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Mössbauer Properties of the Diferric Cluster and the Differential Iron(II)-Binding Affinity of the Iron Sites in Protein R2 of Class Ia *Escherichia coli* Ribonucleotide Reductase: A DFT/Electrostatics Study

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Abstract

The R2 subunit of class-Ia ribonucleotide reductase (RNR) from *Escherichia coli* (*E. coli*) contains a diiron active site. Starting from the apo-protein and Fe(II) in solution at low Fe(II)/apoR2 ratios, mononuclear Fe(II) binding is observed indicating possible different Fe(II) binding affinities for the two alternative sites. Further, based on their Mössbauer spectroscopy and two-iron-isotope reaction experiments, Bollinger *et al.* (*J. Am. Chem. Soc.*, 1997, **119**, 5976–5977) proposed that the site Fe1, which bonds to Asp84, should be associated with the higher observed ⁵⁷Fe Mössbauer quadrupole splitting (2.41 mm s⁻¹) and lower isomer shift (0.45 mm s⁻¹) in the Fe(III)Fe(III) state, site Fe2, which is further from Tyr122, should have a greater affinity for Fe(II) binding than site Fe1, and Fe(IV) in the intermediate X state should reside at site Fe2. In this paper, using density functional theory (DFT) incorporated with the conductor like screening (COSMO) solvation model and with the finite-difference Poisson-Boltzmann self-consistent reaction field (PB-SCRF) methodologies, we have demonstrated that the observed large quadrupole splitting for the diferric state R2 does come from site Fe1(III) and it is mainly caused by the binding position of the carboxylate group of Asp84 sidechain. Further, a series of active site clusters with mononuclear Fe(II) binding at either site Fe1 or Fe2 have been studied, which show that with single dielectric medium outside the active site quantum region, there is no energetic preference for Fe(II) binding at one site over another. However, when including the explicit extended protein environment in the PB-SCRF model, the reaction field favors the Fe(II) binding at site Fe2 rather than at site Fe1 by ~9 kcal mol⁻¹. Therefore our calculations support the proposal of the previous Mössbauer spectroscopy and two-iron-isotope reaction experiments by Bollinger *et al.*

1. Introduction

Ribonucleotide reductases (RNRs) catalyze the reduction of all four ribonucleotides to deoxyribonucleotides, which provide the required building blocks for DNA replication and repair.^{1–3} Three main classes of RNRs have been discovered that display a common reaction

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Supporting Information

The Cartesian coordinates of the OPBE/COSMO optimized geometries for the mono- Fe1(II), mono-Fe2(II), and some of the diferric clusters are given as supporting information.

mechanism using metals and free radical chemistry.⁴ Although these classes differ in composition and cofactor requirements, they all possess a conserved cysteine residue at the active site that is converted (during the catalytic cycle) into a thiyl radical (Cys-S[•]), which initiates substrate turnover by abstracting a hydrogen atom from the ribose ring of the substrate.^{2,3,5} There is a dinuclear metal center in class I RNRs, a cobalt containing cobalamin cofactor (adenosylcobalamin) in class II RNRs, and a 4Fe-4S cluster coupled to S-adenosylmethionine in class III RNRs.³ Each of these cofactors generates a radical that transfers to produce the Cys-S[•]. Class I RNRs have been studied extensively and are found in all eukaryotes as well as in some microorganisms like *Escherichia (E.) coli*. There are two dissimilar protein subunits R1 (α_2 -homodimer) and R2 (β_2 -homodimer) in class I RNRs. R1 contains the conserved cysteine residue and the substrate binding site, and catalyzes the dehydroxylation of the 2'-hydroxyl group of the ribose ring. R2 contains the dinuclear metal cluster that generates a stable radical, which then transfers (through a long-range proton-coupled-electron-transfer propagation mechanism) to create the Cys-S[•] which initiates the ribonucleotide-to-deoxyribonucleotide reaction in R1.

The class I RNRs are further divided into three subclasses, Ia, Ib, and Ic. In class Ia RNRs, a tyrosine residue (Tyr122 in Ia *E. coli* R2) is the radical bearer closest to the diiron center in R2 (Figure 1).⁶⁻⁸ The fairly stable tyrosyl radical is generated by an Fe(III)Fe(IV) intermediate state X following the reaction of the reduced Fe(II)Fe(II) center with molecular O₂.⁹ The active form of class Ia R2 is described as an Fe(III)Fe(III)-Tyr[•] state. The class Ib RNR in *Corynebacterium ammoniagenes (Ca)* was found to be Mn-dependent twenty years ago.^{10,11} However, the role of Mn was unclear, since the enzyme was active in vitro with an iron cofactor (Fe(III)Fe(III)-Tyr[•]).¹²⁻¹⁴ Very recently, it has been discovered that the native class Ib RNR in *Ca* contain a dimanganese cluster in R2, where the Mn(II)Mn(II) center interacts with peroxide to form the Mn(III)Mn(III)-Tyr[•] active state.^{11,15} In addition, both dimanganese and diiron forms of the class Ib RNR of *E. coli* were crystallized.¹⁶ More generally, there is still an open question concerning which metal class Ib RNRs are active with in vivo.¹¹ It is also possible that both dimanganese and diiron forms are physiologically active. On the other hand, the class Ic R2 from *Chlamydia trachomatis (Ct)* was found to have a phenylalanine (Phe127) at the position of the tyrosine residue which bears the radical in class Ia and Ib RNRs.¹⁷ Further, the Bollinger/Krebs group discovered that the *Ct*-R2 contains a mixed-metal Fe-Mn center in its functional form rather than a diiron or dimanganese center, and it uses the Fe(III)Mn(IV) cofactor for radical initiation.¹⁸⁻²³

Currently, there are different proposals for which metal site possesses the Mn or Fe in *Ct*-R2.^{24,25} The metal site that is closer to Phe127 in *Ct*-R2 (or Tyr122 in class Ia *E. coli* R2, see Figure 1) is normally defined as site 1 (or site A),²⁶ while the other metal site is termed site 2 (or site B). Our DFT calculations on the *Ct*-R2 active site models with different oxidation states show better calculated Mössbauer properties compared with experiment when Mn is at site 2.²⁷ Moreover, our Poisson-Boltzmann self-consistent reaction field (PB-SCRF)²⁸⁻³² calculations on four mono-Mn(II) active-site structures indicate that the extended protein environment preferentially stabilizes the structures with Mn(II) binding at site 2.²⁷ Therefore we proposed that the metal site 2 in *Ct*-R2 has higher metal-binding affinity than site 1, and that Mn(II) should first occupy site 2 when two-fold excess Mn(II) is added to the apo-*Ct*-R2 prior to adding Fe(II) in preparing the Fe(II)Mn(II) *Ct*-R2 sample in experiments.^{27,33}

The problems involving the order and location of Mn(II) and Fe(II) binding in class Ic *Ct*-R2 and the Fe(II) binding in class Ia *E. coli* R2 are highly relevant since the ordering of metal binding is connected to the site-specificity of the intermediate states Fe(IV)Mn(IV) and Fe(III)Mn(IV) in *Ct*-R2 and Fe(III)Fe(IV) in *E. coli* R2 (intermediate state X). The basic reasoning is that after Fe(II)Mn(II) or Fe(II)Fe(II) binding to apo *Ct* or *E. coli* R2, the metal

binding sites become locked into place. The site-specificity of binding in the Fe(II)Mn(II) and Fe(II)Fe(II) states are then linked with the site-specificities in the Fe(IV)Mn(IV), Fe(III)Mn(IV), and Fe(III)Fe(IV) states observed after the subsequent reaction with O₂ and the 1e⁻ transfer into the active sites.

