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# Excited-State Proton Transfer of Protonated 1-Aminopyrene Complexed with $\beta$ -Cyclodextrin

John E. Hansen,<sup>†</sup> Ehud Pines,<sup>‡</sup> and Graham R. Fleming\*

Department of Chemistry and the James Franck Institute, The University of Chicago, Chicago, Illinois 60637  
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Excited-state proton transfer in inclusion complexes of 1-aminopyrene and  $\alpha$ -naphthol with cyclodextrins was studied by time-resolved spectroscopy. We find that the kinetic studies are much more sensitive to the complexation than are steady-state spectroscopic measurements. Our data suggest two distinct binding orientations for 1-aminopyrene bound to  $\beta$ -cyclodextrin. The rate of proton transfer is increased by a factor of 2–3 compared to pure water with the inclusion complex resembling a water–ethanol mixture near the 75% by volume alcohol composition. Deuteration enhances the dissociation rate by a factor of 3.5 in both 80% (by volume) ethanol water mixtures and in the complex with  $\beta$ -cyclodextrin. In the case of  $\alpha$ -naphthol the rate of proton transfer slows considerably in the inclusion complex and has the rate expected for an 80% by volume ethanol–water mixture. We suggest that the water near the cavity rim of  $\beta$ -cyclodextrin is modified by the extensive network of OH groups in such a way as to increase its basicity. Similar effects may occur in enzymic systems.

## Introduction

Cyclodextrins are water-soluble molecules containing a hydrophobic cavity that will bind apolar molecules of suitable size to form host–guest enzymelike complexes.<sup>1,2</sup> A considerable amount of interest has been directed toward the nature of the hydrophobic force responsible for the binding since such interactions may provide a model for the active sites of enzymes.<sup>3</sup> In many cases the substrate will undergo a reaction catalyzed by cyclodextrin or one of its derivatives.<sup>4–12</sup> In this study we focus on the influence of cyclodextrins on the acid–base equilibria of their guest molecules.

Cyclodextrins are oligosaccharides comprised of six, seven, or eight glucopyranose units, which are designed as  $\alpha$ ,  $\beta$ , and  $\gamma$ , respectively. These subunits are arranged into a torus structure that resembles a truncated cone. The interior of this torus forms a hydrophobic cavity. The larger rim comprised of secondary hydroxyls attached to C-2 and C-3 carbons of the glucopyranose units we refer to as the secondary hydroxyl rim, while the smaller rim comprised of primary hydroxyls attached to the C-6 carbon we refer to as the primary hydroxyl rim. In our study we used two cyclodextrins,  $\alpha$ -cyclodextrin and a derivative of  $\beta$ -cyclodextrin. The derivative has two hydroxyls of  $\beta$ -cyclodextrin replaced by two methoxys, one hydroxyl on the secondary hydroxyl rim and a second hydroxyl randomly replaced on the primary hydroxyl rim. We refer to this derivative by its commercial name, MMBCD. A sketch showing the dimensions of  $\beta$ -cyclodextrin is given in Figure 1.

Recent quasielastic neutron scattering studies of  $\beta$ -cyclodextrin-11H<sub>2</sub>O<sup>13,14</sup> focus attention on the structure of water inside the cavity and near the secondary hydroxyl rim. These studies reveal an elaborate interconnection of hydrogen bonds between water molecules in an extended structure and the secondary hydroxyl rim. We assume three types of water exist for cyclodextrin in solution, water inside the cavity (which may be displaced by the guest in inclusion complexes), water near the hydroxyl rims, and bulk water. Our focus is on the acid–base properties of water near the hydroxyl rims.

Photoacids are routinely used to probe the environment of aqueous solutions.<sup>15–21</sup> These are large molecules that become more acidic upon electronic excitation<sup>22</sup> and undergo intermolecular proton transfer to water. This reaction is very sensitive to the water environment. The thermodynamic properties of photoacids were described by Förster<sup>23,24</sup> using a thermodynamic cycle.

To probe the water environment of cyclodextrin we measured the rate of excited-state proton transfer of the inclusion complexes of 1-aminopyrene and also of  $\alpha$ -naphthol. Proton transfer is affected by differential solvation of acid and conjugate base, as

well as Coulombic effects generated by ion-pair separation.<sup>25</sup> 1-Aminopyrene is a cationic anilinium-type acid. Use of anilinium acids has been previously suggested<sup>21</sup> to minimize the effects of anion solvation and Coulombic interactions, and so most of our attention is focused on 1-aminopyrene.

## Materials and Methods

$\alpha$ -Cyclodextrin and dimethyl- $\beta$ -cyclodextrin were obtained from Pharmatec, Inc. The average methyl substitution per molecule of the dimethyl derivative of  $\beta$ -cyclodextrin is 1.9. We used the derivatized  $\beta$ -cyclodextrin, MMBCD, due to its high solubility (50% by weight) compared to that of the underivatized  $\beta$ -cyclodextrin (1.8%). MMBCD has the further advantage of having the largest binding constant for a guest molecule of any commercially available  $\beta$ -cyclodextrin derivative.

1-Aminopyrene obtained from Aldrich at 97% purity was twice recrystallized from cyclohexane, and its steady-state excitation and fluorescence spectra were identical with the spectra published in the literature.<sup>26,27</sup> Reagent grade  $\alpha$ -naphthol obtained from Fluka at better than 99% purity was recrystallized from an aqueous solution of 10% ethanol. All other chemicals were at least reagent grade. HClO<sub>4</sub> (60% by weight) and H<sub>2</sub>SO<sub>4</sub> (96% by weight) from Baker and D<sub>2</sub>SO<sub>4</sub> 99% deuterated from Aldrich were added at concentrations of  $2 \times 10^{-3}$  to  $2 \times 10^{-2}$  M in order to protonate 1-aminopyrene in the ground state. H<sub>2</sub>O from Baker was HPLC grade. D<sub>2</sub>O from Aldrich was 99.5% deuterated.

