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

Received March 13, 1997

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).

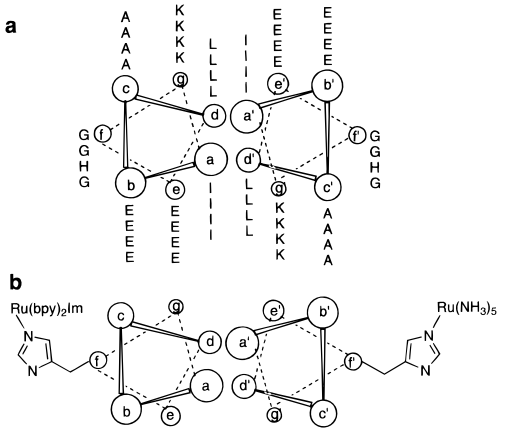

Figure 1. (a) Helical wheel diagram of the dimeric 31-mer polypeptide. A single histidine residue has been incorporated into the most solvent-exposed position of the third heptad repeat. Residues 1, 30, and 31 are omitted from the diagram. (b) Schematic view of the third heptad repeat of the ET heterodimer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

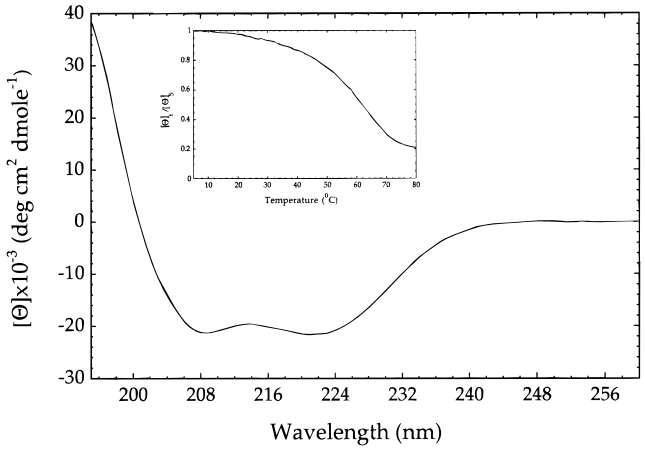

Figure 2. Circular dichroism spectrum of the FMOC-protected 31-mer apodimer (73 μM in 50 mM phosphate buffer, pH 7, 25 °C). Inset: Molar ellipticity at 222 nm measured as a function of temperature. The values are normalized to that observed at 5 °C.

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.

(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.; Millller, 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.

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

S0002-7863(97)00814-7 CCC: $14.00

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.

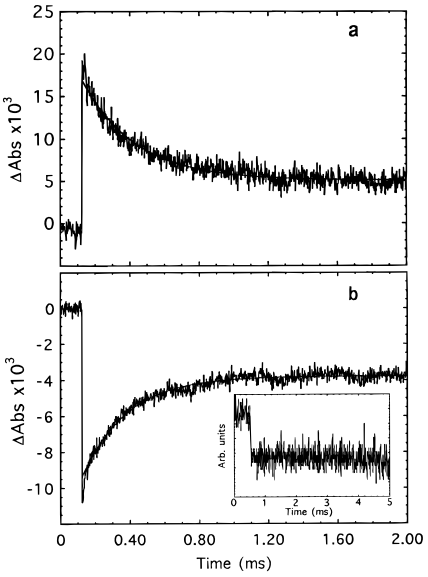

Figure 3. Transient absorption kinetics obtained after photolysis (7 ns laser pulse, Nd:YAG) of a solution of the $[Ru(bpy)_2Im(31\text{-mer})]_2$ homodimer, $[Ru(NH_3)_5Im(31\text{-mer})]_2$ homodimer, and $[Ru(bpy)_2Im(31\text{-mer})/Ru(NH_3)_5(31\text{-mer})]$ heterodimer (total peptide concentration = 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.

Acknowledgment. The authors thank Professor G. S. Bullerjahn for stimulating discussions and assistance in conducting the electrophoresis experiments. Professor M. A. J. Rodgers is also thanked for use of the laser facilities at the BGSU. Ms. Robin Lasey and Dr. D. Y. Chen are acknowledged for their help with the molecular modeling program. This work was partially supported by the National Science Foundation through Grant No. CHE-9307791. The circular dichroism spectrometer was purchased through NSF Grant No. BIR-9208356.

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.

JA970814O

(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.; Gardineer, 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 ($T_m = 70$ °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-Jy.; 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.

(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}.