The differential Fe(II)-binding affinity of the sites of the diiron cluster in class Ia *E. coli* R2 and in mouse R2 has been reported. A single-Fe containing mouse R2 structure was crystallized at pH 4.7 (PDB code: 1XSM), and the Fe was found bound at site 2.³⁴ For the *E. coli* class Ia R2, Bollinger *et al.* had found that Fe(II) was bound in both mononuclear and dinuclear fashions when Fe(II) and apoR2 were complexed at low Fe(II)/R2 ratios (< 2).³⁵ In order to determine whether the two sites in *E. coli* R2 have different Fe(II)-binding affinities, this group subsequently performed Mössbauer spectroscopy and two-iron-isotope (⁵⁷Fe and ⁵⁶Fe) reaction experiments.²⁶ The well resolved and distinct Mössbauer isomer shift (δ) and quadrupole splitting (ΔE_Q) values for the two Fe sites of the diferric state *E. coli* R2 are the important indicators for distinguishing the site where the ⁵⁷Fe or ⁵⁶Fe binds in their experiments.²⁶

The ⁵⁷Fe Mössbauer spectra for the active diferric state of class Ia *E. coli* R2 were first reported 30 years ago with [δ (Fe1) = 0.45 mm s⁻¹, ΔE_Q (Fe1) = -2.45 mm s⁻¹], and [δ (Fe2) = 0.53 mm s⁻¹, ΔE_Q (Fe2) = -1.65 mm s⁻¹] at 4.2 K,³⁶ and were later reexamined by Que's group who reported essentially the same results of [δ (Fe1) = 0.46 mm s⁻¹, ΔE_Q (Fe1) = -2.44 mm s⁻¹, η (Fe1) = 0.2], and [δ (Fe2) = 0.55 mm s⁻¹, ΔE_Q (Fe2) = -1.62 mm s⁻¹, η (Fe2) = 0.6].³⁷ Bollinger *et al.* also obtained very similar Mössbauer data for the diferric *E. coli* R2 with [δ (Fe1) = 0.45 mm s⁻¹, ΔE_Q (Fe1) = 2.41 mm s⁻¹], and [δ (Fe2) = 0.55 mm s⁻¹, ΔE_Q (Fe2) = 1.62 mm s⁻¹] without indicating the ΔE_Q signs.^{26,38} Mössbauer, ¹H NMR, optical and resonance Raman experiments show that the major spectroscopic properties of the active state diferric R2 do not change when the tyrosyl radical is reduced,^{36,37,39} indicating similar structures of the active and inactive (met) diferric center of R2.

In the met Fe1(III)Fe2(III) crystal structure (Figure 1, PDB code: 1MXR, 1.42 Å resolution),⁷ there is one μ -oxo bridge (labeled as O1) between Fe1 and Fe2. Other first-shell ligands include two water molecules (O2 and O3), two histidine (His118 and His241), one aspartic acid (Asp84), and three glutamic acid (Glu115, Glu204, and Glu238) residue sidechains. The tyrosyl radical bearer Tyr122 is close to the Fe1 site and has an H-bonding interaction with O₈₂ of Asp84. The Fe2 site is six-coordinated with O1, O2, Glu115, Glu204, Glu238, and His241. By contrast, the site Fe1 is five-coordinated with O1, O3, Asp84, Glu115, and His118. O₈₁ of Asp84 H-bonds to the water ligand O2. However, in the crystal structure, O₈₁ of Asp84 is also fairly close to Fe1 (2.86 Å), which shows that the Asp84 sidechain has a partial bidentate binding conformation. Based on the met-diferric crystal structure of *E. coli* R2, Bollinger *et al.* proposed that the Fe(III) at site 1 is associated with the higher ΔE_Q and lower δ (as given above) by virtue of its more asymmetric coordination sphere resulting in part from the unique (nearly) bidentate Asp84 sidechain.^{26,42}

In their further two-iron-isotope reaction and Mössbauer spectroscopy experiments, if apoR2 was precomplexed with 0.5 equiv of ⁵⁷Fe(II) and then trapped by the addition of 3 equiv of ⁵⁶Fe(II) and excess O₂, the Mössbauer spectrum (sensitive only to ⁵⁷Fe) of the corresponding diferric state showed 4 to 5-fold greater intensity from site 2 (lower ΔE_Q and higher δ) than from site 1 (higher ΔE_Q and lower δ).²⁶ Conversely, if apoR2 was precomplexed with 2.3 equiv of ⁵⁶Fe(II) and then trapped by addition of 1.1 equiv of ⁵⁷Fe(II) and excess O₂, the diferric state Mössbauer spectrum then showed 3-fold greater intensity from site 1.²⁶ The simple interpretation of these results is that site 2 is preferentially occupied and has greater affinity for Fe(II) binding than site 1.²⁶ If this holds,

their subsequent two-iron-isotope experiments on the intermediate state X (Fe(III)Fe(IV)) showed that the Fe(III) is at site 1 and the Fe(IV) is at site 2, which was supported by our recent DFT calculations.⁴³

In the current paper, we perform DFT and electrostatics calculations to examine whether site 1 (Fe1) of the diferric cluster of *E. coli* R2 is the site that yields a higher ΔE_Q and lower δ , and whether this arises from the unique positioning of the Asp84 sidechain. Further, we examine whether the proposal that site 2 (Fe2) has a greater affinity for Fe(II) binding to the apoR2 is supported by performing calculations on a series mono-Fe(II) active site models of *E. coli* R2.

2. Mössbauer Property Calculations on the Active Site of the Diferric Cluster of Class Ia *E. coli* RNR-R2

2.1. History of Our Mössbauer Calculations on the Fe(III)Fe(III) Active Site Models of R2

DFT calculations and calibrations of the Mössbauer parameters have appeared in recent years.^{44–53} In our previous studies we have performed Mössbauer property calculations on the diferric active-site models of *E. coli* R2.^{44,45,54} In those studies, the initial geometries of our quantum cluster models were taken from a relatively low-resolution (2.2 Å) X-ray crystal structure of met-diferric class Ia *E. coli* R2 (PDB code: 1RIB),⁸ and no constraints were used for the first-shell ligand residue sidechains during the geometry optimizations in ADF^{55,56} (Amsterdam Density Functional Package).

In our initial work, we performed Mössbauer isomer shift parameters fitting for the DFT-PW91⁵⁷ potential.^{44,58} Then a gas-phase single-point calculation directly on the diferric X-ray structure (the quantum model contained only the first-shell ligands and Tyr122 sidechain) yielded very poor Mössbauer properties: [$\delta(\text{Fe1}) = 1.08 \text{ mm s}^{-1}$, $\Delta E_Q(\text{Fe1}) = 0.83 \text{ mm s}^{-1}$], and [$\delta(\text{Fe2}) = 1.05 \text{ mm s}^{-1}$, $\Delta E_Q(\text{Fe2}) = 1.10 \text{ mm s}^{-1}$].⁴⁴ The calculated isomer shifts were slightly improved after the cluster was geometry optimized in the gas-phase: [$\delta(\text{Fe1}) = 0.78 \text{ mm s}^{-1}$, $\Delta E_Q(\text{Fe1}) = -1.20 \text{ mm s}^{-1}$], and [$\delta(\text{Fe2}) = 0.86 \text{ mm s}^{-1}$, $\Delta E_Q(\text{Fe2}) = -1.50 \text{ mm s}^{-1}$].⁴⁴

