Regulation of Electron Transfer Pathways in Proteins with Aromatic Amino Acids
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Abstract
Natural photosynthetic proteins are based upon chains of reactions that start with sub-nanosecond light energy conversion into energy of electrical charges and followed by multi-step electron transfer (ET). Attempts to develop artificial enzymes for photosynthesis have been relatively futile due to the difficulty of generating sufficiently fast primary charge separation even with the smallest proteins. Here we report our results on attempts to accelerate ET by placing aromatic redox-active amino acids along the putative path of ET for the E39C mutant of Pp68, a 3-heme cytochrome. With LC-MS we verified protein purity and successful attachment of Ru(bpy)3 to Cys-39 in E39C and E39C/F41W mutants. Using analytical size exclusion chromatography, we verified successful protein folding and removal of unreacted photoinitiators. With temperature-dependent circular dichroism spectroscopy, we evaluated thermal stability of both mutants. Finally, we observed increasing fluorescence quenching under more alkaline conditions in E39C/F41W suggesting that Trp-41 starts to serve as an intermediate site in the ET pathway responsible for acceleration of ET.

Hypothesis
Slow ET
Fast ET

Figure 1: Structure of E39C mutant of Pp68 with Ru(bpy)3 photosensitizer covalently attached. The observed kinetics of heme oxidation is slow due to long ET pathway. We hypothesize that by inserting an aromatic redox-active amino acid (E39C/F41W) we can create two shorter steps with net fast ET rate.

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Figure 2: Left panel: Jablonski diagram for photoinduced ET in E39C-Ru. Middle panel: Jablonski diagram for E39C-Ru with intermediate ET to a tyrosine or tryptophan residue. Right panel: Redox potential for Tyr and Trp as a function of pH. Figure from Harriman (1987), JPhysChem,91:6104. Photosensitizer Ru(II/II) potential is 0.80V and does not depend on pH.

Figure 3. Left panel: distance between aromatic atoms of Ru(bpy)3 and the closest heme in 3 MD simulations of E39C. Middle panel: Heme-Trp distance for E39C/F41W. Right panel: Ru(bpy)3-Trp distance.

Figure 4. Left panel: relative rate (log10 k/kt) of ET via Trp assuming 0.80V potentials for Ru(II/II) and Trp. Right panel: relative rate (log10 k/kt) of ET via Trp assuming 4.6Å heme-Trp and 10Å Trp-Ru(bpy)3 distances.

Figure 5: LC-MS data shows the expected masses and successful Ru(bpy)3 attachment.

Circular Dichroism Spectroscopy

Figure 7. Left panel: temperature-dependent circular dichroism of E39C-Ru. Right panel: normalized ellipticity for both labeled mutants at 222 nm show that structure is not perturbed by mutations and labeling.

Fluorescence Quenching

Figure 8: Left panel: fluorescence of buffer and E39C-Ru at pH values 7.5, 8.5, 9.5, 10.5, and 11.5. Right panel: fluorescence of E39C/F41W at 7.5, 8.5, 9.5, 10.5, and 11.5.

Conclusions
• Trp provides an alternative pathway with shorter ET steps.
• Rate of ET exponentially depends on distance. According to MD we expect 4.6Å heme-Trp and 10Å Trp-Ru(bpy)3 distances.
• LC-MS identified both the dimers and properly labeled cysteine mutants.
• Analytical size exclusion determined the atomic size and presence of the proteins and other substances, which gave the amount of both dimers and single mutants given by LC-MS.
• Circular dichroism demonstrates that mutations and labeling have not impacted protein structure and stability.
• Fluorescence intensity peaks at 620 nm for E39C-Ru, and fluorescence for E39C/F41W decreases as pH increases, except at pH 9.5 which has shown to be an exception.
• Though no set conclusion has been reached, our research thus far fully support our hypothesis.

Analytical Size Exclusion Chromatography

Figure 6. Left panel: Calibration of a Superdex 200 Increase size-exclusion column. Right panel: size-exclusion chromatography elution profiles for Ru(bpy)3, E39C/F41W, and E39C/F41W-Ru (top to bottom).

Figure 9. Middle panel: Change in the native structure of E39C-Ru monitored by CD at 222 nm with pH. Right panel: Circular dichroism of E39C-Ru at pH 7.5 (39°C) and pH 11.5 (39°C).