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Gated Electron Transfer as a Probe of the Configurational Dynamics of Peptide−Protein Complexes

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We recently reported on the electron-transfer (ET) reactions that occur between a small, negatively charged metallopeptide, [Ru(bpy)$_2$(phen-am)-Cys-(Glu)$_5$-Gly]$^{3-}$ = **RuCE$_5$G**, and ferricytochrome *c* = Cyt *c*, in which an acetamido linker was used to attach the ruthenium polypyridyl complex to the cysteine side chain of the peptide (Figure 1).[1] It was demonstrated that photoinduced electron transfer occurs via parallel pathways that involve the existence of a preformed peptide−protein complex in one case, and the formation of a transient excited-state encounter complex in the other. It was further shown that the rates of both intracomplex ET reactions decrease with increasing solvent viscosity, demonstrating that their kinetics are gated[2−5] by rate-limiting configurational changes[6] occurring within their respective complexes.

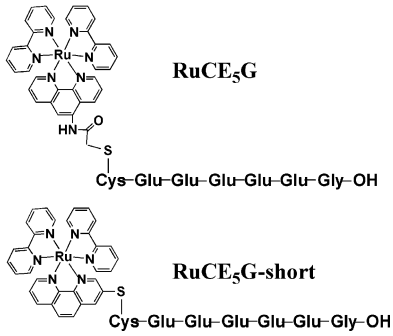

Figure 1. Metal peptides **RuCE$_5$G**, and **RuCE$_5$G-short** which differ by the method of attaching the ruthenium center to the cysteine side chain.

The current work uses gated ET measurements to demonstrate how a small modification of the metal peptide can produce significant changes in the dynamics of its preformed complex. Thus, a ruthenium polypyridyl complex was directly coupled to the CE$_5$G peptide by reacting [(bpy)$_2$Ru(3-bromo-1,10-phenanthroline)](PF$_6$)$_2$[7] with the apopeptide to yield the compound, **RuCE$_5$G-short** (Figure 1). The metallopeptide was purified by reverse-phase HPLC, and its identity was confirmed by ESI-MS (*m*/*z*: calcd for [M − H]$^+$ 1414.4, obsd, 1414.4; calcd for [M]$^{2+}$ 707.7, obsd, 707.8). Emission lifetime measurements show that the triplet state of **RuCE$_5$G-short** decays via first-order kinetics with a rate constant of $k_0 = (8.09 \pm 0.03) \times 10^5$ s^{-1} in 0.5 mM phosphate buffer at 298 K (pH 7). However, when measured in the presence of Cyt *c*, the emission becomes biphasic (eq 1). It is seen that the decay of the shorter-

$$I(t) = A_S \exp(-k_S t) + A_L \exp(-k_L t) \qquad (1)$$

lived component (k_S) is independent of Cyt *c* concentration, having a value of $k_S = (4.07 \pm 0.09) \times 10^6$ s^{-1} at 298 K, and that the value for the longer component (k_L) increases with increasing concentrations of Cyt *c*, saturating at higher protein concentrations. This behavior is qualitatively similar to that previously reported

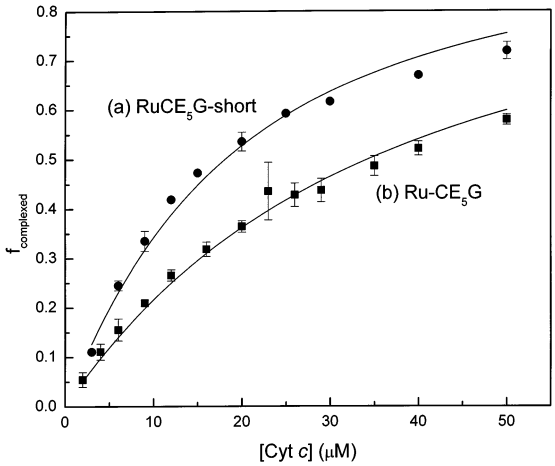

Figure 2. Fractional population of bound (a) **RuCE$_5$G-short** and (b) **RuCE$_5$G** as a function of total Cyt *c* concentration. The solid line represents the fit to eq 2. The error bars reflect the standard deviation of results obtained from the average of three independent experiments taken at 298 K.