Later we improved the fittings of the isomer shift parameters for PW91 potential by optimizing the geometries of the Fe-complexes in the training sets using the conductor-like screening solvation model (COSMO),^{59–62} and by separating the fittings for the $\text{Fe}^{2+,2.5+}$ and $\text{Fe}^{2.5+,3+,3.5+,4+}$ complexes.⁴⁵ The COSMO model is a dielectric solvent continuum model in which the solute molecule is embedded in a molecular-shaped cavity surrounded by a dielectric medium with a given dielectric constant. By using the newly fitted parameters, the Mössbauer calculation within COSMO yielded better results even in a single-point calculations at the X-ray crystal structure: [$\delta(\text{Fe1}) = 0.66 \text{ mm s}^{-1}$, $\Delta E_Q(\text{Fe1}) = 0.08 \text{ mm s}^{-1}$], and [$\delta(\text{Fe2}) = 0.75 \text{ mm s}^{-1}$, $\Delta E_Q(\text{Fe2}) = 0.88 \text{ mm s}^{-1}$].⁴⁵ Optimizing the diferric active site cluster in COSMO model yielded further improvements in the calculated Mössbauer properties: [$\delta(\text{Fe1}) = 0.53 \text{ mm s}^{-1}$, $\Delta E_Q(\text{Fe1}) = -1.70 \text{ mm s}^{-1}$], and [$\delta(\text{Fe2}) = 0.58 \text{ mm s}^{-1}$, $\Delta E_Q(\text{Fe2}) = -1.26 \text{ mm s}^{-1}$].⁴⁵ The negative signs of the quadrupole splittings and the relative values of both δ and ΔE_Q for both sites were correctly predicted. However, the calculated $\delta(\text{Fe1})$ was much larger, and the absolute values of both quadrupole splittings were still much smaller than those observed experimentally.

Later, we also performed similar isomer shift parameter fittings for the DFT-OPBE potential (a combination of Handy's optimized exchange (OPTX)⁶³ and PBE correlation (PBEc)^{64,65} functionals),⁴⁶ and obtained:

$$\delta = \alpha[\rho(0) - A] + C \quad (1)$$

where $A = 11877$ is a constant. α and C were fitted as $\alpha = -0.312$, and $C = 0.373 \text{ mm s}^{-1}$ for the $\text{Fe}^{2.5+,3+,3.5+,4+}$ complexes.⁴⁶ Note that we have been using our own program to calculate the electron density $\rho(0)$ at the iron nucleus based on the TAPE21 file created by an ADF calculation.⁴⁴ Recent versions of ADF (*e.g.* ADF2010) directly output electron densities that are calculated at points on the surface of a small sphere around the nucleus. Our published values for the parameters α and C cannot be combined with the ADF printed electron densities from the output file. Currently we are performing new fittings for α and C in order to use the ADF printed electron densities to calculate isomer shifts.

Very recently, in studying the intermediate and active states of the diiron center with a tryptophan or tyrosyl radical in class Ia *E. coli* R2, we also calculated the Mössbauer properties of the diferric active (with Tyr122*) and met (without Tyr122*) states using the OPBE potential.⁵⁴ The starting geometries of the model clusters were based on the met-diferric X-ray crystal structure 1RIB (2.2 Å resolution),⁸ and were optimized in COSMO. The size of those models was slightly larger than the model shown in Figure 1. Specifically, three additional outer-shell, H-bonding residue sidechains (Asp237, Trp48, and Gln43) were included.⁵⁴ Calculations of the met-diferric state in this model (named as $\text{M}(\text{O}_{\text{br}}, \text{H}_2\text{O}_{\text{t2}})$), which has the same first-shell structural features as Figure 1, yielded: $[\delta(\text{Fe1}) = 0.48 \text{ mm s}^{-1}, \Delta E_{\text{Q}}(\text{Fe1}) = -1.68 \text{ mm s}^{-1}]$, and $[\delta(\text{Fe2}) = 0.54 \text{ mm s}^{-1}, \Delta E_{\text{Q}}(\text{Fe2}) = -1.60 \text{ mm s}^{-1}]$.⁵⁴ For the corresponding active-state ($\text{Fe1(III)Fe2(III)-Tyr122}^*$) model ($\text{A}(\text{O}_{\text{br}}, \text{H}_2\text{O}_{\text{t2}})$), we obtained: $[\delta(\text{Fe1}) = 0.47 \text{ mm s}^{-1}, \Delta E_{\text{Q}}(\text{Fe1}) = -1.72 \text{ mm s}^{-1}]$, and $[\delta(\text{Fe2}) = 0.54 \text{ mm s}^{-1}, \Delta E_{\text{Q}}(\text{Fe2}) = -1.78 \text{ mm s}^{-1}]$.⁵⁴ These calculated isomer shifts now agree well with the experimental values (given in the Introduction section).^{26,36–38} However, the very large observed value of $\Delta E_{\text{Q}}(\text{Fe1})$ (2.41–2.45 mm s^{-1}) and the large difference ($\sim 0.8 \text{ mm s}^{-1}$) between $\Delta E_{\text{Q}}(\text{Fe1})$ and $\Delta E_{\text{Q}}(\text{Fe2})$ were not reproduced. Since the proposal of site 2 having greater Fe(II)-binding affinity than site 1 was based on the proposal that site 1 is associated with the observed higher ΔE_{Q} and lower δ in diferric state, we here revisit the Mössbauer calculations on the diferric active site of R2 to examine whether the observed data can be reproduced and whether it can be verified that site 1 is indeed associated with the higher ΔE_{Q} and lower δ .

2.2. The Current Quantum Cluster of the met-Fe(III)Fe(III) Active Site of Class Ia *E. coli* R2

The initial geometry of our current quantum cluster model is now taken from the met-diferric *E. coli* R2 X-ray crystal structure 1MXR,⁷ which has a higher resolution (1.42 Å) than the 1RIB (2.2 Å),⁸ structure used in our earlier work. The first-shell ligands and the nearby $\text{H}_2\text{O}(3027)$ and residue sidechains of Tyr122 and Trp111 have been shown in Figure 1. In addition to these, some potentially important outer shell H-bonding residue sidechains and water molecules are also included in the quantum model, including Asp237, Trp48, Gln43, $\text{H}_2\text{O}(3010)$, Arg236, and $\text{H}_2\text{O}(3235)$ (see Figure 2). The C_γ of Arg236, C_β of Gln43, and the C_α atoms of all other residues in the quantum cluster are replaced by a hydrogen atom (link-H) to fill the open valence of the terminal carbon atom.⁶⁶ Our previous OPBE geometry optimizations show that the distance between a link-H atom and the terminal carbon atom is normally 1.09–1.10 Å, we therefore set the C(link-H) distance at 1.095 Å along the original C-C direction in the X-ray crystal structure. Since the X-ray structure only shows the coordinates of heavy atoms, we have added the hydrogen atoms using Phenix1.6.⁶⁷ In the quantum cluster, the positions of the added hydrogen atoms are optimized before we optimize the heavy atom positions. During all Cartesian geometry optimizations in the current study, all link-H positions are fixed, in order to partly include the constraining effect of the protein backbone, and to be able to put the optimized active site structure back to the protein to perform the PB-SCRF calculations. The Cartesian

coordinates of the oxygen atoms in Wat3027 and Wat3235 are also fixed during geometry optimizations of the diferric cluster.

All density functional calculations are performed using OPBE functional with integration 4.0 in ADF2009.^{55,56} The two Fe(III) sites in R2 are high-spin antiferromagnetically (AF) coupled, which cannot be obtained directly from the normal DFT calculations. As in previous work,^{27,31,43–45,54} we represent the AF spin-coupled state in DFT by a “broken-symmetry” (BS) state,^{68–70} where a spin-unrestricted determinant is constructed in which one of the Fe sites has spin-up electrons as majority spin and the other site has majority spin-down electrons. Since our quantum cluster (Figure 2) has net charge 0 and its size is relatively large (comparing with models containing only first shell ligands),⁷¹ we use the dielectric constant $\epsilon = 4$ in the COSMO geometry optimizations in the current study. The triple- ζ polarization (TZP) Slater-type basis sets with frozen cores (C(1s), N(1s), O(1s) and Fe(1s,2s,2p)) are applied for geometry optimizations. However, all-electron TZP Slater-type basis sets are then used to obtain the electron densities ($\rho(0)$) and the electric field gradient (EFG) at the Fe nuclei at the optimized geometry in order to calculate the Mössbauer isomer shifts and quadrupole splittings.

