Theoretical Determination of the Reduction Potentials of NQO2 Using Molecular Dynamics Simulations

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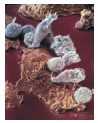
Abstract

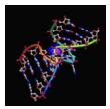
Dihydronicotinamide riboside (NRH): quinone oxidoreductase (NQO2) is a cytosolic quinone reductase (QR). It catalyzes the metabolic reduction of quinones utilizing its cofactor flavin adenosine dinucleotide (FAD) as electron mediator. The redox chemistry of this enzyme is relevant to a number of physiological processes. It promotes natural defense against oxidative and chemical stresses by protecting B-cells (*1*). Furthermore, this enzyme activates anticancer prodrug molecules *in vivo* converting them to DNA-DNA cross linkers (*2*). The core of the catalytic chemistry of QR and prodrug activation involves a reduction of its cofactor flavin adenine dinucleotide (FAD) by nicotinamide riboside (NR) leading to an unfavorable charge separation that must be stabilized by the protein matrix. The detail of the charge stabilization is experimentally inaccessible but extremely important for the binding of prodrugs or other co-substrates (e.g. harmful semiquinone radicals) and their subsequent catalytic conversions. In the current study, we are exploring the reduction process of the enzyme-bound flavin using computational methods. We are using a combined quantum mechanical/molecular mechanical potentials (*3*) to compute the flavin's reduction potential. The reduction of flavin involves additions of two electrons and two protons. Herein we will present the free energy changes for these reduction processes as well as the p*K*as for the flavin ring nitrogens. From the simulated data we will analyze the role of individual residue of the enzyme matrix in stabilizing the charge separation due to reduction steps.

Background of NQO2

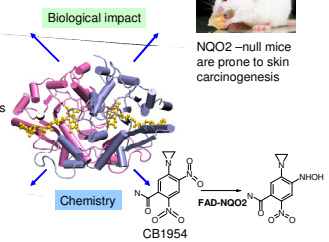


Deletion mutant causes decrease in lymphocytes

NQO2 –null mice are prone to skin carcinogenesis



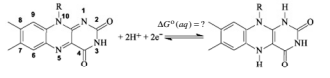
Catalytic products act as DNA inter-strand cross-linker leading to cell death



CB1954
Flavoenzyme: activates pro-drug by catalytic reduction

Questions

- What is the standard reduction potential of FAD-bound NQO2?

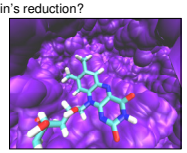


$$E^o = -\frac{\Delta G^o(aq)}{nF} - E^o_H$$

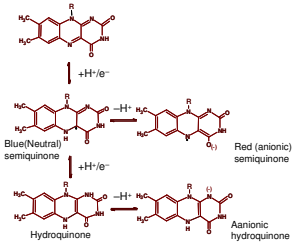
R = 5'-ADP-ribityl-

where '$\Delta G^o(aq)$' is the free energy change for the 2e⁻/2H+ reduction process of FAD in NQO2, E^o is the standard reduction potential, 'F' is the Faraday constant, and n is the number of electrons involved

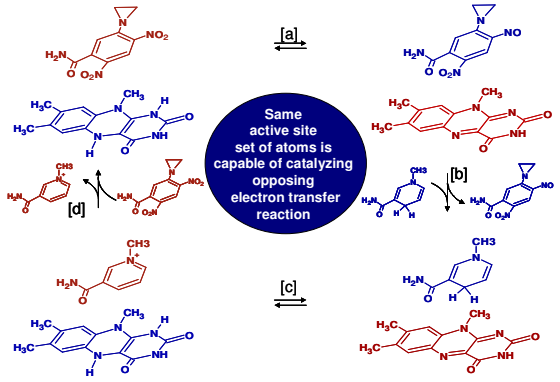
- How enzyme environment stabilizes the charge separation due to flavin's reduction?



Redox States of Flavin



Ping-pong Kinetics



Hybrid QM/MM Method

Basic Molecular Mechanics

$$E = E_{bond} + E_{angle} + E_{dihedral} + E_{vdw} + E_{elec}$$

$$= \frac{1}{2}\sum_{bonds} K_b(b - b_0)^2$$

$$+ \frac{1}{2}\sum_{angles} K_\theta(\theta - \theta_0)^2$$

$$+ \frac{1}{2}\sum_{dihedrals} K_\phi[1 + \cos(n\phi - \gamma)]$$

$$+ \sum_{non\text{-}bonds}\left[4\varepsilon\left[\left(\frac{\sigma}{r}\right)^{12} - \left(\frac{\sigma}{r}\right)^{6}\right] + \frac{q_i q_j}{Dr}\right]$$

(short range)

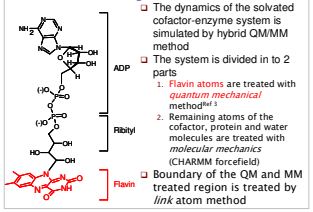
(long range)

