A Chromophore–Quencher-Based Luminescence Probe for DNA

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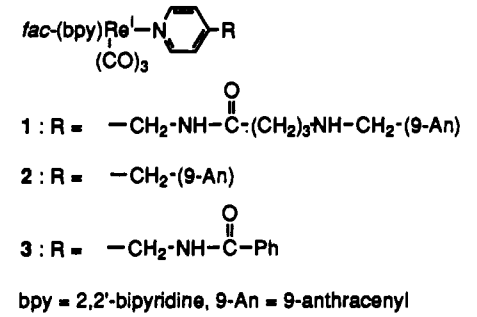
The investigation of intramolecular energy and electron transfer (E_NT and ET, respectively) in chromophore–quencher (C–Q) molecules featuring the *fac*-(b)$Re^I(CO)_3$–L chromophore (b is a bidentate diimine ligand such as 2,2′-bipyridine) covalently linked to either energy acceptor or electron donor/acceptor moieties has been a topic of recent interest.[1,2] Owing to the favorable synthetic and photophysical properties of Re based C–Q compounds, we felt it should be possible to utilize this system as the basis for the rational design of a unique series of spectroscopic and photochemical probes for double-stranded DNA.[3-9] Our approach to the design of C–Q-based DNA probes is to utilize a flexible tether of moderate length to covalently link a Q moiety to the Re complex. The Q moiety is selected such that it binds strongly to the DNA polymer, thereby changing the preferred conformation of the flexible linker, which in turn will influence the through-space and/or through-bond (TS and TB, respectively) electronic coupling between the Re center and Q. Any change in electronic coupling will induce a change in the photophysical properties of the Re chromophore.

The present communication describes the photophysical characterization of C–Q system **1**, which features a Re complex covalently linked to an anthracene (An) quencher unit via a flexible eight-atom spacer. Previous studies show that An (1) quenches the luminescent Re → bpy metal-to-ligand charge transfer (MLCT) excited state via exchange triplet–triplet (TT) E_NT[2] and (2) binds avidly to DNA via intercalation.[10] The present study reveals that MLCT luminescence of **1** undergoes a profound increase in intensity when the complex interacts with DNA in aqueous buffer. The results imply that TS electronic coupling between An and Re is decreased significantly as a result of a change in the preferred conformation of the flexible linker concomitant with intercalation of An into the DNA double helix.

Structures for C–Q complex **1**, C–Q complex **2**, which features a short, inflexible connection between An and Re, and model complex **3** are shown here. All photophysical experiments on these complexes were carried out in aqueous Tris buffer (pH = 7.0) with varying concentrations of double-stranded calf thymus DNA (Sigma, Type I). The reported [DNA] values correspond to [base pair] calculated by assuming $\epsilon_{BP}(280\ nm) = 6.6\ mM^{-1}\ cm^{-1}$.

The absorption spectrum of **1** in the near-UV region is a composite of the Re → bpy MLCT and $S_0 \rightarrow S_1$ An absorptions. The latter feature is clearly discernible owing to the distinct vibronic structure that is characteristic of the anthracene chromophore. Addition of DNA to aqueous solutions of **1** leads



to pronounced hypochromism and a bathochromic shift for the An $S_0 \rightarrow S_1$ transition; these absorption spectral changes are virtually identical with those reported concomitant to binding of 9-aminomethylanthracene to DNA.[10] Addition of DNA to a solution of C–Q complex **2** elicits similar, albeit less dramatic, changes in the An $S_0 \rightarrow S_1$ absorption. Parallel experiments with model complex **3** reveal that the Re MLCT absorption is unaffected by the presence of DNA in this system.[25]

