CHAPTER FIVE

Studying Hopping in Another Pathway of Azurin

5.1 ABSTRACT

Hopping was investigated through an established electron transfer pathway that traverses the interior of azurin. Tyrosine was installed in the 48 site of azurin with the hope that the sequestration would circumvent deprotonation of the radical cation form, thereby allowing for hopping to occur through tyrosine. Two metal-modified proteins were made for the study. Time-resolved UV-VIS spectroscopy measurements indicate that, while the substitution of tyrosine can encourage fast electron transfer between the ruthenium and the copper center, it does not actually participate in hopping.

5.2 INTRODUCTION

In the hope of generating more experimental demonstrations of multistep tunneling at different distances, a hopping system is installed along another known electron transfer pathway in azurin. The system discussed in this chapter is through a network of hydrogen and covalent bonds traversing the protein's interior, ending at the surface residue H83 (**Figure 5.1**).[1] An examination of the electron tunneling pathways between these two centers reveals that the dominant tunneling pathways utilize the backbone of residue 47, close to the wild-type W48. W48 is buried in the protein, theoretically inaccessible from the solvent. The W48 has been demonstrated to be quite important for electron transfer pathways in systems studied in azurin. A previous study

in the group using gold-modified electrodes demonstrated that W48 was important for intermolecular electron transfer.[2] Pulse radiolysis studies on azurin, which study the kinetics of the 27 Å electron transfer between a reduced anion on the disulfide bridge Cys3-Cys26 and the copper center, demonstrated that substitution of W48 with other amino acids at the site diminished electron transfer rates.[3] Substitution at this site, where so much electron transfer research revolves, with tyrosine is only natural.

In fact, it has been done before: Dr. William A. Wehbi made a Y48 mutant and labeled H83 with rhenium. He used flash-quench to observe the neutral radical by EPR.[4] It is hoped that by installing the ruthenium complex at His83, and observing the system using time-resolved spectroscopic techniques, hopping through tyrosine, uncomplicated by deprotonation, will be observed.

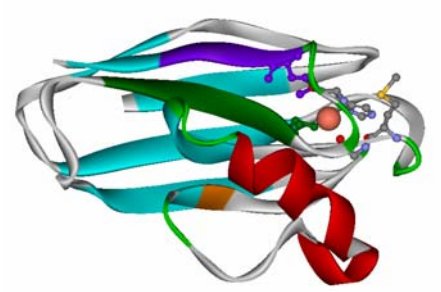


Figure 5.1. *Pseudomonas aeruginosa* azurin (PDB code: 1AZU). His83 is highlighted in orange.

5.3 RESULTS & DISCUSSION

The proteins discussed in this chapter include Ru83/Y48/Az(Cu^{2+}) and Ru83/F48/Az(Cu^{2+}). These proteins were prepared using the methods described in **Chapter 2**.

The time-resolved emission data reveal quenching of the excited state *Ru^{2+} in Ru83/Y48/Az(Cu^{2+}) (**Figure 5.2**). The Ru83/F48/Az(Cu^{2+}) does not demonstrate the

same quenching, which indicates that the tyrosine could be responsible. However, when the studies were repeated on the Ru83/Y48/Az(Cu^{+}) mutant to search for copper oxidation, the emission data belied the hypothesis (**Figure 5.3**).

