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

Gennady V. Kozlov

Michael Y. Ogawa

Bowling Green State University, mogawa@bgsu.edu

Follow this and additional works at: https://scholarworks.bgsu.edu/chem_pub

Part of the Chemistry Commons

How does access to this work benefit you? Let us know!

Repository Citation
https://scholarworks.bgsu.edu/chem_pub/161

This Article is brought to you for free and open access by the College of Arts and Sciences at ScholarWorks@BGSU. It has been accepted for inclusion in Chemistry Faculty Publications by an authorized administrator of ScholarWorks@BGSU.
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 vitro 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 artificial 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 “e” and “g”.7,8 Hydrophilic amino acids occupy positions “e” and “g”.7,8 Hydrophobic 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 fluorenlymethoxycarbonyl 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

\[
(\text{I-E-A-L-E-G-K})-\text{C}^\prime-\text{G}-\text{OH} \quad (\text{I})
\]

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).

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(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 μM in 50 mM phosphate buffer, pH 7, 25 ℃). Inset: Molar ellipticity at 222 nm measured as a function of temperature. The values are normalized to that observed at 5 ℃.

Figure 2 shows the circular dichroism (CD) spectrum of the 31-mer, which consists of a positive signal at 195 nm (θ = +39 000 deg cm² dmol⁻¹) and a pair of negative signals at 208 (θ = −21 800 deg cm² dmol⁻¹) and 222 nm (θ = −22 000 deg cm² dmol⁻¹) indicating that the peptide is >69% α-helical.10 The value of [θ₁₉₅]/[θ₂₂₂] = 1.01 is characteristic of an α-helical coiled-coil.5 In contrast, single α-helices have values of [θ₂₂₂]/[θ₁₉₅] = 0.86.8 The noncovalent assembly is very stable, displaying a noncooperative melting curve with Tₘ = 65 ℃ (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.

The metallohomodimers [Ru(bpy)_2Im(31-mer)]_2 and [Ru(NH_3)_5(31-mer)]_2 (bpy = 2,2'-bipyridine, Im = imidazole) were prepared by methods described previously. Significantly, metalation of the 31-mer does not alter the CD spectrum of the peptide. The absorption spectrum of [Ru(bpy)_2Im(31-mer)]_2 shows maxima at 390, 295, 340, and 486 nm, which is similar to that of [Ru(bpy)_2Im(His)]. The emission properties of the oxidized ruthenium polypyridyl complex. Under these conditions, recombination with the reduced quencher occurred with a second-order rate constant of k_{rec} = 3.5 × 10^5 M\(^{-1}\) s\(^{-1}\). 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.

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-mer)]_2 homodimer following flash quench (2 pages). See any current masthead page for ordering and internet access instructions. JAG70814O

(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.