Communications to the Editor

Photoinduced Electron Transfer Along a *β*-Sheet Mimic

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Electron-transfer (ET) reactions comprise the fundamental steps of many important biological processes. To better understand the mechanisms of these reactions, considerable effort has been made toward the study of ET processes that occur within metal-substituted proteins, protein complexes, and artificial polypeptides.[1] Recent effort has focused about the search for protein[2] or peptide-based[3] ET "pathways". It has been suggested that *β*-pleated sheets may provide an efficient route for mediating long-range electronic coupling.[4−6] However, whereas several *β*-sheet models have recently appeared in the literature, none are amenable to electron-transfer studies.[7−11] Here, we describe the structural and ET properties of the first *β*-sheet-bridged donor/acceptor complex which serves as an archetype to permit future investigation of the ability of *β*-sheets to mediate long-range ET reactions.

Preparation of the *β*-sheet ET complex was initiated by reacting equimolar amounts of the symmetric anhydride of [Ru^{II}-(bpy)$_2$L][12] (bpy = 2,2′-bipyridine, L = 3,5-dicarboxy-2,2′-bipyridine) with NH$_2$-Val-Val-OMe in acetonitrile. The reaction was monitored by reverse-phase HPLC, which showed a new ruthenium polypyridyl complex (**I**) appearing at a longer retention time.[13] The binuclear donor/acceptor complex (**II**) was prepared by coupling **I** to [NH$_2$-Val-Val-Co^{III}(NH$_3$)$_5$],[14] which was subsequently shown by ^{1}H NMR to be attached to the 3-carboxy position of L. The mononuclear *β*-sheet mimic containing no electron acceptor (**III**) was prepared as previously described.[10] Circular dichroism spectroscopy and semiprepara-

Table 1. 400 MHz NMR Parameters for Amide Protons of Δ-*l* **II** taken in 3:1 H$_2$O/D$_2$O

residue	1	2	3	4
δ_{298K} (ppm)	9.70	8.90	8.50	8.45
$^3J_{NH−C^\alpha H}$ (Hz)	6.6	7.5	8.0	8.6
dδ/dT (ppb/K)	−7.2	−8.3	−8.0	−5.8
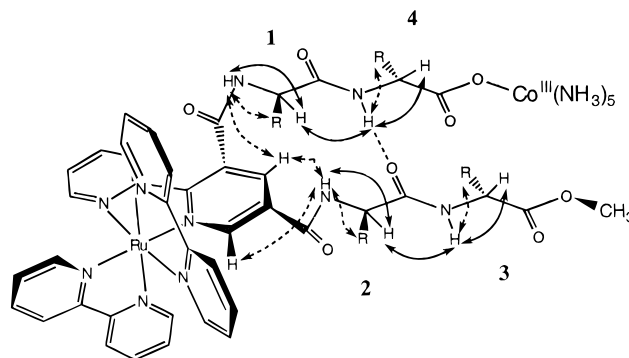
Δ-*l* II

Figure 1. Schematic representation of Δ-*l* **II** including the weak (- - -) and strong (—) NOE interactions observed for the peptide-based protons in 3:1 H$_2$O/D$_2$O (400 MHz, *T* = 10 °C, τ_m = 150 ms, presaturation solvent suppression).

tive C$_{18}$ HPLC were used to identify and separate the Δ-*l* and Λ-*l* diastereomers of **II**.[15] The Δ-*l* **II** was arbitrarily chosen for conformational assignment by 1-D, TOCSY, DQCOSY, and NOESY ^{1}H NMR experiments as performed in H$_2$O/D$_2$O solvents. The one-dimensional spectrum of this compound consists of a single set of sharp, well-defined peaks indicating that this charged (4+) binuclear metallopeptide does not aggregate in aqueous solution but, rather, assumes a single conformation. In contrast, spectra obtained in CD$_3$CN show multiple sets of peptide resonances indicating the presence of multiple conformations in this solvent.

Table 1 summarizes the ^{1}H NMR data used to assign the *β*-sheet conformation to Δ-*l* **II** in aqueous solution.[16] Idealized *β*-sheets exist as extended peptide chains whose amide protons display large backbone coupling constants ($^3J_{NH−C^\alpha H}$ ≥ 7 Hz).[17] The large coupling constant observed for residues 3 and 4 (≥8.0 Hz) show that ϕ = −120° ± 26°, which clearly satisfies the requirement for an extended *β*-strand conformation (ϕ = −119°).[17] The coupling observed for amide 2 (7.5 Hz) is also an acceptable value for this structure. However, the small value of $^3J_{NH−C^\alpha H}$ seen for amide 1 indicates a distortion from the idealized structure. 2-D NOESY was used to confirm the *β*-sheet assignment since they are characterized by close through-space contacts between the C$_i^\alpha$H and N$_{i+1}$H protons of adjacent amino acids ($d_{\alpha N}$ ≤ 2.5 Å).[18] The NOESY spectrum of Δ-*l* **II** clearly shows the existence of strong interresidue cross peaks, in addition to weak intraresidue NOEs (Figure 1). Further conformational data is obtained from the temperature coefficients of the amide proton chemical shifts (dδ/dT). All four amide protons experience a pronounced upfield shift with increasing temperature, indicating that they are in exchange with

