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Inclusion Complexation of 2-Amino-7-bromofluorene by β-Cyclodextrin: Spectral Characteristics and the Effect of pH

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Spectral characteristics of 2-amino-7-bromofluorene (2ABF) have been studied in aqueous β-cyclodextrin (β-CDx) solution. Enhancement in the fluorescence intensity of the neutral from of 2ABF was observed due to the formation of 1:1 complex with β-CDx. The formation of this complex was confirmed by time-resolved fluorescence spectroscopy. The ground state pK_a value for the monocation-neutral equilibrium of 2ABF in β-CDx shows no change with that in aqueous solution, but the excited state pK_a value changes. Based on its photophysical and photoprototropic characteristics in β-CDx, the structure of the 1:1 inclusion complex is proposed.

KEY WORDS: Inclusion complex; 2-amino-7-bromofluorene; β-cyclodextrin; photoprototropism; excited state acidity constant; fluorescence spectroscopy.

INTRODUCTION

Cyclodextrins (CDxs) are water-soluble cyclic oligosaccharides composed of six [α-], seven [β-], or eight (γ-] units of D(+)-glucopyranose units arranged in a truncated cone-shaped structure. They can form inclusion complexes with a large variety of organic and inorganic compounds [1]. The chemical reactivity [2] and the spectroscopic properties [3] of the guest molecules are modified as a result of the inclusion. In particular, ground and excited state protolytic equilibria are influenced because of the variation of the microenvironment experienced by the included guest. For example, an increase in the rate of deprotonation is observed in the case of β CDx bound carbazole [4] and protonated 1-aminopyrene [5] and a decrease in the deprotonation rate in the case of 1- and 2-naphthols [6–9] are observed. Since the fluorescence spectral characteristics of certain molecules are very sensitive to their environment in structured microheterogeneous [10–11] systems, these systems can play a major role in the alteration of these properties.

Our laboratory has been active for years in studying the acid-base properties of amino and hydroxy biphenyls [12–21] in aqueous medium. We have reported the quenching of 2-amino-7-bromofluorene fluorescence by halomethanes in aqueous medium [22]. The present work deals with the spectral and the photoprototropic characteristics of 2ABF in the presence of β-cyclodextrin.

MATERIALS AND METHODS

2-Amino-7-bromofluorene (2ABF) was obtained from Aldrich Chemical Company. This compound was purified by repeated crystallisation from petroleum ether (60–80°). The purity was checked by its sharp melting point and thin layer chromatography. β-Cyclodextrin was purchased from S.D. Fine Chemical Company and used as received. Triply distilled water was used for the preparation of experimental solutions. A modified Hammett's acidity scale (H_0) [23] for the solutions below

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pH 1.5 (using a H_2SO_4–H_2O mixture) was employed. The concentration of 2ABF in the test solutions used was 1.6×10^{-5} M. To measure the fluorescence intensities for the fluorimetric titration, isosbestic wavelength was used for excitation.

The absorption spectra were recorded using JASCO UNIDEC-650 spectrophotometer and the fluorescence measurements were made using JASCO FP-550 spectrofluorimeter. pH values in the range of 1–12 were measured using ELICO LI-1OT model pH meter. Fluorescence lifetimes were measured by means of a time-correlated single photon counting picosecond spectrofluorimeter (TSUNAMI, SPECTRAPHYSICS, USA).

RESULTS AND DISCUSSION

Spectral Characteristics

The absorption maxima, log ε and fluorescence maxima of 2ABF at different concentrations of β-CDx are given in Table I. The absorption spectra are given in the inset of Fig. 1. A small red shift of 2 nm is observed in β-CDx in comparison to that in water. There is no appreciable difference in the molar extinction coefficient by the addition of β-CDx. The red shift in the absorption maxima may be due to the formation of an inclusion complex between 2ABF and β-CDx.

Table I. Absorption and Steady-State Fluorescence Spectral Data of 2ABF

Concentration of β-CDx (M)	Absorption maximum λ_{abs} nm (log ε)	Fluorescence maximum λ_{flu} nm (Excitation wavelength = 312 nm)	I_f
0	287.6 (3.47)	376	152
0.0004	288.0 (3.46)	373	193
0.0008	289.4 (3.46)	373	230
0.0012	289.4 (3.47)	373	267
0.0016	289.6 (3.47)	373	304
0.0020	289.6 (3.48)	373	340

The fluorescence spectra of the neutral form of 2ABF with various concentrations of β-CDx are shown in Fig. 1. The effect of β-CDx on the fluorescence spectra of 2ABF is more pronounced than the corresponding effect on the

