Photoinduced Electron Transfer

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Evidence That a Miniature Cu^I Metalloprotein Undergoes Collisional Electron Transfer in the Inverted Marcus Region**

*Jing Hong, Olesya A. Kharenko, Jiufeng Fan, Fei Xie, Amy K. Petros, Brian R. Gibney, and Michael Y. Ogawa**

The field of protein design seeks to develop synthetic proteins with chemical functions that can be tailored and distinct from those found in natural systems.[1] Much work has thus been directed towards the "bottom-up" design of new proteins that can perform unique chemical tasks[2] and the "top-down" re-engineering of native proteins to enable them to perform new chemical functions.[3] This communication builds on recent work from our group[4–6] to provide evidence that the introduction of a luminescent Cu^I cluster into the hydrophobic interior of a synthetic protein allows collisional photoinduced electron-transfer (ET) reactions to occur in the inverted Marcus region. Whereas the observation of decreasing ET rates with increasing driving force has long been observed in unimolecular,[7] or otherwise diffusionally restricted donor/acceptor systems,[8,9] such behavior has rarely been seen when the donor and acceptor complexes are allowed to diffuse freely in solution.[10,11]

Our group recently reported[5,6] the metal-binding properties of the 32-residue polypeptide C16C19-GGY whose sequence is based on the $(IEALEGK)_n$ heptad repeat previously shown to self-assemble into α-helical coiled coils.[12,13] However, the third heptad repeat of C16C19-GGY was modified by substituting cysteine residues at positions 16 and 19 of the sequence to construct a potential metal-binding site within the hydrophobic core of the coiled coil. It was noted that these apparently simple substitutions were structurally invasive, as the apopeptide exists as a disordered random coil. However, the addition of Cu^I resulted in the self-assembly of a four-stranded coiled coil that encapsulates a cyclic $Cu^I_4S_4(N/O)_4$ cofactor in which each Cu^I atom is trigonally coordinated to two bridging cysteine residues and a terminal nitrogen or oxygen ligand (Figure 1).[5]

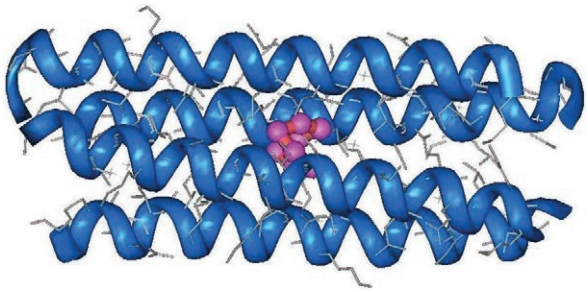

Figure 1. Proposed model of the Cu^I adduct of C16C19-GGY. The metalloprotein exists as a four-stranded helical bundle that contains a cyclic $Cu^I_4S_4(N/O)_4$ cofactor.

The work described herein exploits the fact that Cu^I/C16C19-GGY in aqueous solution displays intense room-temperature luminescence[5] similar to that of multinuclear Cu^I thiolate clusters in both small molecule systems[14] and native metalloproteins.[15] The decay of this emission can be accurately fit to a sum of two single exponential functions [Eq. (1)]

$$I(t) = A_S \exp(\tau_S^{-1} t) + A_L \exp(\tau_L^{-1} t) \quad (1)$$

in which A_S, τ_S, A_L, τ_L are the amplitudes and emission lifetimes of the shorter and longer emission components, respectively (inset, Figure 2). The origin of these two lifetime components is not known and is currently under investigation.

