Of oxidases and peroxidases: new studies using protein film voltammetry

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With a few notable exceptions, the electrochemical characterization of oxidases and peroxidases has been difficult at best. This poster presents our attempts to explore oxidase and peroxidase chemistry at an electrode, as demonstrated by the two enzymes CueO (from E. coli) and NeCCP (from N. europaea), respectively. Both enzymes adsorb to electrodes of pyrolytic graphite edge, and in the case of CueO, alkane-thiol modified gold. In the case of the multi-copper oxidase CueO, this work reveals the reduction potentials of the CueO cofactors, as observed in the absence of substrate, and with inhibitors (azide, cyanide) bound. The context for these findings regarding other multi-copper oxidases will be presented. Regarding NeCCP, protein films of this protein demonstrate highly active catalytic reduction of hydrogen peroxide. The analysis of the wave-shape reveals an n=1 process governs catalysis, and at a reduction potential of approximately 540 mV (vs. NHE). Michaelis-Menton treatment of the substrate dependence gives values of K_m of 55 ∝M, and k_{cat} of ~10000 s^{-1}. The pH-dependence of this feature, along with its relationship to the current understanding of the mechanism of NeCCP will be presented.

Calculation of the Redox Potential of Iron-Sulfur Proteins

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There are many redox proteins having the same redox-active cofactor bound, but differing in redox potential. How the protein modulates the redox potential of the cofactor is not well understood.

We developed a procedure to compute redox potentials in proteins accurately by first principle methods. Our approach is to combine quantum mechanical calculations on the DFT level with continuum electrostatic methods. Our results on the two iron-sulfur proteins, Rubredoxin and Ferredoxin, show excellent agreement with experiments.

For Ferredoxin, it was shown by x-ray crystallography, that a peptide bond rotates comparing oxidized and reduced crystal structure. Our calculations show, that this structural change controls the redox potential of Ferredoxin.

Paramagnetic NMR studies determined the iron of the 2Fe2S center which changes its redox state. Our calculations are in agreement with the experiment, but the two reduced states only differ in energy when a sufficiently large part of the protein is treated quantum-mechanically (see Figure).

The redox potential can be decomposed to gain insight into which contributions modulate the redox potential and what their relative importance is. Hydrogen bond formation close to the 2Fe2S center are found to be of major importance for the redox potential. Charge polarization effects also play an important role.

In Rubredoxin, there was evidence for a leucine modulating the redox potential by permitting solvent access to the center only in the reduced state. Our calculations indicate roughly a cancellation of enthalpic and entropic contributions. Therefore water penetration does not have a strong effect on the redox potential. In agreement, very recent NMRD data indicates the possibility of water penetration also in the oxidized state.

Taking into account the experimental difficulties in measuring an 'absolute' redox potential accurately and the theoretical challenge to compute it, the agreement obtained is striking.