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Inclusion by β-cyclodextrin of a pyrene-labeled dipeptide photoprobe

Guilford Jones, II,* Xin Zhou and Lily N. Lu

Department of Chemistry and the Photonics Center, Boston University, Boston, MA 02215, USA

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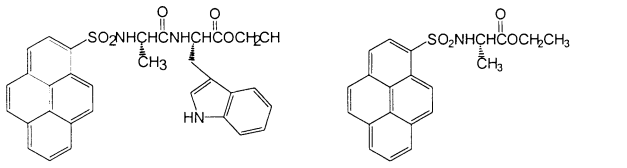
Abstract—An alanyltryptophan dipeptide (**1**), labeled at the N-terminus with a pyrene moiety is bound strongly by β-cyclodextrin in water, leading to enhancement of the fluorescence of the photoprobe. The effect is ascribed to restriction of conformational freedom for the dipeptide and a consequent reduction in the rate of intramolecular electron transfer that occurs between pyrene and Trp/indole moieties. © 2002 Published by Elsevier Science Ltd.

Several prior investigations from our laboratory have employed the amino acid, L-tryptophan (Trp), as a reactive element for photoinduced electron transfer studies. These investigations have included conjugates of one, two, or more amino acid residues with xanthene dye derivatives[1] or with other chromophores that are based on the parent structure, pyrene.[2] Using an array of fluorescence and laser phototransient methods, it was determined that Trp side chains engage in electron transfer events that are 'long range' (> 5 Å) and involve primarily the interaction of the remote chromophore and the Trp residue (indole). In these studies it was possible to measure rate constants for forward electron transfer that leads to formation of radical ion intermediates, and to measure back electron transfer events that occur with rate constants as fast as 10^{10} s^{-1}.[2b]

Photoprobes that are based on electron donor–acceptor interaction are increasingly of interest to photochemists for various applications.[3] Cyclodextrin (CD) microenvironments have been frequently the target of probes that are usually based on sensitive fluorescence measurements. The α-, β- and γ-cyclodextrins consist respectively, of six, seven and eight cyclically-linked pyranose units that give rise to a toroidal structure. Structural features include the formation of a belt of hydrogen bonds around the wider cyclodextrin rim that lends rigidity to the array, especially in the case of β-CD.[4] The primary 6-OH groups located at the narrower rim do not participate in intramolecular hydrogen bonds, and therefore can rotate so as to partially block the cavity.[5]

The interaction of cyclodextrin and the previously reported dipeptide conjugate,[2b] **1** (Scheme 1) has been studied with a focus on four features of host–guest chemistry that have not been properly explored: (1) the role of the microenvironment of cyclodextrins in the control of electron transfer processes for linked donors and acceptors; (2) the complexation by cyclodextrins of low molecular weight modified polypeptides and the assessment of the appropriate binding equilibria; (3) the electron transfer behavior of peptide conjugates as alternative fluorescence probes of the interiors of cyclodextrins; (4) discovery of properties of CD–peptide complexes that may be relevant to understanding drug–carrier interactions. We have also investigated the photophysical properties of pyrene conjugates (Scheme 1) in various solvents in order to determine the effect of solvent polarity, in general, on photoinduced electron transfer. The results, in sum, suggest that β-CD can act as a modulator of rates of electron transfer by 'freezing' conformational mobility for a bound peptide.

At a concentration (<0.5 μM) where **1**[2a] exists predominantly as a monomer,[6] addition of β-cyclodextrin leads to an increase in fluorescence quantum yield (Fig. 1).



Pyr-Ala-TrpOEt (**1**) Pyr-AlaOEt (**2**)

Scheme 1.

Keywords: peptide electron transfer; cyclodextrin–peptide complex.

* Corresponding author.

