Exploring Electron Transfer-Induced Conformational Changes in NRH:Quinone Oxidoreductase

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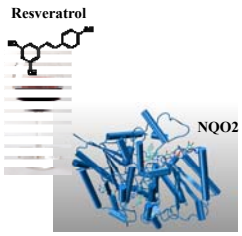
Abstract

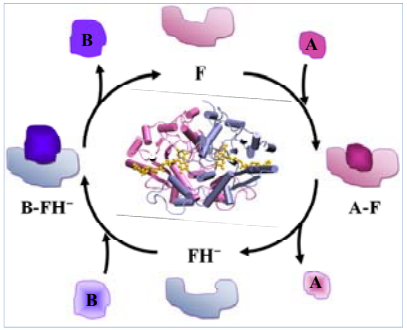
NRH:quinone oxidoreductase is a flavoenzyme that catalyzes the one-step reduction of quinones to hydroquinones using its cofactor, FAD. The enzyme kinetics goes through a 'ping-pong' mechanism, in which changes in the flavin redox state control substrate binding and release. In the reductive half-cycle, the first substrate binds and transfers electrons to the flavin inducing a conformational change of the active site that favors its release. Subsequently, the second substrate binds the same site and accepts electrons from the flavin, thus completing the redox cycle. The redistribution of charges at the active site causes a reversion back to the initial conformation, and thus, the cycle repeats. In previous molecular dynamics simulation study,[1] the observed changes (near the flavin) were in the pico – nanosecond time-scale and cannot account for the oscillatory movements that the active site would need to process the binding and release of the alternate substrates. In this study, we are studying the slower dynamics of the subunit interface (surrounding the active site) using Normal Mode Analysis and exploring its relationship with the faster local motions. The role of coevolving residues on the dynamics[2] is also being examined using Statistical Coevolution Analysis.

Background



- A flavoenzyme that protects our body from harmful semiquinone: performs 2e⁻ reduction of quinones to hydroquinone
- Resveratrol is a strong inhibitor of NQO2, but up-regulates quinone reductase 1, which is another detoxifying enzyme closely similar to NQO2
- The Cofactor FAD is located at the subunit interface
- Redox changes alters substrate binding affinity. Binding and release of the alternate substrates indicates a role of protein dynamics in the regulation of catalysis

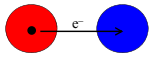
Ping-pong Kinetics



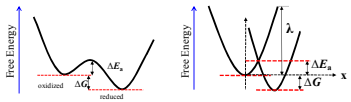
- Change in redox state of flavin alters substrate recognition

Electron Transfer and Reorganization

According to the Marcus theory, an electron transfer reaction involves reorganization of enzyme active site which may undergo substantial conformational change. The free energy of activation (ΔE_a) is related to the reorganization free energy, λ.

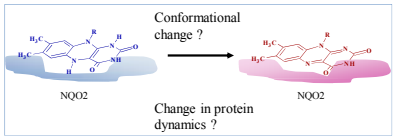


$$\Delta E_a = \frac{(\Delta G + \lambda)^2}{4\lambda}$$



Objectives

- To study the conformational change of NQO2 during the $2H^+$ /2e reduction
- To explore how protein dynamics influence the electron transfer



Methods of Analysis

Normal Mode Simulation

It is believed that slow (> nanosecond) collective motion of the structural elements of a large biomolecule can be identified by normal modes.

Elastic network model

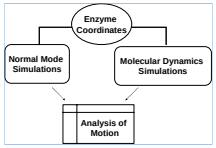
Normal mode calculation is based on the harmonic approximation of the potential energy function around a minimum energy conformation. A coarse-graining approach (the elastic network model) was used, in which the protein residues were represented by only their C_α atoms. The C_α atoms on a protein backbone were considered to be connected by uniform springs whose harmonic potential is given by:

where d_{pq} is the distance between atoms p and q, d^0_{pq} is the distance between these two atoms in the given crystallographic structure, C is the strength of the potential, and R_c is an arbitrary cut-off parameter which defines the maximum interaction range between C_α atoms.

Visual Molecular Dynamics (VMD) was used to calculate residue root mean square derivative (RMSD), superimpose proteins, and mainly for visualization.