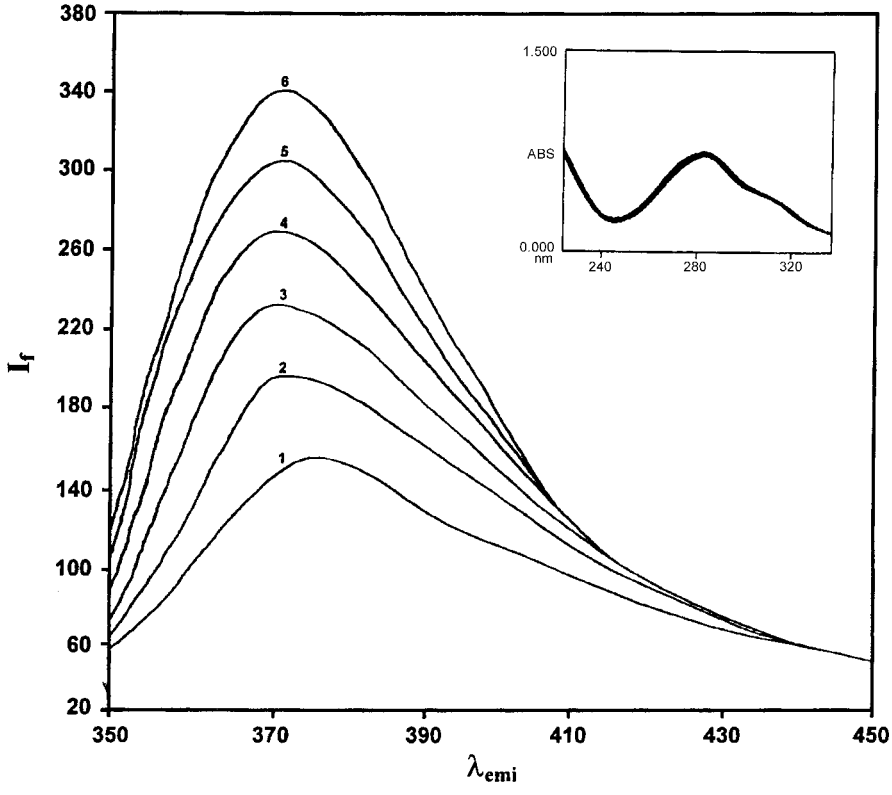

Fig. 1. Fluorescence emission spectra of 2ABF at different concentrations of β-CDx at pH 10. (1) 0 M β-CDx (2) 0.0004 M β-CDx (3) 0.0008 M β-CDx (4) 0.0012 M β-CDx (5) 0.0016 M β-CDx (6) 0.002 M β-CDx. Inset: Absorption spectra of 2ABF at different concentrations of β-CDx.

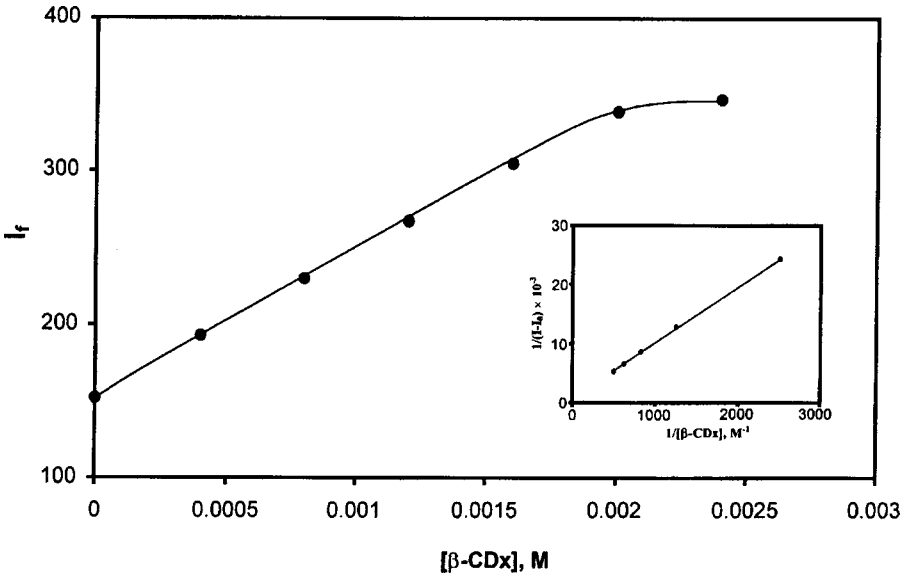

Fig. 2. Fluorescence intensities of 2ABF with various concentrations of β-CDx at pH 10. Inset: Benesi-Hildebrand plot for the complexation of 2ABF with β-CDx.