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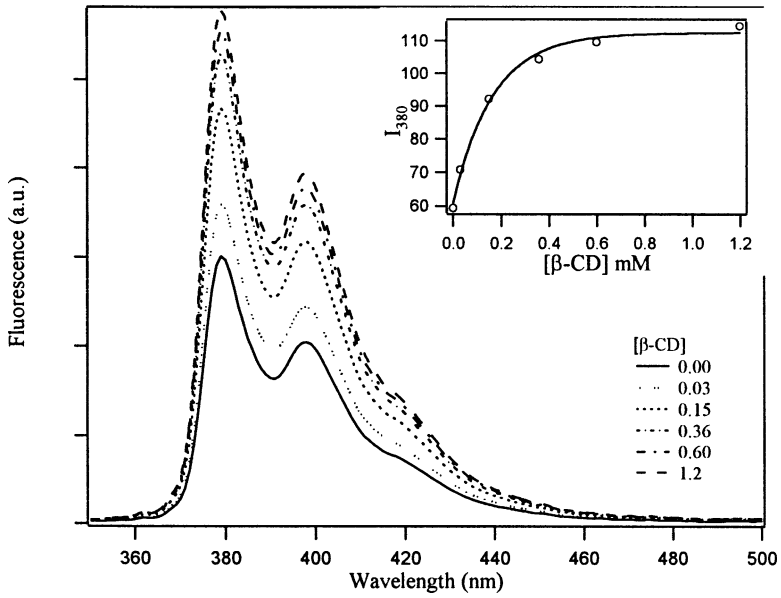

Figure 1. Fluorescence spectra observed for **1** (0.3 μM) upon addition of β-cyclodextrin in water ($\lambda_{ex}=330$ nm). Inset: plot of fluorescence intensity at 380 nm versus the concentration of β-cyclodextrin.

In addition, the emission lifetime of **1** is noted also to increase by about 4-fold (Table 1). This restoration of fluorescence is attributed to the inclusion of the pyrene derivative by β-CD. The inclusion process for low concentrations of **1** (P) can be represented by the equilibrium.

$$P+m\beta\text{-CD} \overset{K_I}{\rightleftharpoons} P{:}\beta\text{-CD}_m$$

Assuming that the emission quantum yield is proportional to the concentration of the fluorescent species and the increase of fluorescence yield is solely due to the increase of the included P, it can be shown that

$$m \ln[\beta\text{-CD}]_0 = \ln\left(\frac{\Delta\Phi}{\Delta\Phi_0-\Delta\Phi}\right)+A \qquad (1)$$

where $[\beta\text{-CD}]_0$ is the analytical concentration of β-CD, A is a constant, $\Delta\Phi$ is the difference of the fluorescence quantum yield between that at any concentration of β-CD and that at $[\beta\text{-CD}]_0=0$ M (free monomer P), $\Delta\Phi_0$ is the difference of quantum yield between that of completely included P and that of free monomer P ($[\beta\text{-CD}]_0=0$ mM); m is the ratio of β-CD to P in the complex.

A plot of $\ln\left(\frac{\Delta\Phi}{\Delta\Phi_0-\Delta\Phi}\right)$ versus $\ln[\beta\text{-CD}]_0$ (Fig. 2) produces the inclusion ratio, $m=1.0\pm0.05$, and a plot of $\frac{\Delta\Phi}{\Delta\Phi_0-\Delta\Phi}$ versus $[\beta\text{-CD}]_0$ (Fig. 2) yields the equilibrium constant $K_I=7.8\pm0.5\times10^3$ M^{-1}. Stoichiometries of 1:1, as shown here, as well as the 1:2 complex of pyrene[7,8] and its derivatives[9] have been reported. In a presumed 2:2 complex, the parallel arrangement of P:β-CD would result in an excimer-like emission (not observed for **1**) such as those found for pyrene and its derivatives included by γ-CD.[9a,9b] For an anti-parallel 2:2 complex, reminiscent of the ternary pyrene–amino acid–CD complex reported by Bohne[8a,8b] and others, a quenching of

Table 1. Fluorescence quantum yields and lifetimes obtained for **1** and **2** in selected solvents