Isomer shifts are computed according to Equation 1. For calculating the quadrupole splittings (ΔE_Q), the EFG tensors V are diagonalized and the eigenvalues are reordered so that $|V_{zz}| \geq |V_{xx}| \geq |V_{yy}|$. The asymmetry parameter η is defined as

$$\eta = (V_{xx} - V_{yy}) / V_{zz} \quad (2)$$

Then the ΔE_Q for ^{57}Fe of the nuclear excited state ($I = 3/2$) can be calculated as

$$\Delta E_Q = (1/2)eQV_{zz}(1 + \eta^2/3)^{1/2} \quad (3)$$

where e is the electrical charge of a positive electron, Q is the nuclear quadrupole moment of Fe. Note that we had used $eQ = 0.15$ electron-barn⁷² in our previous publications.^{44–46} For the current study, we apply a slightly different $eQ = 0.158$ electron-barn taken from the careful quantum chemical calculations (non-relativistic) by Sinnecker *et al.*⁷³

In order to examine if the extended protein field will alter the Mössbauer properties of the diferric active site calculated within the COSMO solvation model, we also put the optimized quantum cluster back to the X-ray crystal structure and performed the PB-SCRF calculations. Three dielectric regions are defined in the PB-SCRF calculations: the quantum cluster region ($\epsilon = 1$), the protein region ($\epsilon = 4$), and the solvent (water) region ($\epsilon = 80$). The PARSE⁷⁴ atomic radii and charges are assigned to atoms that are in the protein, but not in the quantum cluster, and which generate the protein field. During PB-SCRF, the active site model is computed by DFT (OPBE) calculation in the presence of the protein field and reaction field. The protein field acts as a fixed potential. The reaction field of the quantum-cluster charge distribution in the three-dielectric environment is evaluated from a finite-difference solution to the Poisson-Boltzmann (PB) equation, and self-consistency between the reaction field and the electronic structure of the quantum cluster is achieved by iteration.

The DFT/PB-SCRF procedure is described briefly as follows: **1)** A gas-phase DFT broken-symmetry single-point energy calculation (with all-electron TZP Slater-type basis sets) is performed at the COSMO-optimized geometry. **2)** The CHELPG algorithm⁷⁵ combined with singular value decomposition⁷⁶ is then used to fit the point charges of each atom (charges for the link-H atoms are set to zero) from the molecular electrostatic potentials (ESP) calculated by ADF. **3)** The interaction energy of the active site ($\epsilon = 1$) with the

protein ($\epsilon = 4$) and solvent ($\epsilon = 80$) environment is estimated by solving the Poisson-Boltzmann equation using the MEAD (Macroscopic Electrostatics with Atomic Detail) program developed by Bashford.^{77–80} **4**) The reaction field plus protein field potential obtained from step **3** is then added to the Hamiltonian of the DFT single-point energy calculation. The iteration of steps **1** – **4** continues until self-consistency between the reaction field potential and the electronic structure of solute is achieved. This DFT/PB-SCRF procedure has been recently implemented into a developmental version of ADF2009 following up on our work using earlier ADF program versions.^{28–32,81–83}

In COSMO, charge fit and MEAD calculations, the van der Waals radii for atoms Fe, C, O, N, and H were taken as 1.5, 1.7, 1.4, 1.55, and 1.2 Å, respectively.^{30,84}

2.3. The Current Calculated Geometric and Mössbauer Properties of the met-Fe1(III)Fe2(III) Active Site of Class Ia *E. coli* R2

The OPBE/COSMO (broken-symmetry) geometry optimization of the diferric cluster (Figure 2) was converged at cycle 71 (Cyc-71) when the maximum gradient was less than 0.003 Hartree/Å, and the maximum Cartesian displacement was less than 0.01 Å. The Fe...Fe and Fe-ligand distances of this optimized structure is given in Table 1, and its OPBE/COSMO and OPBE/PB-SCRF calculated Mössbauer properties are given in Table 2 in the columns under the label 'Cyc-71'. For comparison, the corresponding results of the original X-ray crystal structure, where only the hydrogen positions are optimized, are also given in Tables 1 and 2, in the columns under the label 'X-ray Opt-H only'. An overlap picture of the first-shell ligand positions in 'Cyc-71' (green) and the X-ray crystal structure (blue) is given in Figure 3.

After geometry optimization, the Fe1...Fe2 distance is shortened by 0.09 Å. The μ -oxo bridge O1 is closer to both Fe1 and Fe2 by more than 0.1 Å. The small changes on other Fe-ligand distances are between 0.0–0.1 Å. From Figure 3, some obvious ligand movement appears on the terminal water O3, Glu238, and Asp84. O3 and the Glu238 sidechain shift toward the His118 direction. The angle $\angle O3-Fe1-N(His118)$ is decreased by 15° from 103.8° in the X-ray structure to 89.3° in 'Cyc-71'. Meanwhile, the Asp84 sidechain moves toward the Fe2 direction, which results in an increase of the Fe1...O₈₁(Asp84) distance by 0.5 Å, and a decrease of the angles $\angle O_{82}(Asp84)-Fe1-Fe2$ and $\angle O_{81}(Asp84)-Fe1-Fe2$ by 20° and 6°, respectively. The partial bidentate binding conformation of the Asp84 sidechain is lost in 'Cyc-71'.

Since all link-H atom positions were fixed, no additional significant movement of the sidechains was observed during the Cartesian geometry optimization. However, the electronic energy of the active site cluster is lowered significantly by 52.8 kcal mol⁻¹ following the OPBE/COSMO geometry optimization. This energy difference is reduced slightly to 49.3 kcal mol⁻¹ when the clusters are inserted into the protein for the OPBE/PB-SCRF calculations.

As mentioned in Section 2.1, our previous calculated Mössbauer properties on the diferric active site of the X-ray crystal structure (1RIB, with resolution 2.2 Å)⁸ of *E. coli* R2 are much worse than the calculated results on the geometry optimized structures,^{44,45} compared with experiments.^{26,36–38} With the current higher resolution X-ray crystal structure (1MXR,⁷ resolution 1.42 Å), an OPBE/COSMO calculation directly on the crystal structure coordinates is found to yield much better Mössbauer properties (see Table 2): [$\delta(Fe1) = 0.53$ mm s⁻¹, $\Delta E_Q(Fe1) = -2.04$ mm s⁻¹], and [$\delta(Fe2) = 0.51$ mm s⁻¹, $\Delta E_Q(Fe2) = -1.28$ mm s⁻¹], than the previous calculations that were based on the lower resolution 1RIB crystal structure. In particular, the large predicted value of $\Delta E_Q(Fe1)$ (2.04 mm s⁻¹) is closer to the experimental value of 2.41–2.45 mm s⁻¹,^{26,36–38} which was never predicted to be larger

than 2.0 mm s^{-1} before.^{44,45,54} However, the predicted isomer shifts of the two iron sites using the crystal structure coordinates are very close to each other with $\delta(\text{Fe1}) > \delta(\text{Fe2})$, which is the opposite to what is found experimentally, where $\delta(\text{Fe1}) = 0.46$ or 0.45 mm s^{-1} is much smaller than $\delta(\text{Fe2}) = 0.55$ or 0.53 mm s^{-1} .^{26,36–38} The predicted value of $\Delta E_Q(\text{Fe2}) = 1.28 \text{ mm s}^{-1}$ is also much smaller than the observed value of 1.62 or 1.65 mm s^{-1} .^{26,36–38}

