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Electron Transfer across a Peptide–Peptide Interface within a Designed Metalloprotein

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Mechanistic studies of biological electron-transfer (ET) reactions have involved the use of surface-derivatized proteins, protein–protein complexes, and polypeptide-bridged donor–acceptor compounds.¹ These latter studies seek to use well-defined model systems to better define the role of the intervening protein matrix in mediating biological electron transfers.^{2–6} However, whereas many *in vivo* ET reactions occur across a *noncovalent* protein–protein interface, the primary role of the peptide spacers found in current model systems is to provide a *covalent* link between the donor and acceptor sites. As such, these systems are poorly suited to probe the mechanisms of ET reactions occurring across a peptide–peptide interface.

Here, we describe the use of an α -helical coiled-coil to design an artificial metalloprotein that is amenable to mechanistic studies of interfacial ET reactions. Recent advances in the field of rational protein design have shown that α -helical coiled-coils can be built from a seven-residue heptad repeat labeled (a-b-c-d-e-f-g) in which hydrophobic residues are located at positions “a” and “d”, and residues able to form interchain salt-bridges occupy positions “e” and “g”.^{7,8} Hydrophilic amino acids occupy the remaining positions of the repeat. As shown in Figure 1a, this sequence produces a situation in which the nonpolar faces of two α -helices can sequester themselves away from the aqueous solvent by forming the coiled-coil structure. This noncovalent peptide assembly is an ideal system in which to study biological electron-transfer reactions as it contains a well-defined peptide–peptide interface.

A 31-residue polypeptide was prepared by solid-phase methods by using fluorenylmethoxycarbonyl N-terminal protection and diisopropylcarbodiimide-hydroxybenzotriazole activation. The amino acid sequence (**I**) was based upon those of existing two-stranded coiled-coils.^{7,8} However, the sequence was also modified to incorporate a single histidine residue at the most highly solvent-exposed position of the third heptad repeat.⁹

After cleavage from the solid support, the crude product was purified by reverse-phase HPLC by using CH₃CN/H₂O gradients (0.1% HTFA) and characterized by MALDI-MS (calcd 3404; found 3405).

(1) For a review, see: (a) Bendall, D. S., Ed. *Protein Electron-Transfer*; Bios Scientific: Oxford, UK, 1996. (b) Bowler, B. E.; Raphael, A. L.; Gray, H. B. *Prog. Inorg. Chem.* **1990**, 38, 259.

(2) Gretchikhine, A. B.; Ogawa, M. Y. *J. Am. Chem. Soc.* **1996**, 118, 1543.

(3) Mutz, M. W.; McLendon, G. L.; Wishart, J. F.; Gaillard, E. R.; Corin, A. F. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, 93, 9521.

(4) (a) Fox, M. A.; Galoppini, E. *J. Am. Chem. Soc.* **1997**, 119, 5277.

(b) Galoppini, E.; Fox, M. A. *J. Am. Chem. Soc.* **1996**, 118, 2299.

(5) Hungerford, G.; Martinez-Insua, M.; Birch, D. J. S.; Moore, B. D. *Angew. Chem., Int. Ed. Engl.* **1996**, 35, 326.

(6) (a) Ogawa, M. Y.; Wishart, J. F.; Young, Z.; Miller, J. R.; Isied, S. S. *J. Phys. Chem.* **1993**, 97, 11456. (b) Ogawa, M. Y.; Moreira, I.; Wishart, J. F.; Isied, S. S. *Chem. Phys.* **1993**, 176, 589.

(7) Betz, S. F.; Bryson, J. W.; DeGrado, W. F. *Curr. Opin. Struct. Biol.* **1995**, 5, 457.

Figure 1. (a) Helical wheel diagram of the dimeric 31-mer polypeptide. A single histidine residue has been incorporated into the most solvent-exposed position of the third heptad repeat. Residues 1, 30, and 31 are omitted from the diagram. (b) Schematic view of the third heptad repeat of the ET heterodimer [Ru(bpy)₂]Im(31-mer)/Ru(NH₃)₅(31-mer), which was prepared as described in the text.

Figure 2. Circular dichroism spectrum of the Fmoc-protected 31-mer apodimer (73 μ M in 50 mM phosphate buffer, pH 7, 25 $^{\circ}$ C). Inset: Molar ellipticity at 222 nm measured as a function of temperature. The values are normalized to that observed at 5 $^{\circ}$ C.