Solvent	Dielectric constant (ε)	**1**			**2**	
		Φ_f[a]	τ (ns)[b]	k_{et} (10^8 s^{-1})[c]	Φ_f[a]	τ (ns)[b]
Water[d] ([β-CD]=0.0 mM)	80.2	0.022	1.7	5.6	0.81	31
Water ([β-CD]=12 mM)	80.2	0.097	8.1	0.91	0.85	38
Cyclohexane	2.03	0.022	1.3	7.3	0.81	29
Benzene	2.28	0.034	1.5	6.4	0.74	32
Ethyl acetate	6.05	0.028	1.3	7.4	0.60	35
Methanol	32.7	0.020	1.2	8.0	0.79	30
Acetonitrile	35.9	0.019	1.5	6.4	0.83	35
DMSO	46.5	0.021	1.2	8.0	0.80	33

[a] 10 μM, Ar-purged sample; coumarin 1 used as standard ($\Phi_f=1$ in acetonitrile, $\lambda_{ex}=330$ nm).
[b] τ values obtained from single exponential fit ($\chi^2=0.999$–1.01) ($\lambda_{ex}=337$ nm).
[c] Rate constants calculated from $k_{et}=\frac{1}{\tau}-\frac{1}{\tau_0}$, where τ is the lifetime of **1** and τ_0 is the lifetime of **2**.
[d] 20% v/v DMF added for increased solubility.

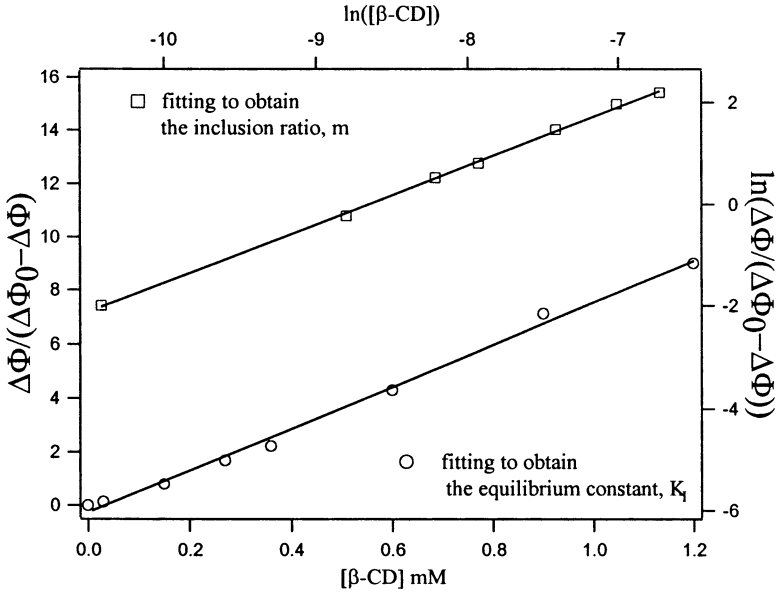

Figure 2. Plots of quantum yield ratios versus $\ln[\beta\text{-CD}]_0$ or $[\beta\text{-CD}]_0$ to obtain values for the stoichiometry and the equilibrium constant for **1**:β-CD complexation.

pyrene fluorescence by tryptophan is expected, a result that is quite opposite to the observed fluorescence enhancement of the included **1** (Table 1). In addition, molecular modeling studies have indicated that the β-CD cavity is not of appropriate size to accommodate two or more pyrene molecules.[7a,7c] The value of K_I for 1:1 complexation of **1** and β-CD is notably larger than values determined for inclusion of unsubstituted pyrene (K_I <500 M^{-1}).[10] The modification of pyrene with additional groups provided by the dipeptide may give rise to H-bond and other 'cavity-fitting' interactions[8a] that are beneficial to binding. A binding model that shows relative sizes of CD host and guest is shown in the graphical abstract.

Interestingly, the dipeptide: β-CD complex exhibited an induced circular dichroism (ICD) ($[\theta] = -81.2\times10^3$ deg cm^2/dmol at 350 nm), which corresponds to the absorption region associated with the L_a, B_b and B_a bands of pyrene (Fig. 3). The observed negative ICD is stronger than that observed for the parent, pyrene,[9b] and appears not to include a component originating from the indole chromophore.[11] Therefore, it is concluded that the pyrene moiety must be specifically included in the complex of **1**; the sulfonamide spacer in Pyr-Ala-TrpOEt may well provide a base for 'tight' hydrogen bond interactions at the rim of β-CD.