for **RuCE$_5$G** in which excited-state electron transfer was shown to occur within both the preformed and encounter peptide−protein complexes.[1] The data collected for the preformed complex with **RuCE$_5$G-short** and Cyt *c* (298 K) shows that the intracomplex ET rate constant is $k_{ET} = (k_S - k_0) = (3.3 \pm 0.1) \times 10^6$ s^{-1}, which is within experimental error of that previously reported for **RuCE$_5$G**.[1]

The binding constant for the preformed complex was determined by using the values of A_S and A_L to calculate the fraction of **RuCE$_5$G-short** that is bound to the protein, $f = (A_S)/(A_S + A_L)$,

$$f = \frac{1/K_b + [\mathrm{Ru}]_0 + [\mathrm{Cyt}] - \sqrt{(1/K_b + [\mathrm{Ru}]_0 + [\mathrm{Cyt}])^2 - 4[\mathrm{Ru}]_0[\mathrm{Cyt}]}}{2[\mathrm{Ru}]_0} \qquad (2)$$

and fitting the data to eq 2 which describes a 1:1 binding isotherm in which K_b is the equilibrium binding constant and $[Ru]_0$ is the initial concentration of the ruthenium peptide. The data obtained at 298 K yield $K_b = (6.8 \pm 0.3) \times 10^4$ M^{-1} which is 2-fold greater than that previously reported for the complex involving the isoionic **RuCE$_5$G** peptide (Figure 2).

The behavior of the transient encounter complex was characterized by fitting the concentration dependence of k_L to eq 3 where $[\mathrm{Cyt\ c}]_{free}$ is the concentration of Cyt *c* that is free in solution, k_{ET}' is the rate constant for electron transfer occurring within the encounter complex, and K_b' is the binding constant for encounter complex. The data obtained at 298 K in 0.5 mM phosphate buffer, yield $k_{ET}' = (9.3 \pm 0.6) \times 10^5$ s^{-1} and $K_b' = (3.1 \pm 0.8) \times 10^4$

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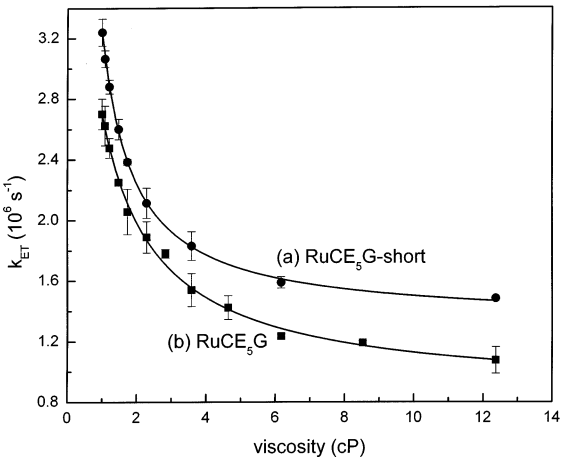

Figure 3. Viscosity dependence of intracomplex electron-transfer rate constants for (a) **$RuCE_5G$-short** and (b) **$RuCE_5G$**. The solid lines indicate the fit of the data to eq 4. The error bars reflect the standard deviation from the average of four independent experiments taken at 298 K.

M^{-1} which are within experimental error to the values reported for the **$RuCE_5G$** complex.[1]

$$k_L = k_0 + \frac{k'_{ET} K'_b [\text{Cyt c}]_{free}}{1 + K'_b [\text{Cyt c}]_{free}} \qquad (3)$$

The dynamics of the preformed complex were first studied by examining the temperature dependence of k_{ET} which gave $\Delta S^{\ddagger} = -94 \pm 2$ J K^{-1} mol^{-1} and $\Delta H^{\ddagger} = 8.3 \pm 0.5$ kJ mol^{-1}. The observation of a negative activation entropy suggests that formation of the transition state may require a reorientation of the complex prior to the electron-transfer event.[6] To investigate this possibility, values of k_{ET} were measured at different solvent viscosities obtained by the addition of sucrose to the buffer solution with care taken to maintain a constant ionic strength (Figure 3). Under these conditions, the emission of **$RuCE_5G$-short** could still be fit to eq 1, and it was verified that the fractional amplitude of the fast component remained unchanged by the addition of sucrose. The data in Figure 3 show that k_{ET} decreases with increasing solvent viscosity to prove that the electron-transfer reaction is indeed gated. The dynamics of the preformed complex was further studied by fitting the viscosity data to eq 4