Figure 2 shows the circular dichroism (CD) spectrum of the 31-mer, which consists of a positive signal at 195 nm ($\theta > +39\,000$ deg cm² dmol^{−1}) and a pair of negative signals at 208 ($\theta = -21\,800$ deg cm² dmol^{−1}) and 222 nm ($\theta = -22\,000$ deg cm² dmol^{−1}) indicating that the peptide is $>69\%$ α -helical.¹⁰ The value of $[\theta_{222}]/[\theta_{208}] = 1.01$ is characteristic of an α -helical coiled-coil.⁸ In contrast, single α -helices have values of $[\theta_{222}]/[\theta_{208}] = 0.86$.⁸ The noncovalent assembly is very stable, displaying a noncooperative melting curve with $T_m = 65$ $^{\circ}$ C (inset, Figure 2).¹¹ Discontinuous SDS polyacrylamide gel electrophoresis showed the existence of two species having molecular weights of ca. 4 and 8 kDa, respectively (data not shown). Thus, a population of peptide monomers and dimers exists in solution.

(8) (a) Zhou, N. E.; Kay, C. M.; Hodges, R. S. *Biochemistry* **1992**, 31, 5739. (b) Zhou, N. E.; Kay, C. M.; Hodges, R. S. *J. Biol. Chem.* **1992**, 267, 2664. (c) Su, J. Y.; Hodges, R. S.; Kay, C. M. *Biochemistry* **1994**, 33, 15501.

(9) The carboxyl end of the 31-mer incorporates a *tert*-butyl protected cysteine residue, which affords the possibility of forming an interchain disulfide cross-link after treatment with hydrofluoric acid. The present study does not take advantage of this feature. However, future work will investigate the ET properties of covalently cross-linked coiled-coils.

(10) Chen, Y.-H.; Yang, J. T.; Chau, K. H. *Biochemistry* **1974**, 13, 3350.

(11) Interestingly, when the 31-mer is dissolved in methanol, the dimer dissociates into two separate α -helices, showing a CD spectrum in which $\theta_{222} = -27\,000$ deg cm² dmol^{−1}, and $[\theta_{222}]/[\theta_{208}] = 0.85$.

Scheme 1

The metallohomodimers $[\text{Ru}(\text{bpy})_2\text{Im}(31\text{-mer})]_2$ and $[\text{Ru}(\text{NH}_3)_5(31\text{-mer})]_2$ (bpy = 2,2'-bipyridine, Im = imidazole) were prepared by methods described previously.¹² Significantly, metalation of the 31-mer does not alter the CD spectrum of the peptide.¹³ The absorption spectrum of $[\text{Ru}(\text{bpy})_2\text{Im}(31\text{-mer})]_2$ shows maxima at $\lambda_{\text{abs}} = 203, 245, 290, 340,$ and 486 nm, which is similar to that of $[\text{Ru}(\text{bpy})_2\text{Im}(\text{His})]$. The emission properties of this homodimer ($\lambda_{\text{em}} = 688$ nm, $\tau = 79$ ns in H_2O) are nearly identical with those of $\text{Ru}(\text{bpy})_2\text{Im}\text{-cyt } c$.¹⁴ The cyclic voltammogram of $[\text{Ru}(\text{NH}_3)_5(31\text{-mer})]_2^{3+}$ shows a single reduction at $E^0 = +0.078$ V vs NHE, which is identical with that reported for $\text{Ru}(\text{NH}_3)_5\text{-modified cyt } c$.¹² The ET heterodimer (Figure 1b) $[\text{Ru}(\text{bpy})_2\text{Im}(31\text{-mer})/\text{Ru}(\text{NH}_3)_5(31\text{-mer})]$ was prepared by heating an approximately equimolar solution of the two homodimers to 80°C for 30 min and allowing the sample to cool back to room temperature. It is noted that this procedure produces a statistical mixture of the homo- and heterodimers. However, only the latter species can display intracomplex electron transfer. The metal-to-metal distance in the heterodimer is roughly estimated to be 23 ± 2 Å, assuming that it remains isostructural to the transcriptional regulator GCN4.¹⁵