Steady-state fluorescence spectra were measured with a Spex Fluorolog spectrofluorimeter. Absorption spectra were measured with a Perkin-Elmer 559A UV/visible spectrometer. pH measurements were made with a Model 9104 Ross pH electrode and an Orion SA520 pH meter.

Fluorescence lifetimes and anisotropies were measured in a time-correlated single-photon counting apparatus, which is described elsewhere.<sup>28</sup> The instrument response function had a full width at half-maximum of 60 ps. Decays were collected at a resolution of 14 and 26 ps/channel. Fluorescence decays and fluorescence anisotropy decays were fit to a sum of exponentials, and acceptable fits had a  $\chi^2$  between 0.90 and 1.20.

Since we are interested in monitoring the time evolution of excited-state acid–base reactions, we collect either the fluorescence decay of the acid form or the fluorescence rise of the conjugate base to determine the rate of proton transfer. The fluorescence of the conjugate base is significantly red shifted with respect to the fluorescence of the acid used, so it is possible to collect fluorescence that originates almost exclusively from the excited-state acid or conjugate base. Decay and rise times determined from fluorescence decays measured for the acid and conjugate base (see Figure 2), respectively, are roughly equal to the inverse of the proton transfer rate. This is because the fluorescence decay rate of protonated 1-aminopyrene in the absence of proton transfer

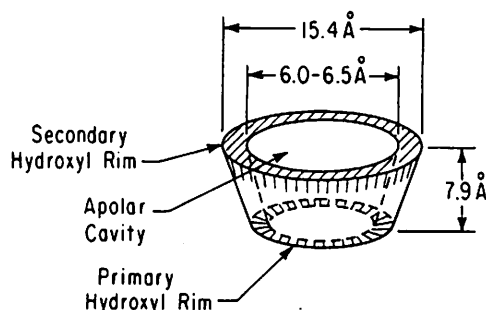
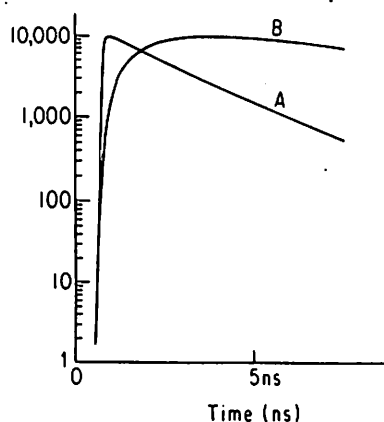
<sup>†</sup> Present address: The University of Michigan, Ann Arbor, MI 48109.

<sup>‡</sup> Present address: Tel Aviv, Israel.

TABLE I: Fluorescence Lifetimes of 1-Aminopyrene in Solution<sup>a</sup>

solvent	cyclodextrin	excited species	$a_1$	rise times of base		decay times of base
				$\tau_1$ , ns	$\tau_2$ , ns	$\tau$ , ns
H <sub>2</sub> O	none	base			immediate	5.18 ± 0.02
HClO <sub>4</sub> /H <sub>2</sub> O	none	acid	0		1.80 ± 0.05	5.10 ± 0.05
H <sub>2</sub> SO <sub>4</sub> /H <sub>2</sub> O	none	acid	0		1.60 ± 0.05	4.97 ± 0.03
H <sub>2</sub> O	MMBCD	base			immediate	5.19 ± 0.03
HClO <sub>4</sub> /H <sub>2</sub> O	MMBCD	acid	0.60	0.85 ± 0.05	1.76 ± 0.05	5.17 ± 0.05
H <sub>2</sub> SO <sub>4</sub> /H <sub>2</sub> O	MMBCD	acid	0.62	0.65 ± 0.05	1.66 ± 0.05	5.08 ± 0.02
H <sub>2</sub> SO <sub>4</sub> /H <sub>2</sub> O	$\alpha$ -cyclodextrin	acid	0		1.60 ± 0.05	5.07 ± 0.02
D <sub>2</sub> O	none	base			immediate	5.11 ± 0.02
D <sub>2</sub> SO <sub>4</sub> /D <sub>2</sub> O	none	acid	0		8.0 ± 0.5	<sup>b</sup>
D <sub>2</sub> O	MMBCD	base			immediate	5.11 ± 0.02
D <sub>2</sub> SO <sub>4</sub> /D <sub>2</sub> O	MMBCD	acid		<sup>c</sup>	2.5 ± 0.5	5.6 ± 0.03

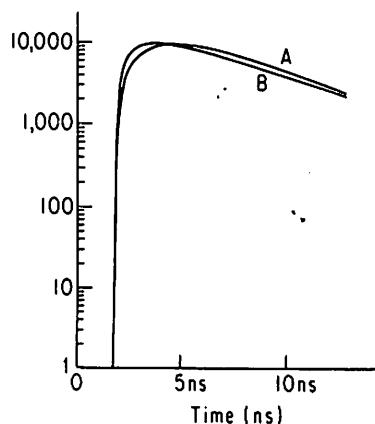
<sup>a</sup>Decays were collected at 440 nm from 295-nm excitation of protonated 1-aminopyrene. Fits given one or two rise times and a decay time. The fluorescence of the base (1-aminopyrene) fit well to a single-exponential decay. <sup>b</sup>An accurate decay time is difficult to determine from fits of fluorescence decays of 1-aminopyrene in D<sub>2</sub>SO<sub>4</sub>/D<sub>2</sub>O, because the rise time is much longer than the decay time. <sup>c</sup>For complexes 1-aminopyrene in D<sub>2</sub>SO<sub>4</sub>/D<sub>2</sub>O there may also be an 8-ns rise time component in the fluorescence decay; however, we are not able to accurately resolve this component because it is much longer than the dominant component.