absorption spectra. Two observations are made. A blue shift and an increase in the fluorescence intensity are observed by the addition of β-CDx upto a concentration of 0.002 M. The increase in the intensity of fluorescence with the addition of β-CDx is shown in Fig. 2. The fluorescence intensity is 2.6 times more in 0.002 M β-CDx than in aqueous solution. The blue shifted fluorescence maximum and the enhancement of fluorescence intensity suggest the formation of an inclusion complex between 2ABF and β-CDx. An increase in the fluorescence intensity for the formation of an inclusion complex has been reported earlier [24–27]. Though the fluorescence maximum is blue shifted it is not so large [3 nm]. This reveals that there is no interaction of the amino group of 2ABF and the —OH groups of β-CDx in the inclusion complex formed. The formation of the inclusion complex is also confirmed by the fluorescence decay curves for 2ABF with different concentrations of β-CDx. The life times and the amplitudes of decay times of 2ABF with and without β-CDx are given in Table II. The time-resolved fluorescence of 2ABF with β-CDx shows a biexponential decay indicating the equilibrium between free and complexed forms. The lifetime of the complexed form and the amplitude increase with the increase in the concentration of β-CDx upto 0.002 M. Above 0.002 M no change in lifetimes and amplitudes of both forms is observed. The χ^2 values for the single and the biexponential fittings are less than 1.37.

The β-CDx dependence of 2ABF fluorescence can be analysed by the Benesi-Hildebrand plot as given by the equation [28–30].

$$\frac{1}{I - I_o} = \frac{1}{I' - I_o} + \frac{1}{K(I' - I_o)[\beta - \text{CDx}]}$$

where K is the binding constant, I_o is the intensity of fluorescence of 2ABF without β-CDx, I is that with a certain concentration of β-CDx, and I' is the fluorescence intensity of 2ABF with the highest concentration of β-CDx. The plot of $\frac{1}{I-I_o}$ Vs $\frac{1}{[\beta-\text{CDx}]}$ is given in the inset of Fig. 2. The linearity in the plot reflects the formation of 1:1 complex between 2ABF and β-CDx. From the slope and intercept, the binding constant K was calculated to be 5.65×10^2 M $^{-1}$.

Table II. Time-Resolved Fluorescence Spectral Data of 2ABF (Excitation Wavelength = 312 nm, Detection Wavelength = 370 nm)

Concentration of β-CDx M	Lifetime (s)	Relative amplitude	χ^2	Standard deviation (s)
0	2.99×10^{-9}	100	1.37	3.07×11^{-11}
0.0004	2.29×10^{-9}	55.12	1.07	4.22×10^{-11}
	6.63×10^{-9}	44.88		7.12×10^{-11}
0.0008	2.50×10^{-9}	38.71	1.09	5.86×10^{-11}
	6.76×10^{-9}	61.29		5.14×10^{-11}
0.0012	2.10×10^{-9}	28.25	1.13	5.49×10^{-11}
	6.56×10^{-9}	71.75		3.49×10^{-11}
0.0016	2.24×10^{-9}	23.90	1.19	7.78×10^{-11}
	6.74×10^{-9}	76.10		3.77×10^{-11}
0.002	1.96×10^{-9}	19.29	1.03	6.48×10^{-11}
	6.55×10^{-9}	80.71		2.81×10^{-11}

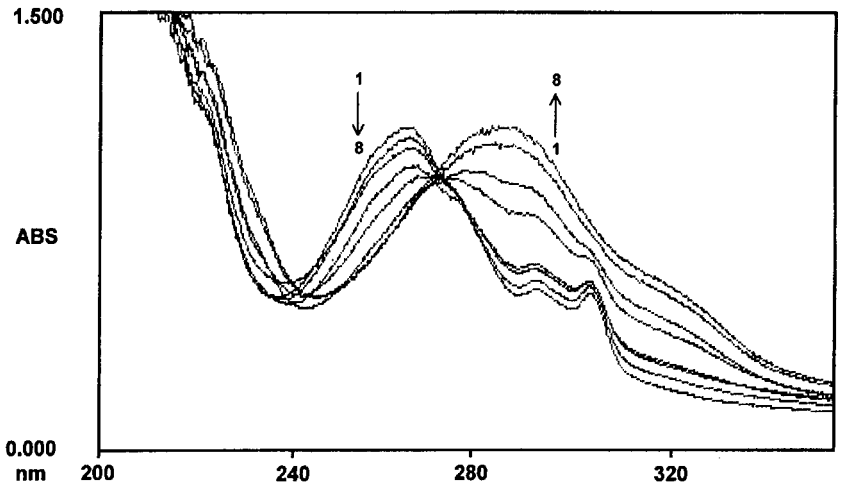

Fig. 3. Absorption spectra of 2ABF with β-CDx at different H_0/pH values (**1.** pH 1.5, **2.** pH 2, **3.** pH 2.5, **4.** pH 3, **5.** pH 3.5, **6.** pH 4, **7.** pH 4.5, **8.** pH 5).