On the other hand, after OPBE/COSMO geometry optimization (Cyc-71, Table 2), the predicted results of $\delta(\text{Fe1}) = 0.45 \text{ mm s}^{-1}$ and $\delta(\text{Fe2}) = 0.53 \text{ mm s}^{-1}$ are in excellent agreement with the experimental data, and are better than our corresponding previously calculated results (see Section 2.1). The predicted value for $\Delta E_Q(\text{Fe2})$ (1.63 mm s^{-1}) also nearly reproduces the observed value. However, the only drawback is the predicted value of $\Delta E_Q(\text{Fe1}) = 1.84 \text{ mm s}^{-1}$, which is still much smaller than the observed value (2.41 – 2.45 mm s^{-1}) and is not as large as the predicted value (2.04 mm s^{-1}) when the crystal structure coordinates are used. To determine whether the extended protein environment would influence the calculated Mössbauer properties, we have inserted the active site clusters into the X-ray crystal structure and performed OPBE/PB-SCRF calculations. The Mössbauer properties obtained from these OPBE/PB-SCRF calculations are essentially the same as the OPBE/COSMO calculations for both the ‘Cyc-71’ and ‘X-ray-Opt-H-Only’ clusters (Table 2). Thus, the extended protein environment does not influence the Mössbauer properties of the diiron center. We further fixed all second- and outer-shell residue sidechains and water molecules at the ‘X-ray-Opt-H-Only’ geometry, and only optimized the first-shell ligand positions (still with link-H positions fixed) using OPBE/COSMO method, we obtained very similar calculated Mössbauer results (not shown here) as those given under ‘Cyc-71’ in Table 2. Therefore, the Mössbauer properties of the iron sites in the active site are dominated by the structures (positions) of the first-shell residues that bind directly with the Fe(III) sites.

As discussed above, the main structural difference around site Fe1 between the geometries of ‘X-ray-Opt-H-Only’ and ‘Cyc-71’ is the different positioning of the terminal water O3 and the Asp84 sidechain. Normally the water ligands are quite mobile since they are not directly constrained by the protein. Therefore, the calculated large $\Delta E_Q(\text{Fe1})$ value (2.04 mm s^{-1}) on the cluster ‘X-ray-Opt-H-Only’ probably results from the orientation of Asp84 sidechain as proposed by Bollinger *et al.*^{26,42}

To examine whether the high $\Delta E_Q(\text{Fe1})$ value can be reproduced in the initial geometry optimization steps where both the geometric and electronic structures of the cluster are relaxed quite a bit on the OPBE/COSMO potential energy surface, and still the Asp84 sidechain is positioned close to that in the ‘X-ray-Opt-H-Only’ conformation, we performed OPBE/COSMO and OPBE/PB-SCRF Mössbauer property calculations on the structures of the 17th and the 23rd cycle obtained from the geometry optimization trajectory. These two structures are named ‘Cyc-17’ and ‘Cyc-23’ in Tables 1 and 2. The geometries and energies fluctuate during the geometry optimization. ‘Cyc-17’ and ‘Cyc-23’ are two local minimums with small maximum gradient of $0.007 \text{ Hartree/\AA}$. Their Asp84 and O3 positions lie between the ‘X-ray-Opt-H-Only’ and ‘Cyc-71’ conformations, while the positions of other first-shell ligand sidechains are found to be close to those found in ‘Cyc-71’. The electronic energies of ‘Cyc-17’ and ‘Cyc-23’ are higher than that of ‘Cyc-71’ by 6.8 and $5.1 \text{ kcal mol}^{-1}$ (see Table 1) in OPBE/COSMO calculations, respectively, and these energy differences are decreased to only 4.1 and $3.1 \text{ kcal mol}^{-1}$, respectively, when the effects of the extended protein field are taken into account in OPBE/PB-SCRF calculations. Therefore, the electronic potential energy surface between the conformations of ‘Cyc-17’ and ‘Cyc-71’ is relatively flat.

After 16 steps of relaxation in the geometry optimization from the X-ray structure, the Mössbauer property calculations on 'Cyc-17' yields very promising results (Table 2). The isomer shifts are almost the same as those obtained for 'Cyc-71' and the results obtained from the experiment. The predicted value of $\Delta E_Q(\text{Fe2})$ (1.54 mm s^{-1}) for 'Cyc-17' is only by about 0.1 mm s^{-1} less than the corresponding value of 'Cyc-71' and the experiments. We note that the calculated value (magnitude) of $\Delta E_Q(\text{Fe1})$ (2.14 mm s^{-1}) for 'Cyc-17' is 0.3 mm s^{-1} larger than that obtained for 'Cyc-71' (1.84 mm s^{-1}) and is much closer to the large value observed experimentally (2.44 mm s^{-1}). Similar results are obtained for 'Cyc-23', where the predicted $\delta(\text{Fe2})$ and $\Delta E_Q(\text{Fe2})$ are a little closer to the corresponding values of 'Cyc-71' and the experiments, but the predicted value of $\Delta E_Q(\text{Fe1})$ drops to 2.04 mm s^{-1} . Again as noticed before, the calculated Mössbauer properties for 'Cyc17' and 'Cyc-23' obtained from OPBE/PB-SCRF calculations are essentially the same as those obtained from OPBE/COSMO calculations.

To determine if it is the relative positions of Fe1 and the Asp84 sidechain in 'Cyc-17' that results in the large $\Delta E_Q(\text{Fe1})$ value, we further optimized the 'Cyc-17' geometry, still with all link-H positions fixed, and with the Cartesian coordinates of Fe1 and $\text{O}_{\delta 1}$, $\text{O}_{\delta 2}$, C_{β} and C_{γ} of the sidechain of Asp84 fixed at the positions in 'Cyc-17'. The newly optimized structure is named 'Fix-Asp84' in both Tables 1 and 2. We find that the large predicted $\Delta E_Q(\text{Fe1})$ value for 'Cyc-17' (2.14 mm s^{-1}) is reproduced with the 'Fix-Asp84' structure (2.13 mm s^{-1}) (Table 2). In addition, the predicted values for $\delta(\text{Fe1})$, $\delta(\text{Fe2})$, and $\Delta E_Q(\text{Fe2})$ are nearly the same as those for the 'Cyc-71' structure. We note that the 'Fix-Asp84' structure is only $2.0 \text{ kcal mol}^{-1}$ higher in energy than 'Cyc-71' (Table 1).

Further, we notice that one of the reasons that the Asp84 sidechain moves toward the Fe2 direction (and the Tyr122 sidechain moves away from Fe1) during the original geometry optimization (toward 'Cyc71') is that, in the crystal structure, the Tyr122 sidechain is very close to the Asp84 sidechain. One of the H_{β} atoms of the Asp84 sidechain is only $1.66 \text{ \AA}$ away from one of the hydrogen atoms in the ring of Tyr122 sidechain. Since our geometry optimizations were performed in Cartesian coordinates with some atom positions fixed, the rotation of the Tyr122 sidechain could not be well optimized. Consequently, we manually rotated the ring of the Tyr122 sidechain in 'Cyc-17' clockwise along the C_{β} - C_{γ} bond (as viewed in Figure 2 or in Figure 1) by 20° so that $\text{O}_{\delta 2}$ of the Asp84 sidechain lies essentially along the same plane with the Tyr122 ring, and the $\text{H}_{\beta}(\text{Asp84}) \cdots \text{H}(\text{Tyr122})$ distance mentioned above is increased to $2.03 \text{ \AA}$. We then performed geometry optimization on this new structure with all link-H positions fixed, and with the Cartesian coordinates of Fe1 and $\text{O}_{\delta 1}$, $\text{O}_{\delta 2}$, C_{β} and C_{γ} of the sidechain of Asp84 fixed at the positions in 'Cyc-17'. We call this optimized structure 'Rotate-Tyr122-Fix-Asp84' in Tables 1 and 2. Again, we produce (Table 2) the large calculated $\Delta E_Q(\text{Fe1})$ value (2.18 mm s^{-1}) on 'Rotate-Tyr122-Fix-Asp84', and the other three predicted values of $\delta(\text{Fe1})$, $\delta(\text{Fe2})$, and $\Delta E_Q(\text{Fe2})$ are all in excellent agreement with the experiment (Table 2). Now the electronic energy of 'Rotate-Tyr122-Fix-Asp84' is almost the same as that of 'Cyc-71', with only $0.8 \text{ kcal mol}^{-1}$ difference in the OPBE/PB-SCRF calculations (Table 1).