In the absence of quencher, a nonlinear least-squares fit of the data to Equation (1) yields values of $\tau_S = 1.02 \pm 0.01$ μs, $\tau_L = 7.75 \pm 0.02$ μs, and $A_S/A_L = 1.0$. The addition of $[Ru(NH_3)_6]^{3+}$ shortens the emission lifetime of the metalloprotein (inset, Figure 2). Importantly, the emission decay remains biexponential in the presence of this and other quenchers (Table 1), and the data can be fit to Equation (1) to yield

[*] J. Hong, O. A. Kharenko, J. Fan, F. Xie, Prof. M. Y. Ogawa
Department of Chemistry and
Center for Photochemical Sciences
Bowling Green State University
Bowling Green, OH 43403 (USA)
Fax: (+1) 419-372-9809
E-mail: mogawa@bgsu.edu
A. K. Petros, Prof. B. R. Gibney
Department of Chemistry
Columbia University
New York, NY 10027 (USA)

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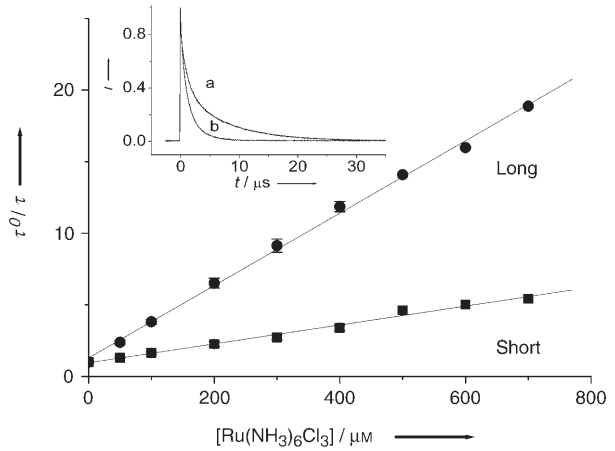

Figure 2. Stern–Volmer plot for the emission quenching of ca. 25 μM Cu^{I}/C16C19-GGY by $[Ru(NH_3)_6]^{3+}$ in 0.2 M acetate buffer (pH 5.5) at 25 °C. Error bars are standard deviations from the mean of triplicate data points and are mostly within the symbols. Inset: Emission lifetime traces of the Cu^{I} adduct of C16C19-GGY taken in the absence (a) and presence (b) of 0.1 mM $[Ru(NH_3)_6]^{3+}$. Solid lines are fits of the data to Equation (1) as described in the text.

Table 1: Electron-transfer driving forces and observed bimolecular quenching constants.

Quencher[a]	$E(Q/Q^-)$[b] [mV vs. NHE]	ΔG [eV]	k_q(short) [10^9 M^{-1}s^{-1}]	k_q(long) [10^9 M^{-1}s^{-1}]
$[RuA_5Cl]^{2+}$	−40	−1.66	3.27 ± 0.14	2.53 ± 0.09
$[RuA_6]^{3+}$	60	−1.76	5.86 ± 0.21	3.47 ± 0.05
$[RuA_5(lut)]^{3+}$	255	−1.95	4.43 ± 0.06	2.44 ± 0.08
$[RuA_5(py)]^{3+}$	300	−2.00	2.46 ± 0.07	1.36 ± 0.05
$[RuA_5(nic)]^{3+}$	353	−2.05	3.46 ± 0.04	1.96 ± 0.06
$[RuA_5(dmpd)]^{3+}$	392	−2.09	1.71 ± 0.21	0.96 ± 0.08

[a] A = NH_3; lut = 3,5-lutidine; py = pyridine; nic = nicotinamide; dmpd = dimethyl 3,5-pyridine dicarboxylate. [b] Determined by cyclic voltammetry in 0.2 M acetate buffer (pH 5.4).

values of $A_S/A_L = 1.0$ under all conditions studied. Thus, the two lifetimes of Cu^{I}/C16C19-GGY were analyzed separately according to the Stern–Volmer equation [Eq. (2)], in which τ^0

$$\tau^0/\tau = 1 + k_q\,\tau^0[Q] \qquad (2)$$

and τ are the emission lifetimes of the short or long emission component measured in the absence or presence of the quencher (Q), respectively, and k_q is the rate constant for the bimolecular quenching reaction. Figure 2 shows that the data for the quenching of both the short and long emission components of Cu^{I}/C16C19-GGY follow linear Stern–Volmer behavior with unity intercepts. Thus, the emission quenching arises from a purely collisional mechanism, and no evidence is seen for a ground-state protein–quencher complex. Analysis of the data obtained for $[Ru(NH_3)_6]^{3+}$ as quencher yields values of $k_q(\text{short}) = (5.86 \pm 0.21) \times 10^9\,\text{M}^{-1}\text{s}^{-1}$ and $k_q(\text{long}) = (3.47 \pm 0.05) \times 10^9\,\text{M}^{-1}\text{s}^{-1}$.