$$k_{ET} = \frac{k_B T(1+\sigma)}{h(\eta+\sigma)} \exp(-\Delta G^{\ddagger}/RT) + k_{\infty} \qquad (4)$$

where η is the solution viscosity determined from tables,[8,9] σ is the internal protein friction, and k_{∞} is the rate constant at infinite viscosity where configurational motions are prohibited.[10−12] The data for the preformed **$RuCE_5G$-short** complex yield values of $\sigma = -0.08 \pm 0.03$ cP, $\Delta G^{\ddagger} = 37.10 \pm 0.02$ kJ mol^{-1}, and $k_{\infty} = (1.3 \pm 0.1) \times 10^6$ s^{-1} (Table 1), and it is noted that the value of $\Delta G^{\ddagger}$ so obtained is consistent with that measured from the Eyring plot ($\Delta G^{\ddagger} = 36.3$ kJ mol^{-1}). For comparison, Figure 3 also shows the results from the preformed complex involving the **$RuCE_5G$** peptide, for which $\sigma = 0.6 \pm 0.1$ cP, $\Delta G^{\ddagger} = 37.0 \pm 0.06$ kJ mol^{-1}, and $k_{\infty} = (8.5 \pm 0.4) \times 10^5$ s^{-1}. Again, the value obtained for the activation free energy was consistent with that measured from the temperature dependence data ($\Delta G^{\ddagger} = 36.0$ kJ mol^{-1}).

Comparison of the internal viscosity values obtained for the two preformed complexes (Table 1) indicates that the absence of the acetamido linker in **$RuCE_5G$-short** results in a negligible value for σ, showing that it forms a more dynamic preformed complex.[13]

Table 1. Results of Internal Viscosity Fits[a]

	$RuCE_5G$-short		$RuCE_5G$	
	preformed	encounter	preformed	encounter
σ (cP)	-0.08 ± 0.03	0.06 ± 0.20[b]	0.60 ± 0.10	-0.02 ± 0.15
$\Delta G^{\ddagger}$ (kJ mol^{-1})	37.10 ± 0.02	40.10 ± 0.02	37.0 ± 0.1	40.70 ± 0.01
k_{∞} (10^6 s^{-1})	1.3 ± 0.1	0.21 ± 0.01	0.85 ± 0.04	0.22 ± 0.04

[a] 298 K. [b] Fit of the data to eq 4 constraining $\sigma = 0$ gives $\Delta G^{\ddagger} = 40.1 \pm 0.1$ kJ mol^{-1} and $k_{\infty} = (0.21 \pm 0.1) \times 10^6$ s^{-1}.

The dynamics of the transient encounter complex involving both **$RuCE_5G$-short** and **$RuCE_5G$** were also studied by fitting the values obtained for k'_{ET} at different viscosities to eq 4 (Supporting Information). Table 1 shows that both types of encounter complexes exhibit negligible internal viscosities and identical values for $\Delta G^{\ddagger}$ and k_{∞}. These results likely reflect the highly dynamic nature of encounter complexes which is not sensitive to small changes of the peptide.

Molecular modeling of the **$RuCE_5G$-short** and **$RuCE_5G$** peptides indicates that they may adopt different conformations. Whereas the short peptide has a roughly linear rodlike geometry, the flexible acetamido linker of **$RuCE_5G$** allows it to form a hairpin-like structure in which the bulky ruthenium polypyridyl cation is placed in closer proximity to the negatively charged glutamate chain. It is speculated that this may lead to the different internal viscosities and binding constants observed for their respective preformed complexes: the higher mobility of the **$RuCE_5G$-short**:Cyt *c* complex may be due to its rodlike conformation, and the lower binding constant of the **$RuCE_5G$** complex may arise from partial charge compensation occurring between the oppositely charged portions of the metal peptide as they are brought closer together in the hairpin structure. Ongoing work in our laboratory is continuing to study the factors which may control the dynamics of biological complexes.

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Supporting Information Available: Plots showing the viscosity dependence of k_{ET}', and molecular modeling results (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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