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)$_3$-Gly]$^{5-}$ = RuCE$_5$G, and ferricytochrome c = Cyt c, in which an acetamido linker was used to attach the ruthenium polymeric complex to the cysteine side chain of the peptide (Figure 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$^2-^3$ 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 polypyrrole complex was directly coupled to the CE$_5$G peptide by reacting [(bpy)$_2$Ru(3-bromo-1,10-phenanthroline)](PF$_6$)$_2$ with the apoprotein 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]$:^{14}$ 1414.4, obsd, 1414.4; calcd for [M]$^{13}$ 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 ± 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-lived component ($k_S$) is independent of Cyt c concentration, having a value of $k_S$ = (4.07 ± 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.

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 $k_{ET} = (k_S - k_0) = (3.3 ± 0.1) \times 10^5$ 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, $f = (A_S)/A_S + A_L$.

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

and fitting the data to eq 2 which describes a 1:1 binding isotherm in which $K_N$ is the equilibrium binding constant and [Ru]$_0$ is the initial concentration of the ruthenium peptide. The data obtained at 298 K yield $K_N = (6.8 ± 0.3) \times 10^4$ 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 $[\text{Cyt c}]_{iso}$ 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 $K_{r}$ is the binding constant for encounter complex. The data obtained at 298 K in 0.5 mM phosphate buffer, yield $k_{ET}' = (9.3 ± 0.6) \times 10^5$ s$^{-1}$ and $K_r = (3.1 ± 0.8) \times 10^4$.
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 $\Delta H^\ddagger = 8.3 \pm 0.5 \ J \ mol^{-1}$. 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. 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 5 G-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

$$k_L = k_0 + \frac{k_{ET} K_q[Cyt c]_{free}}{1 + K_q[Cyt c]_{free}} \tag{3}$$

where $\eta$ is the solution viscosity determined from tables, $\alpha$ is the internal protein friction, and $k_\infty$ is the rate constant at infinite viscosity where configurational motions are prohibited. The data for the preformed RuCE 5 G-short complex yield values of $\alpha = -0.08 \pm 0.03 \ cP$, $\Delta G^\ddagger = 37.10 \pm 0.02 \ kJ \ mol^{-1}$, and $k_\infty = (1.3 \pm 0.1) \times 10^6 \ s^{-1}$ (Table 1), and it is noted that the value of $\Delta G^\ddagger$ 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 5 G peptide, for which $\alpha = 0.6 \pm 0.1 \ cP$, $\Delta G^\ddagger = 37.0 \pm 0.06 \ kJ \ mol^{-1}$, and $k_\infty = (8.5 \pm 0.4) \times 10^6 \ s^{-1}$. 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 5 G-short results in a negligible value for $\alpha$, showing that it forms a more dynamic preformed complex.

The dynamics of the transient encounter complex involving both RuCE 5 G-short and RuCE 5 G 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 $k_\infty$. 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 5 G-short and RuCE 5 G peptides indicates that they may adopt different conformations. Whereas the short peptide has a roughly linear rodlike geometry, the flexible acetamido linker of RuCE 5 G 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 5 G-short:Cyt c complex may be due to its rodlike conformation, and the lower binding constant of the RuCE 5 G 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.

Table 1. Results of Internal Viscosity Fitsa

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<tr>
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<th>RuCE 5 G-short</th>
<th>RuCE 5 G</th>
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<tbody>
<tr>
<td></td>
<td>preformed</td>
<td>encounter</td>
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<tr>
<td>$\sigma$ (cP)</td>
<td>0.08 ± 0.03</td>
<td>0.06 ± 0.20</td>
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<tr>
<td>$\Delta G^\ddagger$ (kJ mol$^{-1}$)</td>
<td>37.10 ± 0.02</td>
<td>40.10 ± 0.02</td>
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<tr>
<td>$k_\infty$ ($10^6 \ s^{-1}$)</td>
<td>1.3 ± 0.1</td>
<td>0.21 ± 0.01</td>
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* 298 K. a Fit of the data to eq 4 constraining $\sigma = 0$ gives $\Delta G^\ddagger = 40.1 \pm 0.1 \ kJ \ mol^{-1}$ and $k_\infty = (0.21 \pm 0.1) \times 10^6 \ s^{-1}$.

References
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