Molecular Dynamics Simulation

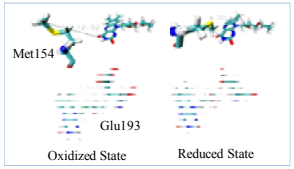
- Molecular dynamics simulation was used to model the protein with four flavin electronic states. In these calculations, molecular mechanical potentials were used to treat protein and solvent atoms, while the flavin atoms were treated with higher-level theory.
- Faster motions (~a few hundred picosecond) were observed analyzing the simulated data

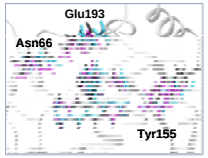


Results

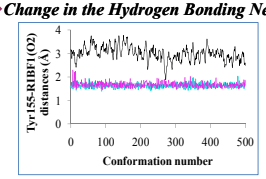
The redox transitions of the flavin-bound enzyme has been simulated by molecular dynamics simulations. The simulated data exhibits local conformational changes. Additionally, they reveal changes in the dynamics of distant part of the enzyme.

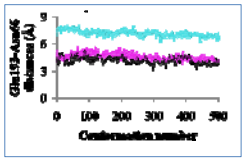
❖*Redox-Induced Conformational Changes*





❖*Change in the Hydrogen Bonding Network*

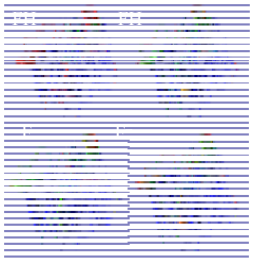




Redox-dependent Protein Motions

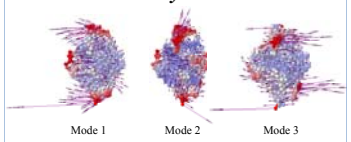
❖*Analysis of Dynamics*

Dynamic regions of the enzyme are extracted from molecular dynamics simulation data by calculating the RMSDs of the C_α atoms. Low, moderate and high dynamics regions are shown in blue, green, and red, respectively. F: a short-hand notation for the flavin-bound NQO2.



Slower and Global Dynamics

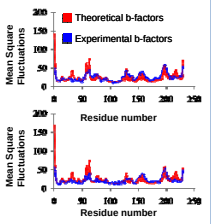
Normal Mode Analysis



Mode	Kappa, κ
1	0.36
2	0.56
3	0.42

κ is a measure of the degree of collectivity of the protein

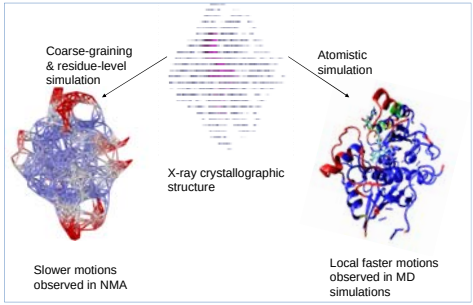
Comparison of B-factors



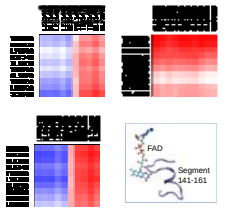
B-factors is the thermal fluctuations of atoms about their average positions

Local and Global Dynamics

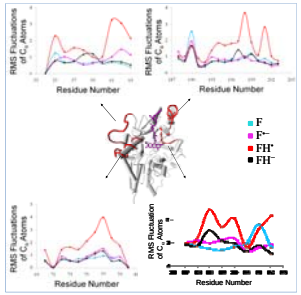
❖*Comparison of Dynamics*



NMA Simulations



Molecular Dynamics Simulations



- Analysis of the normal modes suggests that motion of the loop (144-155) near the redox center is coupled to the motions of the other distant protein segments

Conclusions

- Both Normal mode and Molecular Dynamics simulations show agreement on highly mobile regions.
- MD simulations exhibit unusually high thermal fluctuations of protein segments for the neutral semiquinone.
- Analysis of the MD simulations show redox transition influences the thermal RMS fluctuations near and far the redox center alike.
- NMA shows that dynamics of these near and far sites are indeed coupled and local changes triggered by redox reactions may experience a broader response in the form of an oscillatory protein dynamics.
- This correlated pattern of the local and global dynamics is further being investigated.

Future Directions

- ✓Calculation of the Reorganization Free Energy, λ.
- ✓Identifying co-evolved residues at the subunit interface to explore their role in the redox-induced protein dynamics.

References:

1. **Rauschnot JC Jr., Yang C, Yang V, and Bhattacharyya S. *J. Phys. Chem. A. Revised manuscript submitted.***
2. **Weimer KM, Shane BL, Brunetto M, Bhattacharyay S, and Hati S. *J. Biol. Chem.* (2009) 284, 10088-10099.**