Electron-transfer studies were made by using the laser flash-quench technique (Scheme 1) in which $\text{Q} = \text{Ru}^{\text{III}}(\text{NH}_3)_6$.¹⁴ In a preliminary control experiment, photoexcitation of a solution of the $[\text{Ru}^{\text{II}}(\text{bpy})_2\text{Im}(31\text{-mer})]_2$ homodimer (21 μM) and $\text{Ru}^{\text{III}}(\text{NH}_3)_6$ (9 mM) produced a prompt increase in absorption at 306 nm and a bleach at 480 nm, indicating rapid formation of the oxidized ruthenium polypyridyl complex.¹⁴ Under these conditions, no evidence for recombination was observed on the millisecond time scale (inset, Figure 3). At higher peptide concentrations, recombination with the reduced quencher occurred with a second-order rate constant of $k_{\text{rec}} = 2 \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$. When the flash quench experiment was performed on the statistical mixture of homo- and heterodimers, an identical transient difference spectrum was observed. However, the spectral changes followed biphasic kinetics. Figure 3a shows that the increased absorption at 306 nm decayed to a non-zero baseline with a first-order rate constant of $k_{\text{et}} = 3.5 \times 10^3 \text{ s}^{-1}$. Similar kinetics were observed at 480 nm, except that a persistent bleach was seen at longer times (Figure 3b). The long-lifetime component seen in both curves is consistent with the presence of a population of $[\text{Ru}^{\text{III}}(\text{bpy})_2\text{Im}(31\text{-mer})]_2$ homodimer, consistent with the method of sample preparation, which undergoes slow recombination.¹⁶ However, the fast first-order rate constant, which is observed only in the presence of the heterodimer, is independent of peptide concentration (21–140 μM). This process is therefore assigned to the electron-

Figure 3. Transient absorption kinetics obtained after photolysis (7 ns laser pulse, Nd:YAG) of a solution of the $[\text{Ru}(\text{bpy})_2\text{Im}(31\text{-mer})]_2$ homodimer, $[\text{Ru}(\text{NH}_3)_5\text{Im}(31\text{-mer})]_2$ homodimer, and $[\text{Ru}(\text{bpy})_2\text{Im}(31\text{-mer})/\text{Ru}(\text{NH}_3)_5(31\text{-mer})]$ heterodimer (total peptide concentration = 64 μM) in the presence of a 400-fold excess of $\text{Ru}^{\text{III}}(\text{NH}_3)_6$ at (a) 306 and (b) 480 nm. The solid lines are fits to a single exponential decay expression in which $k_{\text{et}} = 3.5 \times 10^3 \text{ s}^{-1}$. Inset: Results of the flash-quench experiment performed on the $[\text{Ru}(\text{bpy})_2\text{Im}(31\text{-mer})]_2$ homodimer as described in the text.

transfer reaction occurring across the peptide–peptide interface over a distance of ca. 23 Å.

The results described above demonstrate that α -helical coiled-coils can be derivatized to construct a useful model system for studying the mechanisms of biological electron-transfer reactions. To our knowledge, this work provides the first example of an electron-transfer reaction that occurs across the noncovalent peptide–peptide interface of a model protein.

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Supporting Information Available: Polyacrylamide gel electrophoresis results and transient absorption spectrum of the $[\text{Ru}(\text{bpy})_2\text{Im}(31\text{-mer})]_2$ homodimer following flash quench (2 pages). See any current masthead page for ordering and internet access instructions.

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(16) Under these dilute conditions, laser excitation generated $< 10^{-6} \text{ M}$ of the oxidized ruthenium polypyridyl peptide leading to a slow recombination rate. However, when the flash quench experiment was repeated at a larger concentration of heterodimer (138 μM), the recombination rate could be measured. A rapid, first-order decay of ($k = k_{\text{et}} = 3.8 \times 10^3 \text{ s}^{-1}$) was seen, followed by a slower, second-order process having a bimolecular rate constant of $k = k_{\text{rec}} = 3 \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$.

(12) (a) Nocera, D. G.; Winkler, J. R.; Yocom, K. M.; Bordignon, E.; Gray, H. B. *J. Am. Chem. Soc.* **1984**, *106*, 5145. (b) Bechtold, R.; Gardiner, M. B.; Kazmi, A.; van Hemelryck, B.; Isied, S. S. *J. Phys. Chem.* **1986**, *90*, 3800.

(13) Indeed, the metallohomodimers exhibit a slightly higher CD melting temperature ($T_m = 70^\circ\text{C}$), which suggests that the placement of charged metal complexes at the solvent-exposed sites of the helices reinforces the amphipathic nature of the peptide.

(14) Chang, I.-J.; Gray, H. B.; Winkler, J. R. *J. Am. Chem. Soc.* **1991**, *113*, 7056.

(15) O'Shea, E. K.; Klemm, J. D.; Kim, P. S.; Alber, T. *Science* **1991**, *254*, 539.