(1) For a review, see: Bowler, B. E.; Raphael, A. L.; Gray, H. B. *Prog. Inorg. Chem.* **1990**, *38*, 259.
(2) Regan, J. J.; Risser, S. M.; Beratan, D. N.; Onuchic, J. N. *J. Phys. Chem.* **1993**, *97*, 13083.
(3) (a) Isied, S. S.; Ogawa, M. Y.; Wishart, J. F. *Chem. Rev.* **1992**, *92*, 381. (b) Ogawa, M. Y.; Wishart, J. F.; Young, Z.; Miller, J. R.; Isied, S. S. *J. Phys. Chem.* **1993**, *97*, 11456. (c) Ogawa, M. Y.; Moreira, I.; Wishart, J. F.; Isied, S. S. *Chem. Phys.* **1993**, *176*, 589.
(4) Langen, R.; Chang, I.-J.; Germanas, J. P.; Richards, J. H.; Winkler, J. R.; Gray, H. B. *Science* **1995**, *268*, 1733.
(5) Regan, J. J.; Di Bilo, A. J.; Langen, R.; Skov, L. K.; Winkler, J. R.; Gray, H. B.; Onuchic, J. N. *Chem. Biol.* **1995**, *2*, 489.
(6) de Rege, P. J. F.; Williams, S. A.; Therien, M. J. *Science* **1995**, *269*, 1409.
(7) Tsang, K. Y.; Diaz, H.; Graciani, N.; Kelly, J. W. *J. Am. Chem. Soc.* **1994**, *116*, 3988.
(8) Kemp, D. S.; Bowen, B. R.; Muendel, C. C. *J. Org. Chem.* **1990**, *55*, 4650.
(9) Raj, P. A.; Soni, S. D.; Ramasubbu, N.; Bhandary, K. K.; Levine, M. J. *Biopolymers* **1991**, *30*, 73.
(10) Ogawa, M. Y.; Gretchikhine, A. B.; Datta, S. D.; Davis, S. M. *Inorg. Chem.* **1995**, *34*, 6423.
(11) Nowick, J. S.; Smith, E. M.; Noronha, G. *J. Org. Chem.* **1995**, *60*, 7386.
(12) (a) Fernando, S. R. L.; Ogawa, M. Y. *J. Chem. Soc., Chem. Commun.* In press. (b) Fernando, S. R. L.; Maharoof, U. S. M.; Deshayes, K. D.; Kinstle, T. H.; Ogawa, M. Y. Submitted for publication.
(13) The retention times for [Ru(bpy))$_2$L] and **I** were 6.5 and 19.8 min, respectively, under the following isocratic HPLC conditions: YMC-Pack ODS-AQ C$_{18}$ column (250 × 4.6 mm i.d.); 50% CH$_3$OH in 0.1% HTFA; flow rate = 1 mL/min.
(14) Isied, S. S.; Vassilian, A.; Lyon, J. M. *J. Am. Chem. Soc.* **1982**, *104*, 3910.
(15) Milder, S. J.; Gold, J. S.; Kliger, D. S. *Chem. Phys. Lett.* **1988**, *144*, 269.
(16) Comparison of these data with those reported earlier for **III** (ref 10) shows that the present binuclear metallopeptide has somewhat greater *β*-sheet character.
(17) Pardi, A.; Billeter, M.; Wuthrich, K. *J. Mol. Biol.* **1984**, *180*, 741.
(18) Wuthrich, K.; Billeter, M.; Braun, W. *J. Mol. Biol.* **1984**, *180*, 715.
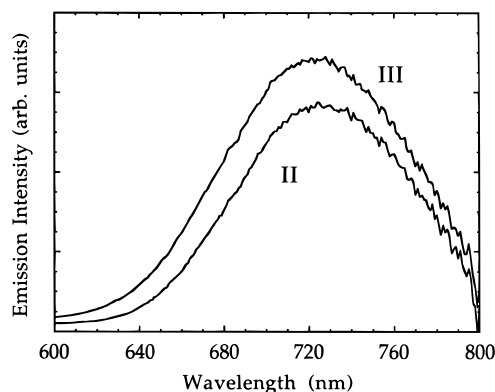
Figure 2. Emission spectra of equimolar samples of **II** and **III** (150 μM) taken in neat D_2O at ambient temperature.

solvent H_2O. However, the lower coefficient for amide 4 ($d\delta/dT = -5.8$ ppb/K) suggests that it is moderately solvent-shielded in a manner suggestive of its participation in an intramolecular hydrogen bond.[19] We note that this residue also displays the largest vicinal coupling constant ($^3J_{NH-C^\alpha H} = 8.6$ Hz).

