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Electron Transfer Across A Peptide-peptide Interface Within A Designed Metalloprotein

Gennady V. Kozlov

Michael Y. Ogawa
Bowling Green State University, mogawa@bgsu.edu

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

Gennady V. Kozlov and Michael Y. Ogawa*

Department of Chemistry and
Center for Photochemical Sciences
Bowling Green State University
Bowling Green, Ohio 43403

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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]

NH_2-K-(I-E-A-L-E-G-K)$_2$-(I-E-A-L-E-**H**-K)-
(I-E-A-L-E-G-K)-C′-G-OH (**I**)

After cleavage from the solid support, the crude product was purified by reverse-phase HPLC by using CH_3CN/H_2O gradients (0.1% HTFA) and characterized by MALDI-MS (calcd 3404; found 3405).

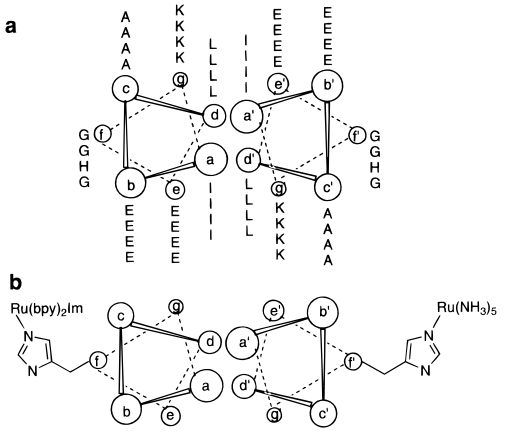

Figure 1. (a) Helical wheel diagram of the dimeric 31-mer polypeptide. A single histidine residue has been incorporated into the most solvent-exposed position of the third heptad repeat. Residues 1, 30, and 31 are omitted from the diagram. (b) Schematic view of the third heptad repeat of the ET heterodimer [$Ru(bpy)_2Im$(31-mer)/$Ru(NH_3)_5$(31-mer)], which was prepared as described in the text.

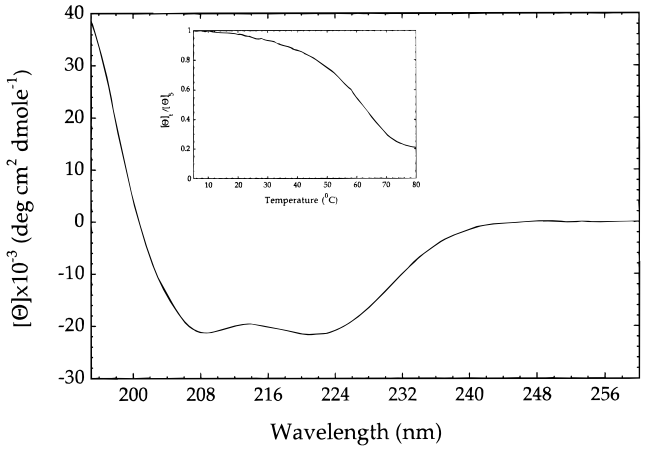

Figure 2. Circular dichroism spectrum of the FMOC-protected 31-mer apodimer (73 μM in 50 mM phosphate buffer, pH 7, 25 °C). Inset: Molar ellipticity at 222 nm measured as a function of temperature. The values are normalized to that observed at 5 °C.

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 cm^2 dmol^{-1}) and a pair of negative signals at 208 ($\theta = -21\,800$ deg cm^2 dmol^{-1}) and 222 nm ($\theta = -22\,000$ deg cm^2 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 $T_m = 65$ °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.

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(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.

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(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 cm^2 dmol^{-1}, and $[\theta_{222}]/[\theta_{208}] = 0.85$.

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Scheme 1

$$[Ru^{II}(bpy)_2Im(31\text{-mer})/Ru^{II}(NH_3)_5(31\text{-mer})] + h\nu \longrightarrow [{}^*Ru^{II}(bpy)_2Im(31\text{-mer})/Ru^{II}(NH_3)_5(31\text{-mer})]$$

$$[{}^*Ru^{II}(bpy)_2Im(31\text{-mer})/Ru^{II}(NH_3)_5(31\text{-mer})] + Q \longrightarrow [Ru^{III}(bpy)_2Im(31\text{-mer})/Ru^{II}(NH_3)_5(31\text{-mer})] + Q^-$$

$$[Ru^{III}(bpy)_2Im(31\text{-mer})/Ru^{II}(NH_3)_5(31\text{-mer})] \xrightarrow{k_{et}} [Ru^{II}(bpy)_2Im(31\text{-mer})/Ru^{III}(NH_3)_5(31\text{-mer})]$$

$$[Ru^{III}(bpy)_2Im(31\text{-mer})/Ru^{II}(NH_3)_5(31\text{-mer})] + Q^- \xrightarrow{k_{rec}} [Ru^{II}(bpy)_2Im(31\text{-mer})/Ru^{II}(NH_3)_5(31\text{-mer})] + Q$$