Figure 1 illustrates a series of emission spectra obtained for complex **1** at various [DNA]/[Re] ratios using 340-nm excitation.[11] The broad, featureless band with $\lambda_{max} \approx 580$ nm is Re → bpy MLCT emission, while the structured emission at shorter wavelength is An fluorescence. In buffer solution without DNA, **1** exhibits very little MLCT emission; comparison of Φ_{em} values for **1** and **3** in aqueous buffer (Table I) indicates that the MLCT emission of **1** is 10 times less intense compared to that of model **3**. Addition of DNA to buffer solutions of **1** leads to a dramatic increase in the intensity of the MLCT emission. This increase occurs at relatively low [DNA]/[Re] ratios and a plateau is reached for [DNA]/[Re] > 4.5 (inset, Figure 1), at which point the emission is approximately 5 times more intense compared to the emission of **1** in buffer without DNA. In sharp contrast to the remarkable behavior of **1**, similar DNA titrations on model complexes **2** and **3** reveal little or no change in MLCT emission intensity or band shape upon addition of DNA. In addition to the changes in the MLCT emission intensity, a significant decrease in intensity and broadening of the vibronic bands of the An fluorescence also occur upon binding of **1** to DNA. While a complete discussion of this effect must await the full paper, it is important to note that the substantial change in An fluorescence is consistent with a strong interaction between the An chrompohore and the DNA polymer.

Binding parameters were determined from the change in the MLCT emission yield as a function of [DNA]/[Re] (inset, Figure 1) by using a Scatchard analysis and assuming a single binding site.[12] The best fit was obtained with the site-covering size, $n = 2 \pm 0.5$ base pairs, and an intrinsic binding constant, $K_b = (4.6 \pm 0.5) \times 10^5\ M^{-1}$.

MLCT emission decay (Em-D) parameters for degassed solutions of **1** and **3** in the absence and presence of DNA are listed in Table I. First, for model complex **3**, the MLCT Em-D is single exponential, and the lifetime is the same in the presence

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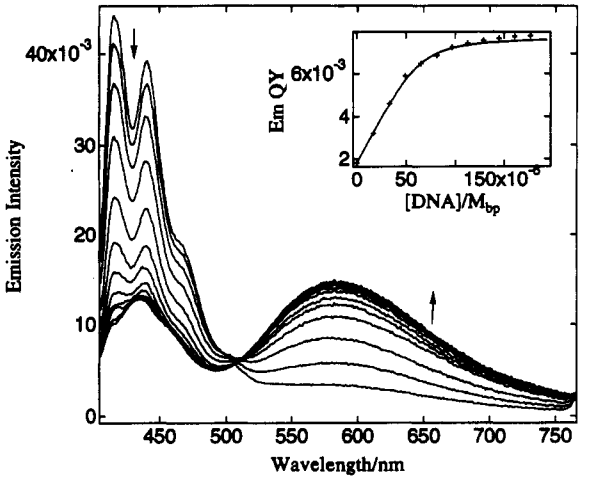

Figure 1. Corrected emission spectra of **1** as a function of [DNA]/[Re]. Arrows indicate direction of change with increasing [DNA]/[Re]. [DNA]/[Re] ratios: 0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5. Inset shows a plot of Φ_{em}^{MLCT} vs [DNA] for the same emission titration experiment. Conditions: Tris buffer; pH = 7.0; [**1**] = 33 μM.

Table I. Photolysis Properties of Complexes **1** and **3**[a,b]

complex	[DNA]/[Re]	Φ_{em}	a_1/%	τ_1/ns	a_2/%	τ_2/ns	a_3/%	τ_3/ns	$\langle\tau\rangle$/ns[c]
1	0	0.002	77	1.9	18	8.3	5	106	8.4
1	11	0.009	42	2.8	28	40	30	84	38
3	0	0.023	100	107					
3	20	0.022	100	107					

[a] Quantum yields for air-saturated solutions relative to $Ru(bpy)_3^{2+}$ in degassed H_2O (Φ_{em} = 0.055). Lifetimes for Ar degassed solutions. [b] a_i and τ_i values are respectively the relative amplitudes and lifetimes of multiexponential fit of emission decay. [c] Mean lifetime.