Figure 1. Sketch showing the dimensions of  $\beta$ -cyclodextrin.Figure 2. Fluorescence decays detected at 374 nm (A) and 440 nm (B) of protonated 1-aminopyrene excited with 295-nm light. H<sub>2</sub>SO<sub>4</sub> is the protonating agent.

is 2 orders of magnitude slower than the decay rate corresponding to proton transfer.

## Results

Figure 3 displays the fluorescence decays collected from the 440-nm emission of 1-aminopyrene following 295-nm excitation of protonated 1-aminopyrene with and without MMBCD present. Table I displays the parameters used to fit fluorescence decays of 1-aminopyrene collected at 440 nm following excitation of the acid. Fluorescence decays were obtained from samples with and without cyclodextrin present. At lower concentrations of MMBCD fluorescence decays of the acid have decay times with relative amplitudes that match the rise times and amplitudes of fluorescence decays of the conjugate base. At higher concentrations of MMBCD background fluorescence resulting from an impurity overlaps with the fluorescence of the acid at 374 nm but is negligible at 440 nm. For this reason we report the fitting parameters

Figure 3. Fluorescence decays collected from the 440-nm emission of protonated 1-aminopyrene with MMBCD present at a concentration of  $5.5 \times 10^{-2}$  M (A) and without MMBCD present (B). Excitation wavelength: 295 nm. H<sub>2</sub>SO<sub>4</sub> is the protonating agent.

of the fluorescence rise of the conjugate base instead of the fluorescence decay of the acid. Several features are apparent from these data:

(1) Solutions of protonated 1-aminopyrene without  $\beta$ -cyclodextrin present have one rising component in the time-resolved fluorescence of the conjugate base. Upon addition of  $\beta$ -cyclodextrin a second, much shorter, rising component appears in the fluorescence of the conjugate base (see Figure 3). In both the presence and absence of  $\beta$ -cyclodextrin the rise times in the fluorescence of the conjugate base are slightly shorter when H<sub>2</sub>SO<sub>4</sub>, rather than HClO<sub>4</sub>, is used to protonate the conjugate base (ca. 5% difference).

(2) The presence of  $\beta$ -cyclodextrin does not affect the fluorescence decay obtained from nonacidic solutions of 1-aminopyrene (i.e., decays resulting from excitation of the unprotonated form of 1-aminopyrene).

(3) The presence of  $\alpha$ -cyclodextrin does not affect the fluorescence decay of the conjugate base after excitation of protonated 1-aminopyrene.

(4) Rise times in the fluorescence of the conjugate base increase by a factor of 3–4 when 1-aminopyrene is dissolved in acidic solutions of D<sub>2</sub>O, rather than in acidic solutions of H<sub>2</sub>O. Addition of  $\beta$ -cyclodextrin to D<sub>2</sub>O solutions has the same relative effect on the fluorescence decay of 1-aminopyrene as it does when added to H<sub>2</sub>O solutions.

In Figure 4 the fluorescence spectrum of a  $10^{-5}$  M solution of 1-aminopyrene acidified to pH 1.4 with H<sub>2</sub>SO<sub>4</sub> is compared to that of the same solution with MMBCD present at a concentration of  $1.4 \times 10^{-2}$  M. Two peaks are present in the spectrum without MMBCD, a very weak fluorescence from the protonated 1-

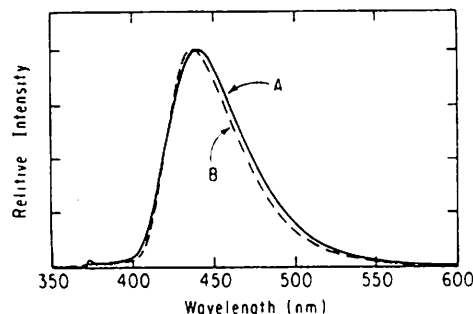


Figure 4. Fluorescence spectra obtained from the 295-nm excitation of protonated 1-aminopyrene with MMBCD present at a concentration of  $1.4 \times 10^{-2}$  M (A) and without MMBCD present (B).

TABLE II: Relative Weights of Fluorescence Rise Times and Fluorescence Anisotropy Measurements of Inclusion Complexes of MMBCD with 1-Aminopyrene<sup>a</sup>

concn of MMBCD, M	amp ratio $a_1/(a_1 + a_2)^b$	$r(0)$	$\phi$ , ps
$8.64 \times 10^{-4}$	$0.098 \pm 0.01$		
$1.73 \times 10^{-3}$	$0.153 \pm 0.005$		
$3.46 \times 10^{-3}$	$0.253 \pm 0.005$	$0.05 \pm 0.02$	$370 \pm 40$
$6.91 \times 10^{-3}$	$0.373 \pm 0.005$	$0.07 \pm 0.02$	$360 \pm 40$
$1.38 \times 10^{-2}$	$0.505 \pm 0.005$	$0.09 \pm 0.02$	$360 \pm 40$
$2.77 \times 10^{-2}$	$0.545 \pm 0.005$	$0.10 \pm 0.02$	$390 \pm 40$
$5.53 \times 10^{-2}$	$0.606 \pm 0.005$	$0.12 \pm 0.02$	$430 \pm 60$

<sup>a</sup>The above data were obtained from fluorescence decays and fluorescence anisotropy decays of  $1.05 \times 10^{-5}$  M solutions of 1-aminopyrene acidified to a pH of 1.4 with  $\text{H}_2\text{SO}_4$  at various concentrations of MMBCD.  $a_1$  and  $a_2$  are the weights of the short and long rise time component, respectively.  $r(0)$  is the initial anisotropy and  $\phi$  is the rotational correlation time. Fluorescence anisotropy decays fit adequately to a single-exponential decay. <sup>b</sup>Fraction of the rising component amplitude originating in the short rising component.