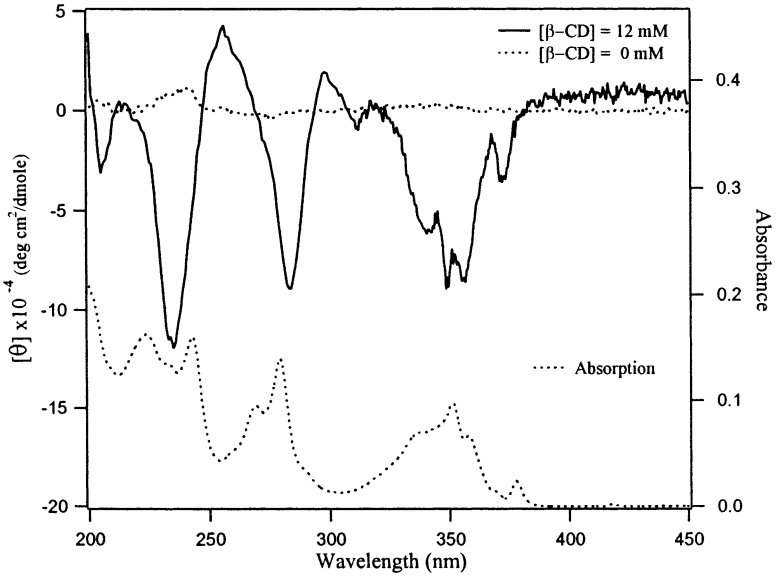

Figure 3. Circular dichroism spectra for **1** (0.3 μM), with and without added β-CD in water, and the absorption spectrum of **1** (1.0 μM) in acetonitrile.

We also investigated the interactions between the two compounds with α- and γ-CD. It is known that α-CD can include tryptophan[12] but not pyrene[7a,7d] due to its small size. No alterations in absorption or fluorescence spectra were observed for **1** (with up to 12 mM of α-CD). The **1**:γ-CD complex exhibited an excimer-like emission ($\lambda_{max}=480$ nm), consistent with the finding that the larger γ-CD can include two pyrene chromophores.[9b]

Fluorescence quantum yields and lifetimes of **1** and the model compound, **2** (a model not containing the redox active Trp residue which shows restored emission yield and lifetime), in various solvents are presented in Table 1. Also presented are computed rate constants that are associated with electron transfer (Trp/indole→pyrene).[2] Somewhat surprisingly, k_{et} values range near 10^8 s^{-1}, and are essentially independent of solvent polarity (Table 1). In contrast, for the β-CD-included **1**, the electron transfer rate constant is decreased by ca. 6-fold (versus any solvent that was investigated). For these various pure solvent media, the rate constants for **1** (an average around 7.3×10^8 s^{-1}) are possibly near a 'maximum' (i.e. where the activation free energy, $\Delta G^{\neq}$, is near zero). This condition applies under circumstances in which $\Delta G^\circ=-\lambda$ and rate constant data fall along the top of a 'Marcus curve' that is a plot of the dependence of log k_{et} and ΔG°.[13] Thus, the effect of increasing solvent polarity is offset, in that an increase in λ roughly mirrors the trend to more negative values of ΔG°, a situation that results in a relatively constant $\Delta G^{\neq}$ and hence, a less varied k_{et}. The fortuitous relationship of ΔG and λ that holds for **1** leads to an assessment of how β-CD complexation affects k_{et} through alternation of long-range electronic coupling between pyrene and Trp/indole moieties. The decelerated rate of electron transfer may thus be ascribed to the restriction of the conformational flexibility of Pyr-Ala-TrpOEt imposed by the cyclodextrin; i.e. the population of conformations that is most effective in through-bond, and possibly through-space, interaction is significantly reduced. We are investigating this proposed 'sampling'[14] of conformational space for short-link peptides in other aqueous self-assembling media.[15]

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