Linear Stern–Volmer behavior is also observed when the different members of a related series of ruthenium(III) ammine complexes ($[Ru(NH_3)_5L]^{n+}$; Table 1) are used to quench the emission of the Cu^{I} protein. Significantly, when L = pyridine or 3,5-lutidine, the emission quenching is accompanied by an increased transient absorption at 400 nm corresponding to the MLCT bands of the reduced $[Ru(NH_3)_5L]^{2+}$ species (inset, Figure 3). The results thus indicate that the emission of Cu^{I}/C16C19-GGY is quenched through a bimolecular electron-transfer reaction.

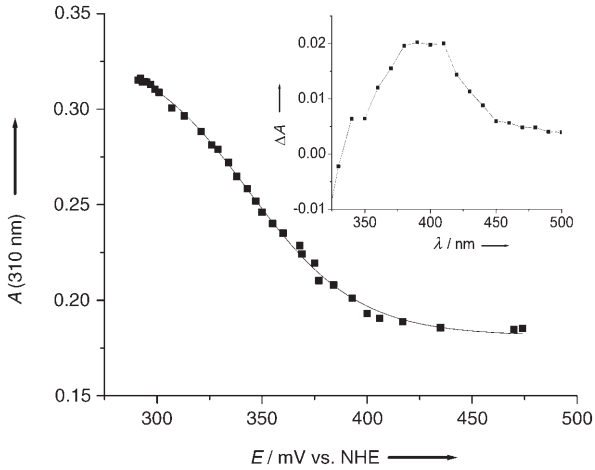

Figure 3. Redox potentiometry of Cu^{I}/C16C19-GGY performed in 0.2 M acetate buffer (pH 5.5) under anaerobic conditions at 25 °C. Inset: Transient absorption spectrum of a solution of Cu^{I}/C16C19-GGY (83 μM) and $[RuA_5(lut)]^{3+}$ (330 μM) measured 20 μs after the laser flash.

The one-electron reduction potential of the Cu^{I}/C16C19-GGY metalloprotein was determined by solution redox potentiometry[16] using $K_2[IrCl_6]$ as an oxidant. Figure 3 plots the absorbance at 310 nm versus the measured solution potential and shows a fit to the Nernst equation ($n = 1$) which yields a single midpoint potential of 343 ± 15 mV versus the normal hydrogen electrode (NHE). Similar results were obtained when the metalloprotein oxidation was monitored by the loss of emission at 600 nm (not shown).

The free-energy change (ΔG) for electron transfer from the Cu^{I}/C16C19-GGY photoexcited donor to the aqueous ruthenium ammine quenchers is calculated by Equation (3)[17], in which $E(Cu^{II}/Cu^{I})$ is the reduction potential of

$$\Delta G = E(Cu^{II}/Cu^{I}) - E(Q/Q^-) - E_{0\text{-}0} \qquad (3)$$

the metalloprotein, $E(Q/Q^-)$ is the reduction potential of the quencher, and $E_{0\text{-}0} = 2.04$ eV (610 nm) is the energy of the excited state as determined by the emission spectrum of Cu^{I}/C16C19-GGY at 77 K measured in 50 % v/v glycerol in 0.1 M acetate buffer (pH 5.4). Table 1 lists the ΔG values and observed bimolecular quenching constants for the reactions studied. Overall, the values of k_q exhibit a greater than threefold decrease with increasingly negative values of ΔG.