aminopyrene peaks at 375 nm and a much stronger peak near 440 nm. There are several differences in the spectrum measured for 1-aminopyrene with MMBCD as compared to that measured for 1-aminopyrene without MMBCD. The peak near 440 nm is slightly narrower and shifted to be blue by 2–3 nm. The very weak peak at 374 nm is now too small to be observed. In Figure 4 the peaks near 440 nm are normalized for comparison purposes. The amplitude of the 440-nm peak of 1-aminopyrene is about 50% greater in the presence of MMBCD. This rules out the possibility that the observed decrease in the quantum yield and the fluorescence lifetime of the acid band at 374 nm is due to quenching, since there is a higher yield of the conjugate base in the presence of MMBCD.

Table II displays the relative weight of the short rising component and the initial fluorescence anisotropy of protonated 1-aminopyrene as a function of MMBCD concentration. The ratio of the weight of the short rising component of the total rising component increases with concentration to an asymptote of ca. 0.62 (see Figure 5). Likewise, the initial fluorescence anisotropy increases with concentration to an asymptote of ca. 0.13. Measured anisotropy decays fit well to a single exponential with an average rotational correlation time of 380 ps.

Figure 6 shows two series of absorption spectra resulting from titrations of 1-aminopyrene with  $\text{HClO}_4$ . The spectra on the left are from titrating a solution with no MMBCD, while those on the right are from titrating a solution with MMBCD present at a concentration of  $5.53 \times 10^{-2}$  M. To measure the pK of the acid in these solutions intensities of absorption peaks at 340 and 358 nm were used to determine at which pH 50% of 1-aminopyrene is protonated (i.e., when  $\text{pK}_a = \text{pH}$ ). The peaks at 340 and 358 nm are associated with the acid and conjugate base, respectively. Since these two peaks overlap it was necessary at each pH to simultaneously solve the two equations:

$$h_1 = i_1 + r_2 i_2 \quad (1)$$

$$h_2 = i_2 + r_1 i_1 \quad (2)$$

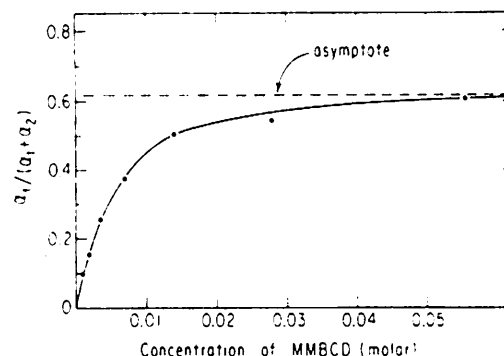


Figure 5. Plot of the fraction of short rising component ( $a_1/(a_1 + a_2)$ ) versus the concentration of MMBCD in a  $1.05 \times 10^{-5}$  M solution of 1-aminopyrene acidified to a pH of 1.4 with  $\text{H}_2\text{SO}_4$ .  $a_1$  and  $a_2$  are the weights (amplitudes) of the short and long rising components, respectively, determined from fits of fluorescence collected from emission at 440 nm of 1-aminopyrene excited with 295-nm light. An asymptote (the dashed line) is approached near 0.62.

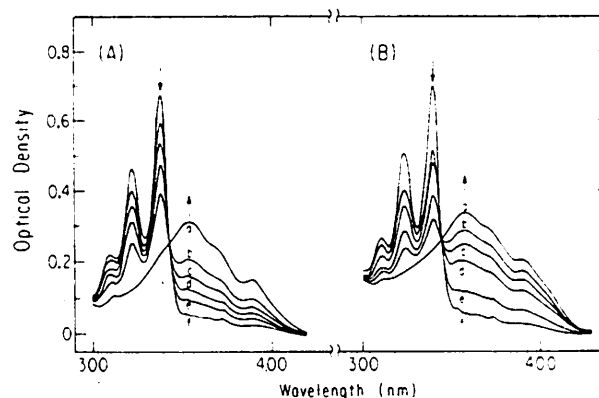


Figure 6. Titration of 1-aminopyrene with and without MMBCD. Displayed are the absorption spectra of 1-aminopyrene without MMBCD (left panel) and with MMBCD at a concentration of  $5.53 \times 10^{-2}$  M (right panel) at various pHs. The arrows above the peaks at 340 and 358 nm indicate decrease and increase, respectively, in intensity with increasing pH. The pHs associated with curves a, b, c, d, e, and f are 6.50, 3.80, 3.29, 2.88, 2.43, and 1.0. The pHs associated with curves a', b', c', d', e', and f' are 6.50, 3.80, 3.29, 2.88, 2.43, and 1.0.

where  $i_1$  and  $i_2$  are the intensities of the 340- and 358-nm peaks, respectively, without contributions from overlap.  $h_1$  and  $h_2$  are the peak heights at 340 and 358 nm measured from the absorption spectrum.  $r_1$  is the ratio of absorbance at 358 nm to that at 340 nm when 1-aminopyrene is completely protonated, while  $r_2$  is the ratio of absorbances at 340 nm to that at 358 nm when 1-aminopyrene is completely unprotonated. We determined a pK of  $3.4 \pm 0.1$  for protonated 1-aminopyrene in water without MMBCD added, while for protonated 1-aminopyrene with MMBCD present at a concentration  $5.53 \times 10^{-2}$  M we determined an apparent pK of  $3.0 \pm 0.1$ . When using  $\text{H}_2\text{SO}_4$  as the titrant we determined an apparent pK at  $2.9 \pm 0.1$  for the 1-aminopyrene solution with MMBCD.