The metallohomodimers $[Ru(bpy)_2Im(31\text{-mer})]_2$ and $[Ru(NH_3)_5(31\text{-mer})]_2$ (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 $[Ru(bpy)_2Im(31\text{-mer})]_2$ shows maxima at $\lambda_{abs} = 203$, 245, 290, 340, and 486 nm, which is similar to that of $[Ru(bpy)_2Im(His)]$. The emission properties of this homodimer ($\lambda_{em} = 688$ nm, $\tau = 79$ ns in H_2O) are nearly identical with those of $Ru(bpy)_2Im$-cyt *c*.[14] The cyclic voltammogram of $[Ru(NH_3)_5(31\text{-mer})]_2^{3+}$ shows a single reduction at $E^0 = +0.078$ V vs NHE, which is identical with that reported for $Ru(NH_3)_5$-modified cyt *c*.[12] The ET heterodimer (Figure 1b) $[Ru(bpy)_2Im(31\text{-mer})/Ru(NH_3)_5(31\text{-mer})]$ 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 $Q = Ru^{III}(NH_3)_6$.[14] In a preliminary control experiment, photoexcitation of a solution of the $[Ru^{II}(bpy)_2Im(31\text{-mer})]_2$ homodimer (21 μM) and $Ru^{III}(NH_3)_6$ (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 $k_{rec} = 2 \times 10^8$ M^{-1} 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 $k_{et} = 3.5 \times 10^3$ 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 $[Ru^{III}(bpy)_2Im(31\text{-mer})]_2$ 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-transfer reaction occurring across the peptide−peptide interface over a distance of ca. 23 Å.

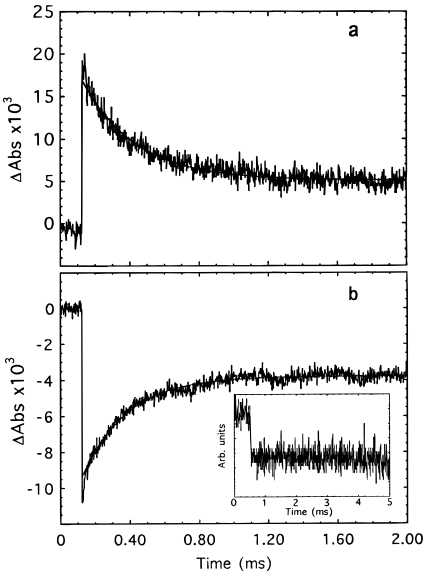

Figure 3. Transient absorption kinetics obtained after photolysis (7 ns laser pulse, Nd:YAG) of a solution of the $[Ru(bpy)_2Im(31\text{-mer})]_2$ homodimer, $[Ru(NH_3)_5Im(31\text{-mer})]_2$ homodimer, and $[Ru(bpy)_2Im(31\text{-mer})/Ru(NH_3)_5(31\text{-mer})]$ heterodimer (total peptide concentration = 64 μM) in the presence of a 400-fold excess of $Ru^{III}(NH_3)_6$ at (a) 306 and (b) 480 nm. The solid lines are fits to a single exponential decay expression in which $k_{et} = 3.5 \times 10^3$ s^{-1}. Inset: Results of the flash-quench experiment performed on the $[Ru(bpy)_2Im(31\text{-mer})]_2$ homodimer as described in the text.

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 $[Ru(bpy)_2Im(31\text{-mer})]_2$ homodimer following flash quench (2 pages). See any current masthead page for ordering and internet access instructions.

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(13) Indeed, the metallohomodimers exhibit a slightly higher CD melting temperature ($T_m = 70$ °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.

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(16) Under these dilute conditions, laser excitation generated $< 10^{-6}$ 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 ($k = k_{et} = 3.8 \times 10^3$ s^{-1}) was seen, followed by a slower, second-order process having a bimolecular rate constant of $k = k_{rec} = 3 \times 10^8$ M^{-1} s^{-1}.