Photoprototropism

The absorption and the fluorescence spectra of 2ABF in 0.002 M β-CDx have been investigated in the H_o/pH range of -5 to 12. The absorption maximum of the neutral form of 2ABF is observed at 289.6 nm (in aqueous solution it is 289 nm) [22]. When the pH is decreased from 7, the absorption maximum of 2ABF is blue shifted (Fig. 3). This blue shifted maximum at 271.4 nm (in aqueous solution it is 269.4 nm) [22] is due to the formation of the monocation. No change in the absorption spectrum occurs below pH 1 and also in the pH range 7–12. A clear isosbestic point is observed at 279 nm. In the pH range 1–7, the absorption spectra of the molecule in β-CDx are found to be similar to those in aqueous solution. This again confirms that there is no interaction between the amino group of the 2ABF molecule and the —OH groups of β-CDx in the complex.

The fluorescence intensities of 2ABF at different pH in β-CDx are shown in Fig. 4. When the pH is decreased from 7, the intensity of fluorescence of 2ABF in β-CDx decreased upto $H_o + 0.44$ and then a new fluorescence band at 320 nm starts ($\lambda_{exci} = 279$ nm) appearing. This is due to the formation of the monocation.

The ground state acidity constant (pK_a) value for the following prototropic equilibrium in β-CDx

$$2ABF + H^+ \rightleftharpoons 2ABFH^+$$

was determined spectrophotometrically. This pK_a value of 3.56 is less than the pK_a value of 4.35 reported in aqueous solution [22]. Because of complexation the protonation may be difficult and requires more acidic conditions.

The fluorimetric titration curves of 2ABF without and with β-CDx meet at the middle of their inflection (see Fig. 5). This reveals that the decrease of the neutral form exactly corresponds to the increase of monocation under both the conditions. The other processes such as proton induced fluorescence quenching are not present. In aqueous solution the pK_a^* value of 4.53 obtained by the fluorimetric titration is close to the ground state pK_a value of 4.33. This indicates that the lifetimes of neutral and monocation are not sufficient for the attainment of equilibrium in the excited state i.e., the excited species loses its energy before undergoing protonation due to its short lifetime [31]. But the fluorimetric titration curves of 2ABF in the presence of β-CDx meet at a point corresponding to pH +0.65. This value is different from the ground state pK_a value of 3.56. If the rates of proton exchange are faster than the rates of fluorescence then proton transfer equilibrium will be established within the lifetime of the excited species and the excited state pK_a determines the shape and position of fluorimetric titration curve. If the rates of excited state proton exchange are slower than the rates of fluorescence then ground state pK_a determines the shape and position of fluorimetric titration curves [31,32]. Decrease of rates of fluorescence increases the lifetimes of the excited species. In this case it is found that the lifetime of the complex is greater than the lifetime of the free species (Table II). Due to the increase in the lifetime of the complexed form the protonation in the excited state occurs and the excited state pK_a^* value of 0.65 is obtained by fluorimetic titration.

From the Benesi-Hildebrand plot the stoichiometry of the 2ABF-β-CDx complex is found to be 1:1. The spectral and the photoprototropic studies show that there is no interaction between the —NH_2 groups of 2ABF and the