## Discussion

**Complexation of 1-Aminopyrene with  $\beta$ -Cyclodextrin.** 1-Aminopyrene is sparingly soluble in water. Addition of  $\beta$ -cyclodextrin increases the solubility of 1-aminopyrene by 1–2 orders of magnitude. This is expected since the aromatic pyrene is stabilized in the hydrophobic core of  $\beta$ -cyclodextrin as previously observed for inclusion complexes of pyrene with  $\beta$ - and  $\gamma$ -cyclodextrin.<sup>29–34</sup> A schematic representation of  $\beta$ -cyclodextrin was displayed in Figure 1, which shows the dimensions of the apolar cavity. Since only half a molecule of 1-aminopyrene can fit into the cavity,<sup>33</sup> it is possible that a 2:1 or 1:1 ( $\beta$ -cyclodextrin/1-aminopyrene) complex can form. We are also interested in how 1-aminopyrene is oriented in the complex. At present definitive structural information on this complex is lacking; however, our

time-resolved and steady-state fluorescence data provide a partial answer to these two questions.

The fluorescence anisotropy decay of 1-aminopyrene in water is faster than the time resolution of our single photon counting system. However, in the presence of  $\beta$ -cyclodextrin we measured a rotational correlation time of around 380 ps for 1-aminopyrene (see Table II). Assuming a spherical rotor, the expression for the rotational diffusion coefficient is<sup>35</sup>

$$D_s = k_B T / 6V\eta \quad (3)$$

and the rotational correlation time,  $\tau_{or}$ , is related to the rotational diffusion coefficient,  $D_s$ , by

$$\tau_{or} = 1/6D_s \quad (4)$$

where  $k_B$  is the Boltzmann constant,  $T$  the temperature (296 K),  $V$  the displaced volume of the rotor, and  $\eta$  the viscosity (ca. 1 cP). The displaced volume calculated from the rotational correlation time is 1552 Å<sup>3</sup>, which is about 10% greater than the volume calculated from the dimensions of a single cyclodextrin molecule.

As can be observed in the fluorescence spectra of 1-aminopyrene in Figure 4, little change occurs upon addition of MMBCD. If the amino group is buried in the hydrophobic cavity, then bulk water is not available to solvate it. This would eliminate the solvation shell around the amino group and thereby create a large blue shift in the fluorescence. We found that when 1-aminopyrene is dissolved in acetone the fluorescence maximum occurs near 400 nm compared to 440 nm for 1-aminopyrene dissolved in water. Absence of a large blue shift indicates that when 1-aminopyrene is complexed with  $\beta$ -cyclodextrin the amino group is exposed to the solvent. To minimize the free energy of complexation the preferred orientation is with the apolar part of the molecule buried in the hydrophobic cavity<sup>36</sup> and the polar amino group exposed to solvent. Even though the fluorescence spectrum of protonated 1-aminopyrene is almost unaffected by complexation with  $\beta$ -cyclodextrin, there is a large effect on the fluorescence decay. We observe similar behavior when 1-aminopyrene is dissolved in water-alcohol solutions<sup>21</sup> that do not approach the pure alcohol limit: fluorescence spectra whose maxima do not differ appreciably from that of 1-aminopyrene in water and a fluorescence decay that is quite different from that of 1-aminopyrene dissolved in water.

Addition of  $\alpha$ -cyclodextrin to a solution of protonated 1-aminopyrene has no effect on the fluorescence decay, fluorescence anisotropy decay, or the fluorescence spectrum. The cavity diameter of  $\alpha$ -cyclodextrin at the secondary rim is between 4.77 and 5.3 Å, while that of  $\beta$ -cyclodextrin is between 6.0 and 6.5 Å. The aromatic portion of 1-aminopyrene is too large to fit into the  $\alpha$ -cyclodextrin cavity but not the amino group, which may fit into it without its solvation shell. This implies that the effect  $\beta$ -cyclodextrin has on the fluorescence decay of 1-aminopyrene requires formation of the inclusion complex with the aromatic residue of 1-aminopyrene, while the amino group remains exposed to the solution.

It should be noted that we protonated 1-aminopyrene with HClO<sub>4</sub>, because it is a better protonating agent for 1-aminopyrene than H<sub>2</sub>SO<sub>4</sub> or other common mineral acids. However, ClO<sub>4</sub><sup>-</sup> has been shown to form complexes with  $\alpha$ - and  $\beta$ -cyclodextrin.<sup>1,37</sup> To test whether the presence of ClO<sub>4</sub><sup>-</sup> ion affected our results, we carried out a parallel study with H<sub>2</sub>SO<sub>4</sub> as the protonating acid (SO<sub>4</sub><sup>2-</sup> ion has been shown to complex appreciably with  $\alpha$ - or  $\beta$ -cyclodextrin<sup>1</sup>). Comparison of the results reveals no significant difference between HClO<sub>4</sub> and H<sub>2</sub>SO<sub>4</sub> as the protonating agent.