In summary, our calculations show that the site Fe1(III) in the diferric class Ia *E. coli* RNR-R2 active site is associated with the higher observed quadrupole splitting value and lower isomer shift value. Therefore, the site with higher Fe(II) binding affinity observed in the two-iron- isotope reaction experiments is site 2.²⁶ The relative positioning between Fe1 and the Asp84 sidechain does influence the Mössbauer quadrupole splitting feature of Fe1. The observed large quadrupole splitting value on Fe1 mainly results from the partial bidentate binding of Asp84, which agrees with the proposal by Bollinger *et al.*^{26,42} Next, with calculations on mono-Fe(II) active site clusters, we will examine the differential affinity of these sites for Fe(II) binding.

3. Apo and Diferrous State of the Active Site of Class Ia *E. coli* RNR-R2

The apo (Fe-free) form of *E. coli* R2 was crystallized 18 years ago, and a summary of the X-ray structure was published.⁸⁶ It was reported that the carboxylate groups and the two histidine sidechains in the Fe-free active site pocket are still very close to each other, and these metal-binding residue sidechains are found in very similar positions in both apo and metal-bound forms.^{86,87} It was therefore proposed that the loss of the four positive charge equivalents of the diferrous site are compensated in the apo protein by the protonation of the two histidine (His118 and His241) and two carboxylate sidechains.^{86,87} The very recent ultraviolet resonance Raman studies also provide evidence that the histidine sidechains in the active site of apo-R2 are protonated.⁸⁸ It is therefore likely that when Fe(II) binds to one of the iron sites, the histidine and one of the three carboxylate sidechains in the other open site remain protonated. Since the apo *E. coli* R2 crystal structure coordinates have not been made publicly available via the Protein Data Bank, we will begin our calculations in the current paper using the diferrous crystal structure to construct the mono-Fe(II) active site models.

The diiron center of the diferrous *E. coli* R2 crystal structure (PDB code: 1XIK, 1.7 Å resolution) is shown in Figure 4.⁶ Compared with the diferric crystal structure (Figure 1), no water molecule is found in the diferrous center (and in the apo Fe-free site),⁸⁶ the two histidine and the Glu115 sidechains are in very similar positions, the carboxylate group of the Glu238 sidechain now binds with both Fe1 and Fe2, the Glu204 sidechain is in partial bidentate binding position to Fe2, and the Asp84 sidechain shifts or rotates but still binds to Fe1 in a partial bidentate mode.

The nearby outer shell H-bonding residue positions (Figure 5) in the diferrous crystal structure are very similar to those in the diferric cluster (Figure 2).

4. Mono-Fe1(II) and Mono-Fe2(II) Quantum Cluster Models and Calculations

The initial geometries of our mono-Fe1(II) and mono-Fe2(II) models were taken from the diferrous X-ray crystal structure 1XIK.⁶ To treat both sites equally, each model contains the same residue sidechains as shown in Figure 5. When constructing the mono-Fe1(II) models, the ion Fe2(II) was deleted from site 2, the His241 sidechain was protonated, and another proton was added to either the Glu204, Glu115, or Glu238 sidechain. Similarly, in constructing the mono-Fe2(II) models, Fe1(II) was deleted from site 1, one proton was added to His118, and another proton was added to either the Asp84, Glu115, or Glu238 sidechain. The geometries were then optimized using OPBE/COSMO with $\epsilon = 4.0$, and with all link-H atom positions fixed. The optimized structures were also inserted to the crystal structure for OPBE/PB-SCRF calculations. The TZP Slater-type basis sets with frozen cores (C(1s), N(1s), O(1s) and Fe(1s,2s,2p)) were applied for these calculations. As explained below, this resulted in four optimized mono-Fe1(II) structures (S1–S4), which are shown in Figure 6, and the three optimized mono-Fe2(II) structures (S5–S7), which are shown in Figure 7. Only the first-shell residues (plus Tyr122 and Trp111) are shown in these figures, but the outer-shell residue sidechains as shown in Figure 5, including Gln43, Trp48, Asp237, and Arg236, and the two water molecules Wat2 and Wat147, were also included in the calculations.

The His241 sidechain was protonated in all mono-Fe1(II) model clusters. In mono-Fe1(II)-S1, the second proton was added to the Glu204 sidechain, which initially formed an H-bond with the carboxylate group of Glu238. However, during geometry optimization, the protonated Glu204 sidechain shifted to form an H-bonding interaction with the Glu115 sidechain. In mono-Fe1(II)-S2, the second proton was added to the carboxylate group of Glu115, which also H-bonded to the Glu238 sidechain in the starting point and which also

shifted to H-bond with the Glu204 sidechain after geometry optimization. The third cluster, mono-Fe1(II)-S3, possesses a protonated Glu238 sidechain that initially forms an H-bond with the carboxylate group of Glu204. However, during geometry optimization, this proton transferred to Glu204, which then forms an H-bond with the Glu238 sidechain. In mono-Fe1(II)-S4, the second proton initially was also added to the Glu238 sidechain but with a direction toward Glu115. This protonated Glu238 kept the H-bonding interaction with Glu115 during the geometry optimization.

The His118 sidechain was protonated in the mono-Fe2(II) clusters and initially formed H-bonding interactions with all the Asp84, Glu115, and Glu238 sidechains. In mono-Fe2(II)-S5, mono-Fe2(II)-S6, and mono-Fe2(II)-S7, the second proton was added to the Asp84, Glu115, and Glu238 sidechains, respectively. For mono-Fe2(II)-S7, the starting position of the protonated Glu238 sidechain H-bonded with both of the Asp84 and Glu115 sidechains. However, during geometry optimization, the carboxylate groups of Glu238 and Glu115 turned away from each other, and the protonated Glu238 only formed an H-bonding interaction with Asp84 in the optimized structure.

The energies for each cluster obtained from the OPBE/COSMO geometry optimization and from OPBE/PB-SCRF calculations are given in Table 3. Among the four mono-Fe1(II) clusters, mono-Fe1(II)-S1 has the lowest OPBE/COSMO and OPBE/PB-SCRF energies. The energies of mono-Fe1(II)-S4 and mono-Fe1(II)-S2 are by 25 and 17 kcal mol⁻¹ higher than that of mono-Fe1(II)-S1 after geometry optimization. Therefore it is unlikely that these structures represent the mono-Fe1(II) active site. Calculations in single-dielectric medium (COSMO) show that the electronic energies of mono-Fe1(II)-S1 and mono-Fe1(II)-S3 differ by only 4 kcal mol⁻¹. In the OPBE/PB-SCRF calculations, their polarized gas-phase electronic energies (E_{el}^{f}) and the protein field energies ($E_{\text{protein}}^{\text{f}}$) are very similar to each other. However, their reaction field energies ($E_{\text{reaction}}^{\text{f}}$) are 15 kcal mol⁻¹ apart, resulting in a final 14 kcal mol⁻¹ $E_{\text{OPBE/PB-SCRF}}$ energy difference, with S1 still being the lowest in energy. Therefore, the different charge distributions in mono-Fe1(II)-S1 and mono-Fe1(II)-S3, which result in minor energy difference in the single-dielectric medium solvation calculations, can produce a large reaction field energy difference in the two-dielectric media calculations.