Marcus theory[18] describes how the rates of electron-transfer reactions are controlled by the interplay between the thermodynamic driving force ($-\Delta G^0$) and reorganization energy (λ) for the reaction. Equation (4) predicts that

$$k_{ET} = \sqrt{\frac{4\pi^3}{h^2 \lambda R T}} H_{DA}^2(r) \exp\left\{-\frac{(\Delta G^0 + \lambda)^2}{4\lambda R T}\right\} \quad (4)$$

electron-transfer rates should increase with increasing driving force, reach a maximum value determined by the magnitude of the electronic coupling matrix element (H_{DA}) when $-\Delta G = \lambda$, and then decrease with further increases in driving force as the system enters the Marcus inverted region.

Although numerous examples of inverted Marcus behavior exist for unimolecular ET reactions,[7] there are only a few reports of such behavior for forward charge-separation reactions involving a collisional mechanism.[10,11] Bimolecular ET processes typically occur in a series of steps [Eqs. (5)–(7)]

$$D + A \underset{k_{-d}}{\overset{k_{+d}}{\rightleftharpoons}} [DA] \quad (5)$$

$$[DA] \xrightarrow{k_{ET}} [D^+A^-] \quad (6)$$

$$[D^+A^-] \underset{k'_{+d}}{\overset{k'_{-d}}{\rightleftharpoons}} D^+ + A^- \quad (7)$$

consisting of 1) the formation of a diffusional encounter complex [Eq. (5)]; 2) a unimolecular electron-transfer event within this complex [Eq. (6)]; and 3) the subsequent dissociation of the successor complex [Eq. (7)]. Under the steady-state approximation, Equation (8) is obtained for the

$$k_{obs} = k_q = \frac{k_{+d}\, k_{ET}}{k_{-d} + k_{ET}} \quad (8)$$

observed second-order rate constant for electron transfer. Thus, in cases of relatively slow electron-transfer reactions ($k_{-d} \gg k_{ET}$), the expression simplifies to $k_{obs} = K_{eq} k_{ET}$, in which $K_{eq} = k_{+d}/k_{-d}$ is the equilibrium constant for the formation of the diffusional encounter complex. However, at higher driving forces, the magnitude of k_{ET} will increase according to Equation (4), and when $k_{ET} \gg k_{-d}$, the reaction will become diffusion-controlled ($k_{obs} = k_d$). Under these conditions, the values of k_{obs} will remain invariant with increases in driving force, thus prohibiting the observation of inverted Marcus behavior. Such is typically the case for bimolecular ET reactions occurring at high driving force.

The observed free-energy dependence of k_q(short) and k_q(long) can be interpreted in terms of inverted Marcus behavior given the reasonable assumptions that the values of K_{eq} remain relatively constant throughout this series of reactions and that the related ruthenium quenchers bind to the Cu^I protein in a similar manner. Figure 4 shows how the values of k_q(short) and k_q(long) decrease with increasing driving force. Fits of the data to Equation (4) yield values of $\lambda = 1.7 \pm 0.1$ eV and $H_{DA} = 4.8 \pm 0.4$ cm^{-1} for the short component, and $\lambda = 1.7 \pm 0.1$ eV and $H_{DA} = 3.9 \pm 0.4$ cm^{-1} for the long component. The source of the scatter in the data is not presently known but, in light of the previous assumptions, might reflect small differences in either the K_{eq} values or relative binding sites of the different acceptors.[19]

Whereas interpretation of H_{DA} is problematic because the equilibrium constant is unknown, further insights into the redox properties of the Cu^I metalloprotein can be obtained by a cross-relation[18] analysis of the λ values obtained above. Assuming $\lambda_{11} = 1.2$ eV for the ruthenium pentaammine acceptors,[20] a value of $\lambda_{22} = 2.2$ eV is obtained for Cu^I/C16C19-GGY which is very similar to that reported for $[Cu(phen)_2]^{2+/+}$ ($\lambda = 2.4$ eV).[21] These results suggest that one-electron oxidation causes a significant conformational change of the $Cu^I_4S_4(N/O)_4$ cluster.

