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DISSERTATION SUMMARY

Protein electron transfer. Spectroscopy and modeling

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Redox (electron transfer) reactions are widespread in living organisms and are of utmost importance in biological energy conversion. Most life forms depend on either photosynthetic or respiratory ATP production. Both of these are intimately linked to long range intra- and interprotein electron transfer processes coupled to transmembrane proton transport, which results in the energized state of the membranes hosting the ATP synthase enzyme. Electron transfer in proteins takes place between redox cofactors separated by distances well above 10 Å in some cases and yet, the electron transfer is sufficiently rapid for efficient energy conversion (usually faster than milliseconds).

Marcus theory describes the rate of electron transfer in the non-adiabatic, outer shell case applicable to redox proteins, with parameters characteristic of the physical properties of the medium. These parameters can be viewed in two different ways (Jones et al. 2005)

One intepretation consideres the protein as a semi-homogeneous and semi-isotropic medium whose actual structure can be characterized solely by its packing density as far as electron transfer efficiency is concerned. In the other interpretation the chemical composition and secondary and tertiary structural elements are explicitely taken into account in calculating the optimal electron transfer pathway connecting donor and acceptor.

We are experimentally studying the electron transfer rate by kinetic spectroscopy, using the photoactive covalent redox label TUPS (thiouredopyrene-trisulfonate; Kotlyar et al. 1997). Two forms of TUPS can bind to either lysine or cysteine side chains. Photoexcitation of the label by a 355 nm laser pulse (Nd-YAG third harmonic) yields the triplet excited state of TUPS, which is a good electron donor or acceptor – depending on the redox partner (Kotlyar et al. 2004). We have attached TUPS to genetically engineered cysteine side chains of horse heart cytochrome c. In collaboration we have also measured electron transfer on TUPS labeled flavodoxin, as well as in the complex of TUPS labeled cytochrome and cytochrome c oxidase.

We have mapped the entire surface of horse heart cytochrome c in terms of electron transfer coupling leading to the heme redox cofactor using available pdb structures and the program HARLEM (www.kurnikov.org). In this way we can identify potential "cold" and "hot" regions in the protein for electron transfer, and assist future site directed mutagenesis for positioning the redox label at locations of particular interest.

Both on cytochrome and on flavodoxin the kinetics of electron transfer between TUPS and the respective redox cofactors (heme and flavin) are more complex than expected. We have performed molecular dynamics calculations to demonstrate that TUPS can occupy several equilibrium positions relative to the protein surface. Electron transfer pathway (and packing density) calculations have been carried out for these conformations. It appears that the optimal electron transfer pathways do not necessarily follow the covalent link between TUPS and the redox cofactors, but may involve through space jumps from the label to the protein surface. The correlation between the measured electron transfer rates and the pathways so calculated turns out to be much better than the correlation between the rates and the covalent distance between donor and acceptor (Tenger et al. 2005).

References

Jones ML, Kurnikov IV, Beratan DN (2002) The nature of tunneling pathway and average packing density models for protein-mediated electron transfer. J Phys Chem A 106:2002-2006.

Kotlyar AB, Borovok N, Hazani M (1997) Photoinduced electron transfer in singly labeled thiouredopyrenetrisulfonate cytochrome c derivatives. Biochemistry 36(50):15828-15833.

Kotlyar AB, Borovok N, Khoroshyy P, Tenger K, Zimányi L (2004) Redox photochemistry of thiouredopyrenetrisulfonate. Photochem Photobiol 79(6):489-493.

Tenger K, Khoroshyy P, Leitgeb B, Rákhely G, Borovok N, Kotlyar A, Dolgikh DA, Zimányi L (2005) Complex kinetics of the electron transfer between the photoactive redox label TUPS and the heme of cytochrome c. J Chem Inf Mod 45(6):1520-1526.

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