For the three mono-Fe2(II) clusters, after OPBE/COSMO geometry optimizations, the energy of mono-Fe2(II)-S5 is about 4 kcal mol⁻¹ lower than that of mono-Fe2(II)-S7 (see ΔE in Table 3), and this difference is approximately the same in the OPBE/PB-SCRF calculations. The energy of mono-Fe2(II)-S6 is much higher (at 12–17 kcal mol⁻¹). So both OPBE/COSMO and OPBE/PB-SCRF calculations yield the same energy order of $E(\text{mono-Fe2(II)-S5}) < E(\text{mono-Fe2(II)-S7}) < E(\text{mono-Fe2(II)-S6})$ (Table 3). Unlike the relative total energies of these three structures, the calculated E_{strain} , $E_{\text{protein}}^{\text{f}}$, and $E_{\text{reaction}}^{\text{f}}$ energies of these three structures are very similar with each other.

As for the preference of Fe(II) for site 1 or site 2 in the monoferrous state, the OPBE/COSMO energies do not provide a distinction: the electronic energies of mono-Fe1(II)-S1 and mono-Fe2(II)-S5 are almost the same (−21601 kcal mol⁻¹). However, in the OPBE/PB-SCRF calculations, the final reaction field energies ($E_{\text{reaction}}^{\text{f}}$) of mono-Fe2(II)-S5 and mono-Fe2(II)-S7 are much more negative (stabilizing) than that of mono-Fe1(II)-S1 by ~12 kcal mol⁻¹. As a result, the final OPBE/PB-SCRF energies ($E_{\text{OPBE/PB-SCRF}}$) of mono-Fe2(II)-S5 and mono-Fe2(II)-S7 are lower than the corresponding energy of mono-Fe1(II)-S1 by about 9 and 5 kcal mol⁻¹, respectively. The dipole moments obtained from OPBE/PB-SCRF calculations of the mono-Fe1(II)-S1 (56.4 D) and mono-Fe2(II)-S5 (55.4 D) clusters are almost identical in magnitude. However, as shown in Figures 8 and 9, their directions are different. Once again, we see that the different charge distributions, which may not yield

different reaction field solvation energies within a single dielectric medium, may receive different dielectric response in the two-dielectric media environment in the PB-SCRF calculations. Our calculations thus show that the protein plus solvent environment around the *E. coli* R2 active site favors the Fe(II) binding to site 2, which agrees the proposal of the two-iron-isotope reaction experiments.²⁶

5. Conclusions

Our current DFT calculations have produced the distinct Mössbauer isomer shifts and quadrupole splittings of the two Fe(III) sites in the diferric state of class Ia *E. coli* RNR-R2, as observed in experiments.^{26,36–38} The iron site with the higher quadrupole splitting and lower isomer shift has been demonstrated to be site 1, which binds with Asp84 and is closer to Tyr122. The first-shell ligand binding positions are the main factors that determine the ⁵⁷Fe Mössbauer properties. The rotation or oscillation of the Tyr122 sidechain influences the position of the Asp84 sidechain. The subtle movement of the Asp84 sidechain, which is on a flat potential energy surface, does influence the electric field gradient surrounding the Fe1(III) nucleus, and the partial bidentate binding conformation of the Asp84 sidechain is the main factor for the large observed quadrupole splitting value of Fe1(III) in *E. coli* R2.

When a single dielectric continuum (COSMO) solvation model is used in calculations, the active site model cluster of *E. coli* R2 does not have an obvious energetic preference for Fe(II) binding to site 1 or site 2. However, when the extended protein (with explicit atomic charges and van der Waals radii) and solvent environment is represented using two dielectric media, the reaction field energies in the OPBE/PB-SCRF calculations reveal that Fe(II) binding at site 2 is energetically preferred. This supports the previous Mössbauer spectroscopy and two-iron- isotope reaction experiments²⁶ that suggest site 2 should have greater affinity for Fe(II) binding to apoR2 than site 1 and the Fe(IV) in the Fe(III)Fe(IV) intermediate state X is at site 2, agreeing with our previous DFT calculations.⁴³

Supplementary Material

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Figure 1.

The diferric center of the X-ray crystal structure of class Ia *E. coli* RNR-R2. Its PDB code is 1MXR with 1.42 Å resolution.⁷ The C_α atoms of the residues in this figure are replaced with hydrogens. Figure was prepared with Molden4.7 and Xfig3.2.4.^{40,41}

Figure 2. The quantum cluster of our current met-diferric *E. coli* RNR-R2 active site model. The initial geometry is taken from the met-diferric *E. coli* R2 X-ray crystal structure 1MXR.⁷ A closer look at the upper diiron center is given in Figure 1.

Figure 3. Overlap of the first-shell ligands of the optimized (Cyc-71, in green) and the X-ray crystal structure (in blue)⁷ of the met-diferric *E. coli* RNR-R2 active site. Figure was prepared with VMD1.8.6 and Xfig3.2.4.^{41,85}

Figure 4. The diferrous center of the X-ray crystal structure of class Ia *E. coli* RNR-R2. Its PDB code is 1XIK with 1.7 Å resolution.⁶ The C_α atoms of the residues in this figure are replaced with hydrogens.

Figure 5.

The active site of the diferrous center of the X-ray crystal structure 1XIK⁶ of class Ia *E. coli* RNR-R2 which shows more outer-shell H-bonding residue sidechains than Figure 4, and has the same size as the corresponding diferric cluster shown in Figure 2.

Figure 6.

The four OPBE/COSMO optimized mono-Fe1(II) model structures (S1–S4). Other outer-shell residue sidechains shown in Figure 5, including Gln43, Trp48, Asp237, and Arg236, and the two water molecules Wat2 and Wat147, were included in the calculations but are not shown here.

Figure 7.

The three OPBE/COSMO optimized mono-Fe2(II) model structures (S5–S7). Other outer-shell residue sidechains shown in Figure 5, including Gln43, Trp48, Asp237, and Arg236, and the two water molecules Wat2 and Wat147, were included in the calculations but are not shown here.

Figure 8. The direction of the dipole moment (56.4 D) of the mono-Fe1(II)-S1 cluster. Figure was prepared with Molekel4.3 and Xfig3.2.4.^{41,89}

Figure 9.
The direction of the dipole moment (55.4 D) of the mono-Fe2(II)-S5 cluster.

Table 1

Comparing the Central Geometric Parameters ($\text{\AA}$ or $^\circ$) and Relative Energies (ΔE , kcal mol $^{-1}$) Between the X-ray Crystal Structure and the DFT/COSMO Optimized Structures of the Met-Diferric Active Site (Figure 2) of Class Ia *E. coli* R2.

	X-ray ^d	OPBE/COSMO Optimized Structures					
	Opt-H Only	Cyc-17 ^b	Cyc-23 ^c	Cyc-71 ^d	Fix-Asp84 ^e	Rotate-Tyr122- Fix-Asp84 ^f	
Fe1...Fe2 ^g	3.39	3.25	3.28	3.30	3.29	3.29	
Fe1...O ₆₁ (Asp84)	2.86	3.22	3.30	3.37	3.22	3.22	
Fe1-O ₆₂ (Asp84)	1.98	1.99	1.97	1.96	1.99	1.99	
∠O ₆₂ (Asp84)-Fe1-Fe2	139.5	131.5	128.2	119.9	130.8	130.4	
∠O ₆₁ (Asp84)-Fe1-Fe2	89.6	88.6	87.6	83.2	88.0	87.6	
Fe1-O1	1.93	1.77	1.77	1.76	1.77	1.76	
Fe1-N(His118)	2.12	2.06	2.05	2.05	2.07	2.08	
Fe1-O(Glu115)	2.02	2.02	2.03	2.01	2.02	2.02	
Fe1-O3	2.30	2.29	2.31	2.39	2.30	2.31	
∠O3-Fe1-N(His118)	103.8	98.9	97.7	89.3	95.2	93.4	
Fe2-O1	1.94	1.82	1.83	1.83	1.83	1.83	
Fe2-O2	2.30	2.35	2.36	2.30	2.36	2.35	
Fe2-N(His241)	2.22	2.19	2.18	2.18	2.18	2.18	
Fe2-O(Glu115)	2.04	2.09	2.10	2.14	2.13	2.13	
Fe2-O(Glu204)	1.95	2.05	2.03	2.02	2.02	2.01	
Fe2-O(Glu238)	2.06	2.05	2.05	2.07	2.05	2.06	
ΔE(COSMO)	52.8	6.8	5.1	0.0	2.9	1.8	
ΔE(PB-SCRF)	49.3	4.1	3.1	0.0	2.0	0.8	

^aThe cluster (Figure 2) taken directly from the X-ray crystal structure IMXR (1.42 Å resolution). ⁷ Only the hydrogen positions were optimized with OPBE/COSMO ($\epsilon = 4.0$) method.