For hybrid system the total potential energy

$E_{(total)} = E_{QM} + E_{QM/MM} + E_{MM}$

E_{QM} and $E_{QM/MM}$ are calculated as given in ref 3

QM and MM Regions



- The dynamics of the solvated cofactor-enzyme system is simulated by hybrid QM/MM method
- The system is divided in to 2 parts
  1. Flavin atoms are treated with *quantum mechanical* method[Ref 3]
  2. Remaining atoms of the cofactor, protein and water molecules are treated with *molecular mechanics* (CHARMM forcefield)
- Boundary of the QM and MM treated region is treated by *link* atom method

Free Energy Perturbation

The free energy change for both electron and proton addition processes is computed by 'Thermodynamic Integration' method

Potential energy of a hybrid system: $E_{hybrid} = (1-\lambda)E_A + \lambda E_B$

λ is a coupling parameter varied from 0 to 1 (0.1, 0.2, etc)

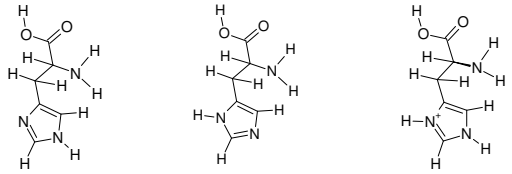
Free energy change $\Delta G = \int_0^1 \langle \partial G(\lambda)/\partial\lambda \rangle d\lambda = \int_0^1 \langle E_A - E_B \rangle_\lambda$

The main assumption is that change of chemical state of the system occurs without any major change of the cartesian coordinate

Modeling of Polar Residues

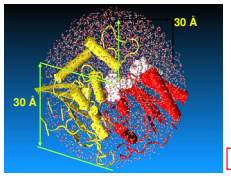
Residues	Charges
Lys, Arg	+1
Glu, Asp	-1
Ser, Cys	0
Asn, Gln, Tyr	0

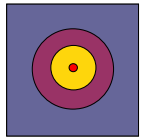
'His' residues are modeled based on their local environment



Charge = 0 Charge = 0 Charge = +1

QM/MM Calculation Setup





1. 30 Å water sphere added around the active site center
2. Deleting all atoms beyond 30 Å
3. Stochastic boundary condition
4. Explicitly treated water molecules are modeled by TIP3P
5. The charge of the 30 Å solvated enzyme was made 0 by putting counter ions

Reaction center : Quantum mechanics

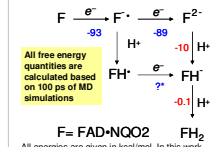
Reaction zone : Newtonian mechanics

Buffer zone: Langevin's equation of motion

Reservoir zone (extended to infinity) : Generalized Born's model for solvation using continuum electrostatics

Results

Computation of Free Energy Changes for the Entire 2e⁻/ 2H⁺ Reduction



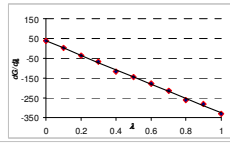
F= FAD•NQO2

All energies are given in kcal/mol. In this work we have computed the free energy changes for the two electron transfer processes and two proton transfer processes.
*computation of this step is currently being carried out

Quantifying the Effect of Enzyme Environment

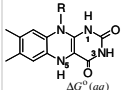
Linear Variation of $\delta G/\delta\lambda$

The calculation demonstrates that the variation of $\delta G/\delta\lambda$ in all perturbation calculations are completely linear with λ.
λ is the parameter by which the electronic state of the flavin is perturbed and $\delta G/\delta\lambda$ is the response of the active site environment to that change.



All free energies are calculated from the area under the curve obtained in the ensemble-averaged partial derivative of the free energy plotted against λ. Other correction terms used to obtain the final free energy values are as discussed in ref 3.

Stabilization of $FADH^-$ by NQO2 Active Site



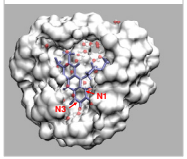
3 pK_as of the Flavin Ring

Acidic protons on flavin atom	$\Delta G^o(aq)$ (kcal/mol)	pK_a
N1	–0.10	0.1
N3	–5.5	4
N5	–29	22

$$pK_a = \frac{\Delta G^o(aq)}{2.303RT}$$

The protons attached to N1 and N3 on the flavin ring are acidic

N1 and N3 Protons Exposed to Solvent



Conclusions

- The standard reduction potential of the Dihydronicotinamide riboside (NRH): quinone oxidoreductase (NQO2), computed for a $2e^-/2H^+$ reduction is -117 mV.
- The calculated potential is 100 and 160 mV positive, respectively, to the experimentally determined standard reduction potentials for similar quinone reductases from Aspartate oxidase (E^o = -216 mV) and Lipoamide dehydrogenase (E^o = -280 mV).
- The pK_a of N1 and N3 protons of the hydroquinone state ($FADH_2$) are less than 7, suggesting that the NQO2 active site will stabilize the $FADH^-$ state at physiological pH.
- Future work will include visualization and analysis of the influence of active site residues on redox potentials and pK_as.

References
1. Iskander et al. (2006) *J. Biol. Chem., 281*, 30917–30924.
2. AbuKhader et al. (2005) *J. Med. Chem. 48*, 7714-7719.
3. Bhattacharyya et al. (2007) *J. Phys. Chem. A 111*, 5729-5742.

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