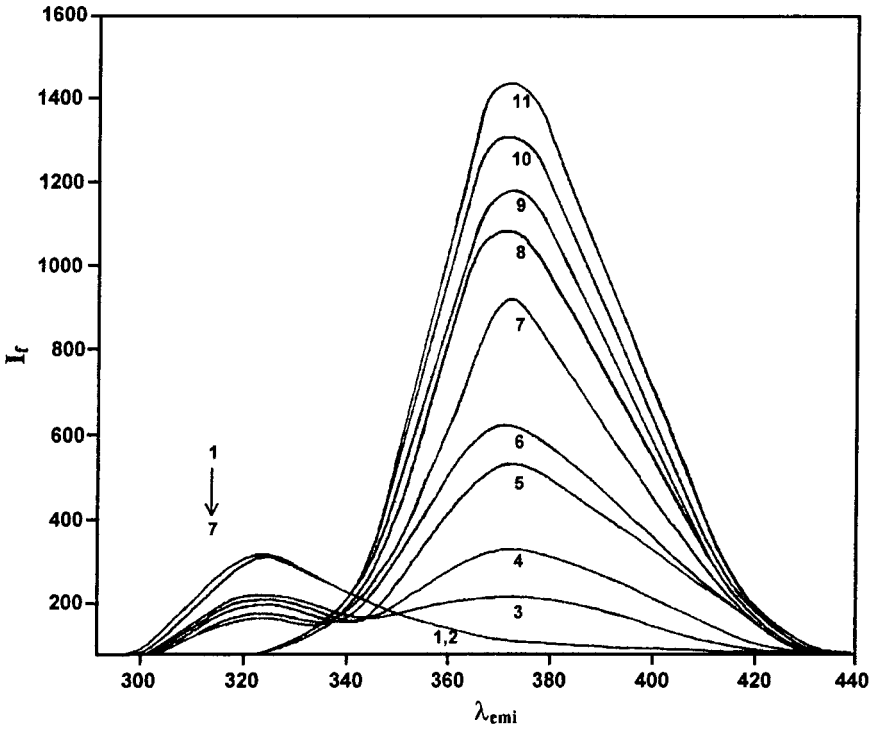

Fig. 4. Fluorescence spectra of 2ABF with β-CDx at different H_0/pH values (**1.** H_0 −1.85, **2.** H_0 −1.62, **3.** H_0 −1.38, **4.** H_0 −0.26, **5.** H_0 +0.13, **6.** H_0 +0.44, **7.** H_0 +0.83, **8.** pH 1.4, **9.** pH 1.9, **10.** pH 2.5, **11.** pH 3.5).

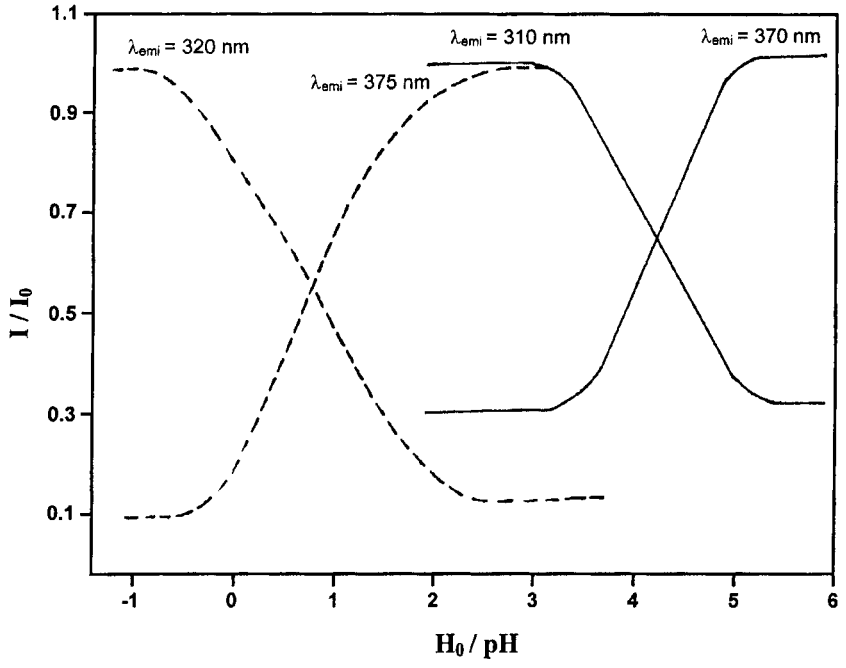

Fig. 5. Fluorimetric titration curves for 2ABF without and with β-CDx (___ without β-CDx, – – – with 0.002 M β-CDx).

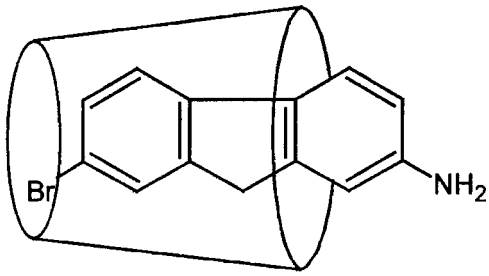

Fig. 6. Schematic diagram of the inclusion complex (1:1) of 2ABF with β-CDx.

—OH groups of β-CDx. This indicates that the —NH_2 group of 2ABF lies outside the cavity of β-CDx and it is in an aqueous environment. Further there is no possibility of accommodation of 2ABF molecule completely in the cavity of β-CDx having a length of 7.8 Å. So the complex formed is the axial inclusion complex with the fluorene inside the β-CDx cavity as shown in Fig. 6.

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