**Calculation of the Binding Constant of 1-Aminopyrene and  $\beta$ -Cyclodextrin.** The weight of the short rising component,  $a_1$ , of 1-aminopyrene increases with increasing concentration of  $\beta$ -cyclodextrin. It would appear that the relative weight of the short component is equal to the fraction of 1-aminopyrene complexed with  $\beta$ -cyclodextrin. However, an asymptote is reached where 38% of the total rising amplitude ( $a_1 + a_2$ ) is contributed by  $a_2$ , a rising component similar to that of the unbound 1-aminopyrene. We conclude that some of the 1-aminopyrene is not affected kinetically by complexation (see below). For acidic solutions of

TABLE III: Binding Constants Determined for Inclusion Complexes of MMBCD with 1-Aminopyrene<sup>a</sup>

concn of MMBCD, M	bound:total 1-aminopyrene	binding const <sup>b</sup>	$r(0)/r_\infty(0)$	binding const, <sup>c</sup> M
$8.64 \times 10^{-4}$	0.16	220		
$1.73 \times 10^{-3}$	0.25	194		
$3.46 \times 10^{-3}$	0.41	202	0.42	206
$6.91 \times 10^{-3}$	0.60	217	0.58	202
$1.38 \times 10^{-2}$	0.82	330	0.75	217
$2.77 \times 10^{-2}$	0.88	266	0.83	176
$5.53 \times 10^{-2}$	0.98			

<sup>a</sup> The values listed above are derived from data displayed in Table II. The column labeled bound:total 1-aminopyrene is the fraction of the total 1-aminopyrene bound determined from rise times in the fluorescence decays of 1-aminopyrene, as described in the text. The column labeled  $r(0)/r_\infty(0)$  is the ratio of the initial fluorescence anisotropy to the initial fluorescence anisotropy determined for infinite concentration of MMBCD. <sup>b</sup> Binding constants determined from values listed in the column labeled bound:total 1-aminopyrene. <sup>c</sup> Binding constants determined from values listed in the column labeled  $r(0)/r_\infty(0)$ .

1-aminopyrene with MMBCD concentrations greater than  $2.76 \times 10^{-2}$  M changes in both the relative weight of the short rise time component and the initial fluorescence anisotropy are relatively small. This suggests that most of the 1-aminopyrene is bound at a concentration of  $2.76 \times 10^{-2}$  M MMBCD. Further it indicates that 1-aminopyrene binds to  $\beta$ -cyclodextrin in at least two different orientations. One orientation produces the faster fluorescence rise time, while the other orientation has a fluorescence rise time similar to that of unbound 1-aminopyrene. The relative weight of these two orientations can be determined directly from the asymptote of the curve shown in Figure 5. From the relative weights of the two rising components—taking into consideration that 38% of the bound species contributes to the long rising component and assuming only a 1:1 complex forms—we determined a binding constant for complexation of  $237 \pm 47$  M<sup>-1</sup> (see Table III). We also obtained the fraction of bound 1-aminopyrene in acidic solutions at a particular concentration of MMBCD from the ratio of the initial fluorescence anisotropy to the asymptotic value of the initial fluorescence anisotropy (see Table III). This assumes no contribution to the fluorescence anisotropy from unbound 1-aminopyrene, which decays at least 1 order of magnitude faster. A binding constant determined from these ratios yielded a value of  $200 \pm 23$  M<sup>-1</sup>, in good agreement with the value obtained from the fluorescence rise times. The low initial anisotropy observed for totally bound 1-aminopyrene may result from internal conversion between low-lying singlet excited states, as has been observed for indole.<sup>38,39</sup>

We also carried out a double-reciprocal analysis for the binding constant of the complex plotting

$$\frac{1}{K[\beta\text{-CD}]} = \frac{[1\text{-aminopyrene}]_0}{[\text{inclusion complex}]}$$

against the inverse of the concentration of  $\beta$ -cyclodextrin (see Figure 7). Our data do not indicate a transition from a 1:1 to a 2:1 ( $\beta$ -cyclodextrin/1-aminopyrene) complex. The equilibrium constant that is extracted from the slope of Figure 7 is  $214 \pm 5$  M<sup>-1</sup>, in good agreement with the previous determinations of the binding constant. We conclude that our data suggest that only 1:1 complexes are formed.

**Orientation of 1-Aminopyrene in the Complex with  $\beta$ -Cyclodextrin.** We suggest possible orientations of 1-aminopyrene complexed to  $\beta$ -cyclodextrin that are consistent with our results and provide a basis for a model that explains the increased rate of excited-state proton transfer observed for protonated 1-aminopyrene complexed with  $\beta$ -cyclodextrin. As previously mentioned, the proton transfer rate of 1-aminopyrene is affected by the solvent environment. Recent work<sup>40</sup> on proton transfer rates in aqueous binary mixtures gives a linear correlation of the proton transfer rate with the gas-phase basicity (proton affinity) of organic cosolvents that contain oxy-groups capable of forming hydrogen bonds with water. The faster of the two proton transfer

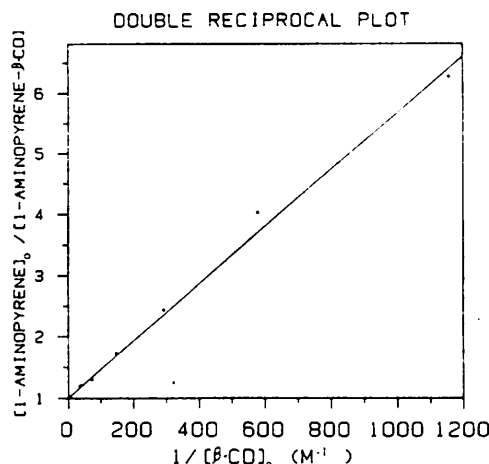


Figure 7. Double-reciprocal plot for the 1-aminopyrene  $\beta$ -CD complex. The fraction for the complexed  $\beta$ -CD out of its total concentration was taken from Table III. Assuming that  $\beta$ -CD is present in excess and in the case of simple 1:1 complexation the following double-reciprocal relation holds:  $1/K[\beta\text{-CD}] = [1\text{-aminopyrene}]_0/[\text{inclusion complex}]$ , where  $K$  is the  $\beta$ -CD inclusion complex equilibrium constant. The full line shown has a  $[\text{slope}]^{-1}$  of 215 M.