The driving force for photoinduced ET from the [*Ru(bpy)$_2$L] donor to the [Co^{III}(NH$_3$)$_5$] acceptor of **II** can be calculated according to eq 1.[20] The metal-based oxidation of **III** was

$$\Delta G^\circ = -[E_{1/2}(Co^{III/II}) - E_{1/2}(Ru^{III/II})] - E_{MLCT} \qquad (1)$$

measured as $E^\circ = +1.13$ V vs Ag/AgCl ($E^\circ = +1.34$ V vs NHE) in 0.1 M NaCl, which is similar to that reported for related compounds.[21] Using the estimated values of $E_{MLCT} = +1.7$ V, as taken from the room temperature emission maximum ($\lambda_{em} = 725$ nm in D_2O), and $E_{1/2}(Co^{III/II}) = -0.1$ V vs NHE[22] gives a driving force for photoinduced ET of $\Delta G^\circ = -0.27$ eV.

Figure 2 shows that the steady-state luminescence of **II** is quenched relative to that of the mononuclear species **III**. This can be analyzed in terms of eq 2 to give $k_q(\Phi) = 4.3 \times 10^6$

$$k_q(\Phi) = (\Phi_0/\Phi - 1)1/\tau_0 \qquad (2)$$

s^{-1}, where Φ and Φ_0 are the emission quantum yields of **II** and **III**, respectively, and the emission lifetime of **III** is $\tau_0 = 45 \pm 1$ ns, as measured in D_2O.[23,24] This result is confirmed by emission lifetime measurements from which k_q was calculated according to eq 3 to give $k_q(\tau) = 2.8 \times 10^6$ s^{-1}. In this treatment, $\tau = 40 \pm 1$ ns, which is the observed lifetime of **II**.

$$k_q(\tau) = (1/\tau - 1/\tau_0) \qquad (3)$$

The lifetime results were found to be independent of concentration (50–250 μM), indicating that an intramolecular quenching mechanism obtains. To confirm that k_q represents an electron-transfer rate and is not due to an energy-transfer mechanism, a dilute sample of **II** (50 μM in D_2O) was subjected to steady-state photolysis using a quartz-tungsten lamp. Reverse-phase HPLC of the resulting solution showed no evidence of the binuclear starting material. The lifetime of the resulting photoproduct closely resembled that of the mononuclear ruthenium compound, which indicates that aquation of the reduced cobalt center has occurred.

The photoinduced electron-transfer rate of **II** can be compared to those reported for the polyproline-bridged series, [*Ru(bpy)$_2$-(cmbpy)-Pro$_n$-Co(NH$_3$)$_5$]$^{4+}$ where cmbpy = 4-carboxy-4′-methyl-2,2′-bipyridine and $n = 1-3$.[3a,25] In that series, adjacent proline residues are believed to reside in the polyproline II conformation and, like β-pleated sheets, to exist as extended peptide chains. The excited-state ruthenium complex [*Ru(bpy)$_2$(cmbpy)] is a better reductant by ca. 200 mV than [*Ru(bpy)$_2$L] by virtue of its higher emission energy ($\lambda_{em} = 648$ nm). However, the rate of photoinduced ET for the $n = 2$ proline case was several-fold smaller than that observed for **II** ($k_{ET} = 1.1 \times 10^6$ s^{-1}). The apparent enhancement of k_{ET} for **II** may be attributable to several factors including the intrinsic photophysical properties of the ruthenium donor,[26] the possibility of a slightly shorter through-space distance to the Co(NH$_3$)$_5$ center, or the presence of an efficient coupling pathway along the β-sheet. In order to discriminate between these possibilities, ongoing work in our laboratory seeks to determine the distance dependence of k_{et} in related β-sheet systems.

(19) Imperiali, B.; Fisher, S. L.; Moats, R. A.; Prins, T. J. *J. Am. Chem. Soc.* **1992**, *114*, 3182.

(20) Rehm. D.; Weller, A. *Isr. J. Chem.* **1970**, *8*, 259.

(21) Kelly, L. A.; Rodgers, M. A. J. *J. Phys. Chem.* **1995**, *99*, 13132.

(22) Isied, S. S. *Prog. Inorg. Chem.* **1984**, *32*, 443.

(23) Emission lifetimes are taken from transients collected as an average of five laser shots acquired from two separate samples of material obtained from two separate syntheses. Excitation was achieved using the 532 nm output from a Q-switched Nd:YAG laser (10 ns pulse). In all cases, the decay profiles remained single exponential for at least 8 half-lives.

(24) All emission measurements were performed in D_2O to enhance the emission lifetimes (see ref 12b).

(25) Electron-transfer rates in the oligoproline series in ref 3a were originally reported on the basis of transient emission data. In all cases, these results have been subsequently confirmed by transient absorption experiments (Isied, S., private communication).

(26) Cooley, L. F.; Larson, S. L.; Elliott, C. M.; Kelly, D. F. *J. Phys. Chem.* **1991**, *95*, 10694.