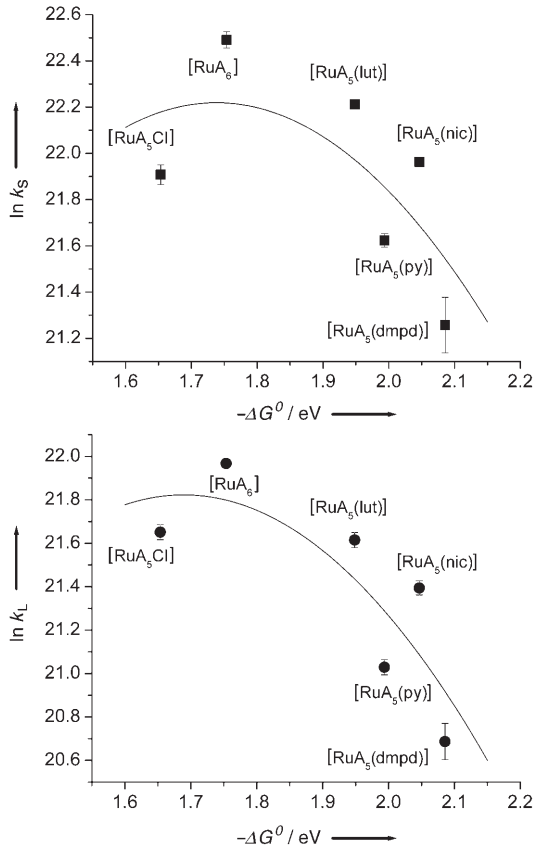

Figure 4. Free-energy plot of k_{obs} values for the short (top) and long (bottom) components to the emission lifetime. Error bars represent the deviation from the mean of triplicate experiments. The solid lines are fits of the data to Equation (4).

In summary, evidence is presented to indicate that the miniature Cu^I/C16C19-GGY metalloprotein undergoes collisional photoinduced electron transfer to a variety of ruthenium(III) acceptors in solution. Inverted Marcus behavior is observed as the buried $Cu^I_4S_4(N/O)_4$ cofactor has a very high reorganization energy and experiences weak electronic coupling to the aqueous acceptors. This latter effect might be due to the positioning of the cofactor within the hydrophobic core of the protein which prohibits close approach between the donor and acceptor to lower the value of H_{DA}.

Experimental Section

The peptide C16C19-GGY and its Cu^I derivative were prepared as previously described.[5,12] Emission-lifetime measurements[22] were performed on argon-saturated solutions containing 100 μM $[Cu(CH_3CN)_4]^+$, 100 μM C16C19-GGY, and 0–700 μM $[Ru(NH_3)_5L]^{n+}$ (L = Cl, NH_3, 3,5-lutidine, nicotinamide, pyridine, and dimethyl 3,5-pyridinedicarboxylate) dissolved in 0.2 M acetate buffer (pH 5.4). The concentration of the C16C19-GGY stock solutions (ca. 200 μM in 0.2 M acetate buffer) were determined by UV/Vis spectroscopy by

using a value of $\varepsilon_{275} = 1450\,\text{M}^{-1}\text{cm}^{-1}$. These solutions were freshly prepared and stored under argon before each experiment. The concentrations (0.01–0.05 M) of the stock solutions of the $[Ru(NH_3)_5L]^{3+}$ (L = 3,5-lutidine, nicotinamide, pyridine, and dimethyl 3,5-pyridinedicarboxylate) quenchers were obtained by reducing the sample with excess ascorbic acid and measuring the absorption of their MLCT bands. The concentrations (0.01–0.05 M) of the stock solution of the $[Ru(NH_3)_5L]^{n+}$ (L = Cl and NH_3) quenchers were measured directly by UV/Vis spectroscopy. The $[Cu(CH_3CN)_4]PF_6$ stock solution (0.01 M) in acetonitrile was prepared anaerobically by syringe techniques. In the presence of metallopeptide, the concentration of the higher-potential quenchers was corrected for ground-state reduction as determined by measuring the MLCT absorption bands.

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