rates measured for complexed 1-aminopyrene is similar to the proton transfer rate of 1-aminopyrene in aqueous binary mixtures of simple alcohols when present in comparable molar ratios to water. The slower of the two proton transfer rates of complexed 1-aminopyrene is the same as that measured for 1-aminopyrene in bulk water. We suggest that the hydroxyl rims of  $\beta$ -cyclodextrin have a similar affect on their immediate water environment as do the simple alcohols in binary aqueous mixtures. To be more specific, in those orientations where the amino group of the complexed 1-aminopyrene is farther away from the hydroxyl rims and is completely surrounded by bulk water molecules the rate of proton transfer is the same as that of uncomplexed 1-aminopyrene. In those orientations where the amino group is near a hydroxyl rim-water interface, it is affected by the microenvironment created by the  $\beta$ -CD cavity and the rate of proton transfer is accelerated by roughly a factor of 2. Figure 8 shows two possible orientations. Our data indicate that the latter orientation is preferred by a ratio of 1.5:1. We conclude that the environment near the end of the cavity of  $\beta$ -cyclodextrin resembles the solvent environment in binary aqueous mixtures with simple alcohols. This conclusion is similar to the one reached by several authors using different types of probe molecules complexed with  $\beta$ -CD.<sup>41-43</sup>

**Free Energy Relation in the Proton Dissociation of the 1-Aminopyrene- $\beta$ -Cyclodextrin Complex.** As noted above, the  $pK$  of 1-aminopyrene determined from titration with  $\text{HClO}_4$  is 3.4 and noticeably lowered when complexed with  $\beta$ -cyclodextrin. A similar effect on the  $pK_a$  of 1-*d*-naphthyl-3-(dimethylamino)propane was reported by Cox et al.<sup>43</sup> The apparent value of the  $pK$  for the complex was determined to be 3.0, with a titration end point not as clearly defined as that for the uncomplexed 1-aminopyrene. Assuming there are two sets of orientations for 1-aminopyrene complexed to  $\beta$ -cyclodextrin, which affect proton transfer rates differently, we also expect the  $pK$  to depend on orientation (see below). It is reasonable to expect that for the orientation where the amino group is farther away from the hydroxyl rim and toward the bulk water the  $pK$  of the complex is similar to that for uncomplexed 1-aminopyrene. We assume that the  $pK$  determined for complexed 1-aminopyrene is an average of two  $pK$ s resulting from the two sets of orientations. If we weight the contribution of the  $pK$  from each set of orientations based on the weight determined for that orientation in the fluorescence decay, we calculate a  $pK$  of  $2.6 \pm 0.1$  for 1-aminopyrene when the amino group is nearer to the  $\beta$ -CD cavity.

The rate of proton transfer between a donor acid and acceptor base,  $k_p$ , is given by the Brønsted relation<sup>25,44</sup>

$$\log k_p = \log \alpha + \beta \Delta pK_{AB} \quad (5)$$

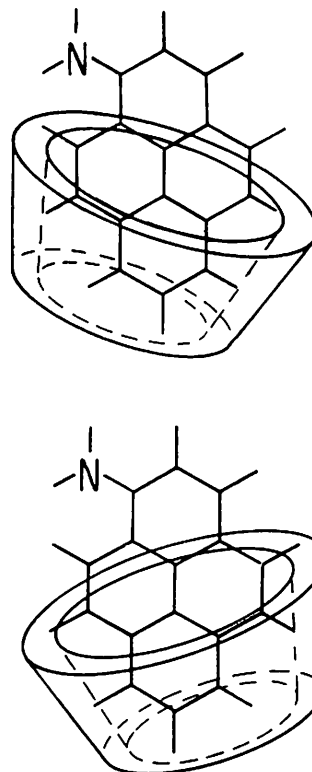


Figure 8. Two possible orientations for 1-aminopyrene complexed to  $\beta$ -cyclodextrin. The top figure shows an orientation where the amino group is directed toward the secondary hydroxyl rim of  $\beta$ -CD. The bottom figure shows an orientation where the amino group is further away from the  $\beta$ -CD cavity.

where  $\alpha$  and  $\beta$  are constants for a series of similar reactions.  $\Delta pK_{AB}$  is the difference in  $pK$ s of the donor and acceptor acids. For a fixed acceptor (water) the Brønsted relation reduces to

$$\log k_p = \log \alpha + \beta pK_A$$

Thus the Brønsted relation suggests that the rate of proton transfer to water is a linear function of the acidity constant within a family of acids. Deviation from this empirical relation occurs in very exothermic reactions. However, even in this region roughly linear correlations are found over a small range of  $pK_a$  values. This is indeed the case, as shown by Pines and Fleming,<sup>21</sup> for 1-aminopyrene in various aqueous binary mixtures. Assuming that complexation produces similar changes in both the ground- and the excited-state  $pK_a$  of 1-aminopyrene, one finds that the free energy correlation between  $\Delta pK$  as measured in the ground state and the fast component in the rate of the excited-state proton transfer falls in the region for aqueous binary mixtures of simple alcohols<sup>21</sup> near the 75% (vol) ethanol data point (see Figure 9). A similar conclusion was reached by Heredia et al. by considering the  $Z$  values of  $\beta$ -CD complexes.<sup>42</sup> This again supports our conclusion that the water environment near the cavity of  $\beta$ -cyclodextrin resembles the one found in water-alcohol mixtures.

