



Computing Redox Potentials of Type-1 Copper Sites Using Combined Quantum Mechanical/Molecular Mechanical Method



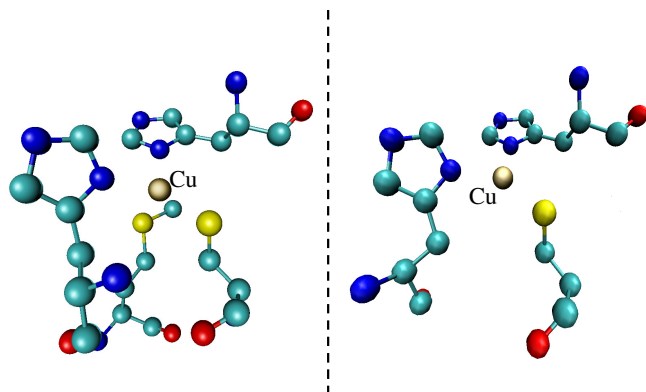
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Abstract

Type-1 copper centers belong to the family of metal-containing oxidoreductases, where the redox active moiety is a copper ion bound in a nitrogen-sulfur donor environment. These metalloproteins, commonly known as blue-copper proteins, act as electron mediators within electron transport chains as the copper shuttles between Cu(I) and Cu(II) states. One critical element of the redox chemistry of type-1 copper sites is the display of a large variation (between 200 -1000 mV) in the Cu(I/II) redox potential, which translates into a Gibbs free energy difference of 18 kcal/mol. The cause of this huge fluctuation is unclear and only speculated to be due to active site hydrophobicity, axial ligation, and outer sphere coordination. In order to gain an insight into the role of protein matrix on the redox potentials of the copper center, we are using combined quantum mechanical/molecular mechanical simulation. The copper center and the atoms within its primary coordination sphere are treated with density functional theory, while embedded in a molecular mechanically treated region. Results of these studies on model systems as well as type-I proteins will be presented.

Background

Type I sites are a broad class of proteins characterized by a Cu-N₂S copper containing active site, and are important in biological oxidation/reduction reactions and electron transport.



Plastocyanin
Redox Potential= 363 mV

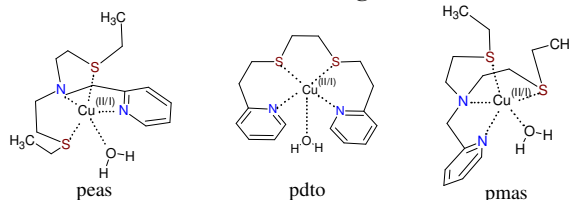
Rusticyanin
Redox Potential= 798 mV

Hypothesis

The protein matrix plays an important role in tuning of the redox potential. With combined quantum mechanical/molecular mechanical simulations, it is possible to quantify the contributions of individual protein functionalities on redox energetics.

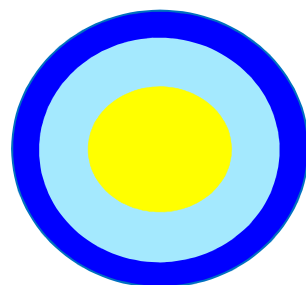
Methods

DFT studies with Analog Molecules



Three model systems with known structure and redox potentials^[2] were used for building and standardizing molecular models

QM/MM Scheme



Water layer — MM Layer
Protein Layer — MM Layer
Copper center: QM Layer

Water Layer- A 17Å water layer around the protein
Protein Layer- A 12 Å truncation of the protein
Copper Center- The site of highest electron movement and direct copper coordination

Calculations were performed using 2 layers of theory, DFT was used in modeling copper and the coordinating functional groups. Amber was used in modeling the surrounding protein.

Kohn-Sham DFT Scheme

The partition of the electronic energy according to Kohn-Sham equation: $E = E^T + E^V + E^I + E^{XC}$, where E^T = kinetic energy of electrons, E^V = nuclear-electron and nuclear-nuclear interactions; E^I = electron-electron repulsion; E^{XC} = electron correlation represented by the *quantum mechanical exchange energy*, which accounts for electron spin and the dynamic correlation energy due to concerted motion of electrons.

M06L and M06-2X, employs a new exchange-correlation functional with improved medium-range (2-6 Å) correlation energy that can appreciate the electron correlation in a significantly better way.

Free Energy Calculations

- Electronic structure calculations were performed using density functional theory
- Aqueous-state free energies were computed following standard procedures^[3]
- ONIOM calculations were performed using a 6-31+G(d,p) basis set and M06L functional in the quantum mechanical region and Amber molecular mechanics in the molecular mechanical region
- Effect of solvent beyond 17 Å was taken into account with Born's solvation correction
- Gas-phase vibrational-rotational free energies at 298 K, were calculated with the harmonic oscillator-rigid rotor approximation
- The electronic free energy was approximated as $-RT \ln d$, where R is the gas constant, T is the temperature, and d is the electronic degeneracy of the ground state. Its contribution is < 0.5 kcal/mol and was neglected

Results

Results of Analog Calculations

Functional	$\Delta G^\circ(aq)$ (kcal/mol)	Standard reduction potential (mV)	* Δ from experimental (mV)
peas (pmas) [pdto]			
B3LYP	-120.5 (-116.1) [-123.7]	943 (754) [1084]	344 (360) [492]
M06-2X	-114.5 (-112.1) [-117.5]	687 (579) [815]	88 (185) [223]
M06L	-114.7 (-110.8) [-112.5]	694 (526) [600]	95 (132) [9]
M06	-125.5 (-123.5) [-125.2]	1162 (1077) [1151]	563 (683) [559]
LC-WPBE	-127.8 (-125.0) [-128.9]	1261 (1142) [1309]	662 (748) [717]
WB97X-D	-122.5 (-118.0) [-121.1]	1033 (836) [971]	434(442)[379]

— * | Experimental Redox — Calculated Redox |

— Results are calculated with the analogs and a water molecule coordinated

Results of ONIOM Calculations

Energies	Plastocyanin	Rusticyanin
Δ Energies QM region (kcal/mol)	-114.5	-118.6
Δ Thermal Correction (kcal/mol)	-2.1	-2.7
Δ Conversion to Gibbs free energy (kcal/mol)	-1.7	-7.2
Δ Born Correction for a 17Å simulated spherical system (kcal/mol)	9.7	9.7
Total Gibbs free energy Difference (kcal/mol)	-108.6	-118.8
Redox Potential (mV)	428 (experimental: 363) ^[4]	873 (experimental: 798) ^[4]

Conclusion/Future

- M06L is the most appropriate functional for modeling Cu-N₂S₂ systems
- The average deviation from experimental values for the copper analogs for the three functionals were 78.4 mV for M06L, 166 mV for M06-2X, and a high 399 mV for B3LYP
- ONIOM type QM/MM calculations have been used to calculate the redox potentials of copper proteins

Continuing Studies

- Developing a new method of calculation using DFT and CHARMM molecular dynamics
- The role of protein matrix in charge-separation upon reduction
- Perturbing the active site to observe changes in the redox energetics

We would like to thank:

- The American Chemical Society
- XSEDE & SDSC for computational resources
- University of Wisconsin- Eau Claire and it's staff and organizations for supporting our work

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