^bGeometry optimization started from the X-ray crystal structure with only link-H positions fixed. 'Cyc-17' is the geometry of the cluster at the 17th cycle during the optimization.

^cThe geometry of the cluster at the 23rd cycle of the geometry optimization.

^dThe final optimized (at the 71st cycle) geometry of the active site cluster.

^eStructure obtained from the optimization starting from the geometry of 'Cyc-17' with all link-H atoms fixed, and also with the Cartesian coordinates of Fe1 and O₆₁, O₆₂, C₆₁ and C₆₂ of the sidechain of Asp84 fixed at the positions in 'Cyc-17'.

^fStarting from the geometry of 'Cyc-17', the ring of Tyr122 sidechain was rotated 20° clockwise (facing Figure 2) along the C β -C γ bond. Then the structure was optimized with link-H atoms fixed, and also with the Cartesian coordinates of Fe1 and O δ 1, O δ 2, C β and C γ of the sidechain of Asp84 fixed at the positions in 'Cyc-17'.

^gResidues and atoms are labeled in Figure 1.

Table 2

Mössbauer Isomer Shifts δ (mm s^{-1}), Quadrupole Splittings (ΔE_Q) (mm s^{-1}), and η for the X-ray Crystal Structure and the OPBE/COSMO Optimized Structures of the Met-Diferric Active Site (Figure 2) of Class Ia *E. coli* R2, Obtained from OPBE/COSMO and OPBE/PB-SCRF Calculations, and Compared with Experiment (Exp).

Structures ^d							Exp ^b
X-ray	OPBE/COSMO Optimized						
Opt-H Only	Cyc-17	Cyc-23	Cyc-71	Fix-Asp84	Rotate-Tyr122-Fix-Asp84		
OPBE/COSMO							
$\delta(\text{Fe1})$	0.53	0.45	0.44	0.45	0.45	0.45	0.46
$\delta(\text{Fe2})$	0.51	0.52	0.53	0.54	0.53	0.53	0.55
$\Delta E_Q(\text{Fe1})$	-2.04	-2.14	-2.04	-1.84	-2.13	-2.18	-2.44
$\Delta E_Q(\text{Fe2})$	-1.28	-1.54	-1.57	-1.63	-1.66	-1.67	-1.62
$\eta(\text{Fe1})$	0.21	0.15	0.18	0.29	0.16	0.17	0.2
$\eta(\text{Fe2})$	0.67	0.45	0.39	0.21	0.32	0.31	0.6
OPBE/PB-SCRF							
$\delta(\text{Fe1})$	0.54	0.45	0.44	0.45	0.45	0.45	0.46
$\delta(\text{Fe2})$	0.51	0.52	0.53	0.54	0.53	0.53	0.55
$\Delta E_Q(\text{Fe1})$	-2.05	-2.14	-2.03	-1.84	-2.13	-2.17	-2.44
$\Delta E_Q(\text{Fe2})$	-1.25	-1.52	-1.55	-1.60	-1.63	-1.64	-1.62
$\eta(\text{Fe1})$	0.23	0.16	0.18	0.28	0.17	0.19	0.2
$\eta(\text{Fe2})$	0.69	0.46	0.40	0.22	0.33	0.32	0.6

^a See notes a-f under Table 1.

^b Taken from Ref. ³⁷, at 4.2K. Other Mössbauer experiments reported very similar results. In Ref. ³⁶: [$\delta(\text{Fe1}) = 0.45 \text{ mm s}^{-1}$, $\Delta E_Q(\text{Fe1}) = -2.45 \text{ mm s}^{-1}$], and [$\delta(\text{Fe2}) = 0.53 \text{ mm s}^{-1}$, $\Delta E_Q(\text{Fe2}) = -1.65 \text{ mm s}^{-1}$]. In Refs. ^{26,38} (without indicating the ΔE_Q signs): [$\delta(\text{Fe1}) = 0.45 \text{ mm s}^{-1}$, $\Delta E_Q(\text{Fe1}) = 2.41 \text{ mm s}^{-1}$], and [$\delta(\text{Fe2}) = 0.55 \text{ mm s}^{-1}$, $\Delta E_Q(\text{Fe2}) = 1.62 \text{ mm s}^{-1}$].

Table 3

Energy (E ; kcal mol⁻¹) and Relative Energy (ΔE ; kcal mol⁻¹) Comparisons between mono-Fe1(II) and mono-Fe2(II) Model Clusters Obtained from OPBE/COSMO and OPBE/PB-SCRF Calculations.

	Mono-Fe1(II)				Mono-Fe2(II)		
	S1	S2	S3	S4	S5	S6	S7
OPBE/COSMO							
$E_{\text{OPBE/COSMO}}^a$	-21601.2	-21584.0	-21597.2	-21576.3	-21601.4	-21589.2	-21596.9
$\Delta E_{\text{OPBE/COSMO}}$	0.2	17.4	4.2	25.1	0.0	12.2	4.5
OPBE/PB-SCRF							
E_0^b	-21523.1	-21499.3	-21521.4	-21486.5	-21521.7	-21504.5	-21515.9
E_{strain}^c	20.3	21.1	18.3	20.7	22.8	24.6	23.6
E_0^d	-21502.8	-21478.2	-21503.1	-21465.8	-21498.9	-21479.9	-21492.3
E_{protein}^e	-9.7	-10.9	-10.3	-10.2	-9.8	-11.7	-12.1
E_{reaction}^f	-118.5	-120.9	-103.5	-120.0	-130.9	-131.4	-131.3
$E_{\text{OPBE/PB-SCRF}}^g$	-21631.0	-21610.0	-21616.9	-21596.0	-21639.6	-21623.0	-21635.7
$\Delta E_{\text{OPBE/PB-SCRF}}$	8.6	29.6	22.7	43.6	0.0	16.6	3.9

^aElectronic energies after OPBE/COSMO geometry optimizations.

^bInitial (i) gas-phase electronic energy of the quantum cluster from DFT at the COSMO optimized geometry.

^cThe electronic energy change during the whole SCRF cycle, $E_{\text{strain}} = E_0^f - E_0^i$, which is the energy cost of cluster polarization.

^dThe quantum cluster electronic energy from DFT at the end of the OPBE/PB-SCRF cycle, where the electron orbitals and density are polarized by the protein and solvent (f denotes final orbitals).

^eThe final protein field energy, obtained from the charge-charge interactions between the active site and the protein charges, screened by the different dielectric media ($\epsilon = 1.0$ in quantum cluster, $\epsilon = 4.0$ in protein, and $\epsilon = 80$ in solvent).

^fThe final reaction field energy, obtained from the interaction between the active site charges (where $\epsilon = 1.0$) and the dielectric solvent ($\epsilon = 80.0$) and protein ($\epsilon = 4.0$) environment.

^gThe total energy after OPBE/PB-SCRF calculations: $E_{\text{OPBE/PB-SCRF}} = E_0^f + E_{\text{protein}}^f + E_{\text{reaction}}^f$.