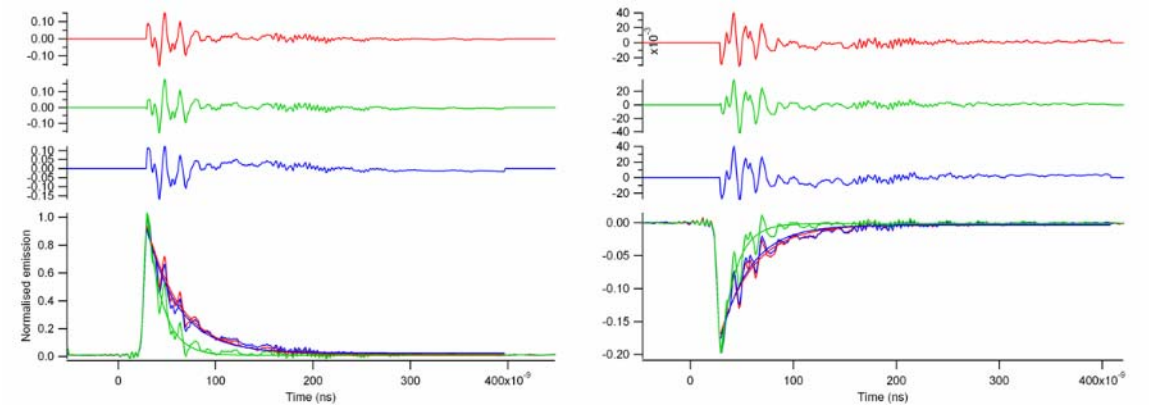


Figure 5.2. Time-resolved UV-VIS spectroscopy of Ru83/Y48/Az(Cu^{2+}). Green trace is 30 μM Ru83/Y48/Az(Cu^{2+}), 25 mM NaP_i, pH 7.4. Red trace is Ru model compound, 30 μM [Ru(trpy)(tfmbpy)(im)]$(PF_6)_2$ in 25 mM NaP_i, pH 7.4. Blue trace is 30 μM Ru83/F48/Az(Cu^{2+}) **Left:** λ_{ex} = 490 nm, λ_{em} = 700 nm. Each are fit to single exponential decay. Label τ = 35 ns, Ru83/F48/Az(Cu^{2+}) τ = 33 ns, Ru83/Y48/Az(Cu^{2+}) τ = 17 ns. **Right:** λ_{ex} = 510 nm, λ_{obs} = 480 nm. Label τ = 34 ns, Ru83/F48/Az(Cu^{2+}) τ = 30 ns, Ru83/Y48/Az(Cu^{2+}) τ = 15 ns

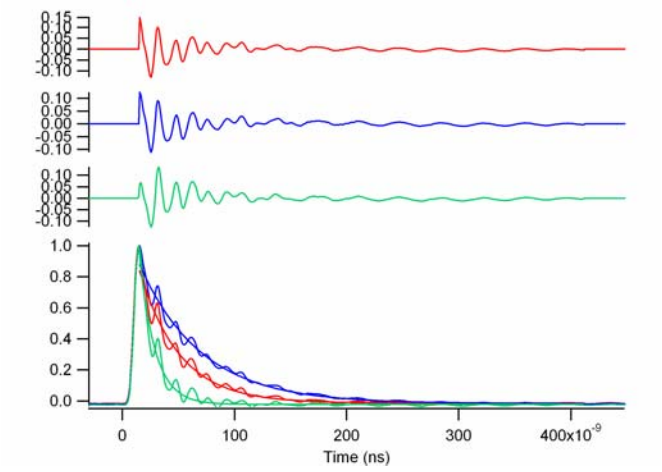
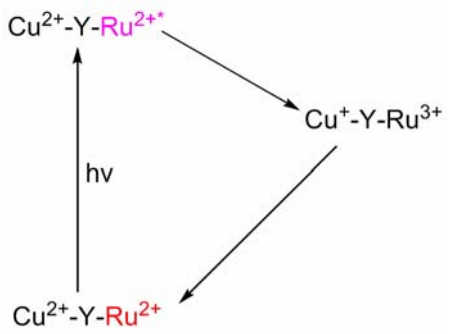


Figure 5.3. Time-resolved emission of Ru83/Y48/Az(Cu^{+}). Green trace is 30 μM Ru83/Y48/Az(Cu^{2+}), 25 mM NaP_i, pH 7.4. Red trace is Ru model compound, 25 μM [Ru(trpy)(tfmbpy)(im)]$(PF_6)_2$ in 25 mM NaP_i, pH 7.4. Blue trace is Ru83/F48/Az(Cu^{+}) λ_{ex} = 490 nm, λ_{em} = 700 nm. Each are fit to single exponential decay. Label τ = 35 ns, Ru83/Y48/Az(Cu^{2+}), τ = 17 ns, Ru83/Y48/Az(Cu^{+}), τ = 40 ns



Scheme 5.1. Events after sample excitation in Ru83/Y48/Az(Cu^{2+}). Colors indicate species observed spectroscopically.

From this data, it appears that the Cu^{2+}, and *not* Y48, is participating in electron transfer with the ruthenium (**Scheme 5.1**). This is not unfamiliar behavior for a *Ru^{2+}-Cu^{2+} system; it was observed in the study of ruthenium-modified plastocyanins.[5] The difference in kinetics in the F48 and Y48 mutants is likely due to more efficient tunneling pathways in one mutant over the other, allowing for increased electronic coupling, and so faster electron transfer. Given that electron transfer is so efficient in these studies, it is likely that hopping does not need to be accessed for efficient electron transfer.

5.4 CONCLUSIONS

While the kinetics on the Ru83/Y48/Az(Cu^{2+}) system do not demonstrate multistep tunneling behavior, they do demonstrate that the 48 site is an important site in determining the electron transfer kinetics between Ru83 and the copper center. They also demonstrate that electron transfer between the Ru83 and copper center in azurin is already quite efficient in the system.

5.5 EXPERIMENTALS

The metal-modified proteins were prepared as described in **Chapter 2**. Laser spectroscopy studies were carried out as described in **Chapter 2**.

5.6 REFERENCES

(1) Regan, J. J. *Chem. & Biol.* **1995**, *2*, 489.
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