**The Rate of Deuteron Dissociation of Deuterated 1-Aminopyrene Complexed with  $\beta$ -Cyclodextrin.** The rate of deuteron dissociation of deuterated 1-aminopyrene (deuterated with  $\text{D}_2\text{SO}_4$  at a concentration of  $5 \times 10^{-2}$  M) is accelerated by a factor of 3 upon complexation with MMBCD (see Table I). This is a similar effect to that observed for the rate of proton dissociation of protonated 1-aminopyrene complexed with MMBCD. Having used the rate of proton transfer of protonated 1-aminopyrene in binary mixtures of water-alcohol solutions as a model to explain the rate of proton transfer of protonated 1-aminopyrene complexed with  $\beta$ -cyclodextrin, we now extend this analogy to deuterated systems. We measured a rate of deuterium dissociation for deuterated 1-aminopyrene in aqueous mixtures of 80% (by volume) ethanol (acidified with  $\text{D}_2\text{SO}_4$  at a concentration of  $5 \times 10^{-2}$  M) of 2.6

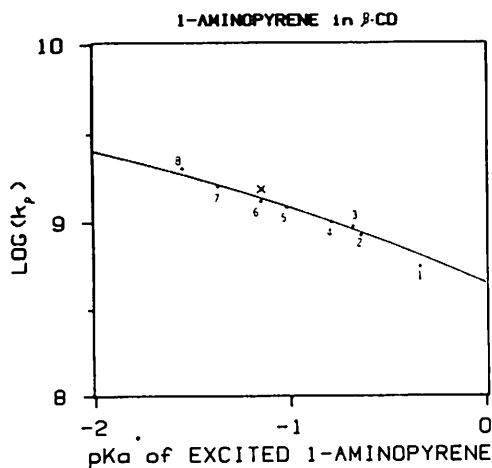


Figure 9. Free energy correlation between  $pK_a$  and the fast component in the excited-state proton transfer rate of 1-aminopyrene complexed with  $\beta$ -cyclodextrin. The data point for the complex is shown as "X". Also shown are the proton transfer rates vs  $pK_a$  in several water-organic solvent mixtures: (1) pure  $H_2O$ ; (2) 40% (vol) methanol; (3) 35% (vol) ethanol; (4) 60% (vol) methanol; (5) 55% (vol) ethanol; (6) 75% (vol) ethanol; (7) 70% (m) dioxane; (8) 70% (m) DMSO. The free-energy correlation (full line) was taken from ref 21.

ns, in close agreement to that measured for deuterated 1-aminopyrene complexed with MMBCD (2.5 ns). Thus for both free and complexed systems we observe a deuterium effect of around 3.5.

**Proton Transfer Rates of  $\alpha$ -Naphthol Complexed with  $\beta$ -Cyclodextrin.** Complexes of  $\alpha$ -naphthol (a neutral photoacid) with  $\beta$ -cyclodextrin have been previously studied.<sup>45</sup> It was found that the OH group lies close to the secondary hydroxyl rim of  $\beta$ -cyclodextrin. The excited-state proton transfer rate of  $\alpha$ -naphthol complexed with  $\beta$ -cyclodextrin was studied via fluorescence decay measurements. We were able to adequately fit the measured fluorescence decay of the acid collected at 370 nm with two decay components. Values determined for the decay times of these two decay components were  $700 \pm 100$  ps (75%) and  $1600 \pm 100$  ps (25%). In pure water  $\alpha$ -naphthol had a fluorescence decay that was fit to a single exponential with a decay time of  $36 \text{ ps} \pm 5 \text{ ps}$ . Consequently, the rate determined for deprotonation of  $\alpha$ -naphthol complexed to  $\beta$ -cyclodextrin is observed to be much slower than that of uncomplexed  $\alpha$ -naphthol. Adhering to the analogy that the interfacial water of  $\beta$ -cyclodextrin has the properties of water in binary mixtures with simple alcohols, this decreased rate in deprotonation of complexed  $\alpha$ -naphthol is indeed similar to that for  $\alpha$ -naphthol in water-alcohol mixtures. At 80% (by volume) ethanol this rate was measured by Robinson et al.<sup>47</sup> to be ca. 1.1 ns, which is similar to the average rate found by us in  $\beta$ -cyclodextrin- $\alpha$ -naphthol complexes (0.93 ns).

## Conclusion

A significant aspect of this study is the finding that the sensitivity of the kinetic measurements is greater than that of the corresponding spectral steady-state measurements in revealing ground-state structural information. While only a 2–3-nm blue shift is observed in the fluorescence spectrum of 1-aminopyrene when complexed with  $\beta$ -cyclodextrin, the rate of proton transfer is affected by as much as a factor of 3. Using proton transfer as a probe, we were able to detect some structural heterogeneity among the complexes and not merely a structural average. Our study provides evidence that interfacial interactions around cyclodextrins may be modeled by corresponding interactions in suitable homogeneous mixtures of water and organic solvents. In the particular case of a photoacid probe, the hydrogen bond interactions around the cavity of these cyclodextrins influence the proton transfer rate. Interestingly, both increased (1-aminopyrene) and decreased ( $\beta$ -naphthol) rates are generated by complexation and both are consistent with the same ethanol-water ratio (75–80%

alcohol by volume). The effect of deuteration of 1-aminopyrene is also quantitatively consistent with the value at the same ethanol-water composition. We suggest that the chemical nature of water around the cavity rim is greatly influenced by the strength and nature of hydrogen bond interactions with varying polar groups present on the cavity surface. This effect may play an important role in acid-base catalytic activity of enzymes. As for  $\beta$ -CD, our data indicate that the microenvironment near its cavity rim resembles the one present in water-ethanol mixtures near the 75% (vol) alcohol composition. The transition to a bulk waterlike environment probably occurs within two or three solvation layers of water, as indicated by the considerable fraction of the complexed 1-aminopyrene that is unaffected kinetically. The ethanol-like characteristic of the  $\beta$ -CD rim is probably due to the extensive network of OH and methoxy groups that is present at the rim. A similar conclusion was reached by Cox et al.<sup>43</sup> who found that the polarity near the  $\beta$ -CD rim is slightly higher than that of pure ethanol.<sup>43</sup>

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