transition state TS\textsubscript{2}(L), in which the distance of the breaking Fe\textsuperscript{3+}–S bonds is 4.413 Å and the two fragments are quite similar to the final daughter anions P(L). We note that the electron densities and spin densities of the ferric centers from the intermediate I\textsubscript{2}(L) through TS\textsubscript{2}(L) to the daughter anions P(L) do not significantly change. The reaction from I\textsubscript{1}(L) to P(L) is slightly exothermic by 0.24 eV with a barrier of 1.48 eV. Taking the SIE correction into account, the fission barrier is estimated to be ~1.2 eV. In the late stage of the fission reaction, with the decreasing Coulomb repulsion and increasing Fe–S bonding interactions, the total energy goes down. Overall, the reaction via the low-spin fission channel from the parent cluster R through the intermediates I\textsubscript{1}(L) and I\textsubscript{2}(L) to the daughter anions P(L) (Scheme 3) is almost thermoneutral with an endothermicity of only 0.09 eV with a lower barrier of 1.8 eV and, taking the SIE and spin projection corrections into account, is exothermic by 0.53 eV with a low barrier of 1.51 eV. Thus, this channel should be more favorable than the high-spin channel.

The proposal of conversion to an Fe\textsuperscript{II} before breaking the Fe–S bond is also consistent with previous studies of the [1Fe] complexes.\textsuperscript{25} In these studies, we found that the thiolate association of [Fe(SCH\textsubscript{3})\textsubscript{3}]\textsuperscript{2–} and the thiolate dissociation of
show that the spin-coupling interaction of both the reduced electron exchange interaction. The DFT optimized geometries competition between HDvV spin coupling interaction and high-spin states of [2FeS] 2− of [4FeS] 2− play an important role in the symmetric fission of the [4FeS] 2− analogue complexes. Studies show that the fission [Fe 2S 2Cl 2 ] 2− daughter cluster is a low-spin ground state in which the minority α spin has Fe(d[3])−Cl antibonding character, as is consistent with most findings for [2Fe−2S] clusters in previous experimental and theoretical studies. Further confirmation of the DFT results is made by the PES measurements. The B3LYP/6-31(+(++))G** calculations show that the calculated ADE and relaxation energy (λreax) of the low-spin [Fe 2S 2Cl 2 ] 2− are 3.97 and 0.15 eV, respectively, in very good agreement with the experimental values of 3.80 and 0.13 eV (Table 2). However, the high-spin [Fe 2S 2Cl 2 ] 2−, with significant geometry distortions upon photodetachment of the delocalized electron, has a larger ADE of 4.09 and a much larger λreax of 0.64 eV, which is not in agreement with the PES measurements.

Full geometry optimizations and energy calculations of the [Fe 2S 2Cl 2 ] 2− fission along the high-spin and low-spin fission pathways were performed at the B3LYP/6-31G** and B3LYP/6-31+(++)(++++)G** levels. We found that the symmetric fission along the high-spin fission channel is endo- thermic by 1.34 eV with a very high barrier of 2.65 eV whereas the reaction along the low-spin fission channel in the gas phase is exothermic by 0.53 eV with a relatively low barrier of 1.51 eV. Significantly, the low barrier along the low-spin fission channel is due to a pathway through a spin-localized cubelike [Fe 2S 2Cl 2 ] 2− intermediate, which contains two valence-localized Fe 3+ sites and two valence-localized Fe 2+ sites and is only 0.13 eV higher in energy than the parent. Further fission can easily proceed through breaking two tetrahedral Fe 2+−S bonds to generate a half-opened intermediate. Thus, a low-energy conversion from sublayers with Fe 2.5+−Fe 2.5+ to Fe 2+−Fe 3+ reduces the strength of the Fe−S bonds within each sublayer even below the Fe−S bond between the sublayers so that the fission occurs by breaking both sublayers rather than occurring between the sublayers.

The current study provides valuable insight into iron−sulfur cluster assembly/disassembly mechanisms. The results show that symmetric fission of the isolated cluster occurs via spin localization of iron valences before fission. This implies that the mechanism can be tested experimentally (in conjunction with theoretical study) by altering the terminal ligands and/or second shell to change spin localization of [4Fe−4S] sites. Moreover, this implies a protein may be able to alter the favorability of different modes of fission by changes the degree of spin localization via altering the second shell.

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References and Notes


[Fe(SCH 3 ) 4 ] 2− are exothermic by 2.6 and 1.8 eV, respectively, and the oxidation energies of [Fe(SCH 3 ) 4 ] 2+ and [Fe(SCH 3 ) 3 ] − are −1.7 and 2.7 eV, respectively, at the B3LYP/6-31(+(++))G** level of calculation. These results indicate that [Fe(SCH 3 ) 4 ] 2+ favors a high-spin d 6 electron configuration of Fe III, whereas [Fe(SCH 3 ) 3 ] − favors a high-spin d 5 electron configuration of Fe II, which means that there must be a conversion from Fe III to Fe II upon dissociation of a thiolate ligand. The DFT calculations and experimental observations here show that in the gas phase the Fe II ↔ Fe III conversion through spin-state crossovers with or without spin flipping, which would significantly lower the reaction barrier, may be involved in iron−sulfur cluster assembly/disassembly. Although a tetrahedral ferric center has a very low reduction potential in the gas phase, resulting in a ferrous center with high energy, the electrostatic effects in solvent or protein environments may significantly increase the reduction potentials of the tetrahedral ferric center, facilitating the Fe III ↔ Fe II conversion. This means that the cluster can make contact quickly with a reactant or other redox site and provide or absorb electrons, making iron−sulfur cluster assembly/disassembly easier than in the gas phase.

Conclusions

The electronic structures, energetics, and fission mechanisms of the [4Fe−4S] analogue complexes have been investigated by DFT calculations and PES measurements. The results of these studies show that the spin crossover of the spin-delocalized the FeII−FeII conversion. This means that the cluster can make contact quickly with a reactant or other redox site and provide or absorb electrons, making iron−sulfur cluster assembly/disassembly easier than in the gas phase.

Conclusions

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Fission of [4Fe−4S] Analogue Complexes