and absence of DNA, which implies that this complex binds weakly, if at all, to DNA.[13] In sharp contrast to the behavior of **3**, the Em-D of **1** is markedly inhomogeneous: in both the absence and presence of DNA, a triple-exponential function is required to simulate the experimental Em-D. In aqueous buffer, the Em-D of **1** is dominated by comparatively short lifetime components with ≈95% of the total Em-D occurring in less than 10 ns (note $\langle\tau\rangle$, Table I). Addition of DNA has two predominant effects on the Em-D of **1** (1) the lifetimes of the fast and middle decay components increase; (2) the amplitude of the fast component is decreased while the amplitudes of the middle and slow components increase. These changes combine to produce a substantial increase in the $\langle\tau\rangle$ for **1** in the presence of DNA.

The hypochromism and bathochromic shift of the An $S_0 \rightarrow S_1$ transition upon binding of **1** and **2** to DNA provide unequivocal evidence that An interacts strongly with DNA. Similar absorption changes have been observed upon addition of DNA to other substituted anthracenes, and these effects have been explained using an intercalation model for the binding interaction.[10,15] By analogy, we suggest that the predominant mode for binding of C–Q complex **1** to DNA is intercalation of An. Note that the K_b and n values for binding of **1** to DNA are consistent with values typical of other intercalating molecules.[10,15] In addition, studies of model complex **3** indicate that the Re chromophore interacts little, if at all, with DNA.

The origin of the heterogeneous Em-D kinetics and the strong effect of DNA on the MLCT emission properties of **1** can be explained if the underlying mechanism for intramolecular quenching is considered. This quenching process involves TT E_NT from the Re MLCT state to produce $^3An^*$ via the Dexter exchange mechanism.[2] Since it is well-established that the rate of exchange E_NT (k_{E_NT}) by either TB or TS pathways depends strongly upon distance,[16] it is expected that k_{E_NT} in **1** will depend strongly on the conformation of the flexible eight-atom tether that links Re to An.

Modeling studies suggest that in fluid solution the tether in **1** can easily adopt "closed" conformations that allow Re and An to come within van der Waals separation.[17] Furthermore, owing to the hydrophobicity of the two chromophores, closed conformations may be energetically preferred in aqueous solution. Thus, the comparatively weak MLCT emission intensity and predominance of short-lived Em-D components for **1** in aqueous buffer are consistent with the notion that, on the average, the Re and An chromophores are comparatively close together, allowing relatively strong interchromophore electronic coupling and large k_{E_NT}. By contrast, the increase in MLCT emission intensity and in the amplitudes of comparatively slow Em-D components concomitant with binding of **1** to DNA signals that intercalation of An forces the tether to adopt more extended conformations. As a result, electronic coupling between Re and An is diminished, leading to a substantial decrease in k_{E_NT}. Note that this conformational model is supported by the observation that the MLCT emission of **2**, in which the tether that links Re to An is relatively constrained, is virtually unaffected by binding to DNA.

The DNA-induced conformation change model is also supported by computer based modeling studies which indicate that if An is intercalated, unfavorable steric interactions between the spherical Re complex and the surface of the DNA polymer cause the tether to adopt a rather extended conformation with a minimum Re to An separation distance of ≈16 Å (center-to-center).[17] However, despite the overall difference in the conformation of the tether when **1** is free in solution or bound to DNA, the observation of heterogeneous Em-D kinetics for **1** in both circumstances indicates that the flexible tether can adopt many different conformations in which the distance and relative orientation of the chromophores vary.

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Supplementary Material Available: A textual presentation of the synthetic details and NMR data for **1** (2 pages). Ordering information is given on any current masthead page.

(13) Two other experiments strongly imply that **3** binds at best only weakly to DNA. (1) The emission of **1** is completely depolarized in both the absence and the presence of DNA. (2) The efficiency for Stern–Volmer quenching of **1** by $Fe(CN)_6^{4-}$ is the same in the absence and presence of DNA.[29]

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