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Inclusion Complexes of Cyclodextrins with 4-Amino-1,8-Naphthalimides

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Abstract

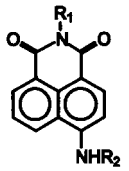
The formation of inclusion complexes between 4-amino-1,8-naphthalimides and cyclodextrins (CDs) was investigated. The naphthalimides used in the study were 4-amino-1,8-naphthalimide (**I**) and 4-(2-phosphonoethylamino)-N-(2-phosphonoethyl)-1,8-naphthalimide, tetraethylester (**II**). The CDs employed were α-CD, β-CD, γ-CD, HP-α-CD, HP-β-CD and HP-γ-CD (HP = hydroxypropyl). Evidence for complex formation was obtained from the changes in the fluorescence spectra of the dyes in the presence of increasing amounts of the CDs. The most striking changes were observed with HP-β-CD and HP-γ-CD. Treatment of the data using Benesi–Hildebrand plots was consistent with a 1:1 inclusion model. The determined stability constants were (K_{eq}, M^{-1}): 106 (**I**:HP-β-CD, pH = 2.0), 193 (**I**:HP-β-CD, pH = 7.0), 113 (**I**:HP-γ-CD, pH = 7.0), 155 (**II**:HP-β-CD, pH = 2.0), 121 (**II**:HP-β-CD, pH = 7.0), 301 (**II**:HP-γ-CD, pH = 7.0). It can be concluded that compound **I** forms a more stable complex with HP-β-CD than with HP-γ-CD. Compound **II**, on the other hand, forms a more stable complex with HP-γ-CD than with HP-β-CD.

Introduction

4-Amino-1,8-naphthalimides (ANI) constitute a class of strongly fluorescent dyes with very interesting photophysical properties [1, 2]. Owing to these properties, ANIs have been used in a series of important applications, ranging from the development of advanced materials to the pharmaceutical area. Examples of technological applications include electroluminescent (EL) devices [3, 4], sensors [5–7], switchers [8] and artificial photosynthetical systems [9, 10]. Regarding biomedical applications, ANIs have been used as fluorescent labeling agents for biological tissues [11, 12] and as antiviral agents [13, 14].

The importance of ANIs in different research fields urges a study of their interaction with cyclodextrins (CDs). CDs are cyclic oligossacharides that form inclusion complexes with a great variety of organic compounds in aqueous solution [15]. Complex formation between ANIs and CDs is expected to improve the properties of the dye in view of the above applications. For instance, increased fluorescence quantum yields, which are usually observed in CD complexes, could improve the performance of ANIs in EL devices and switchers. In the case of pharmaceutical applications, the increased water solubility of CD-included drugs should improve the bioavailability of ANIs. Complexes of ANIs with CDs could also be used to deliver the dye to specific target tissues in the human body.

In this article, we present studies of complex formation between two different ANI derivatives and a variety of cyclodextrins. The structures of the dyes employed in this study are shown in Scheme 1. The CDs tested were α, β and γ-cyclodextrins, as well as their highly water soluble hydroxypropyl (HP) derivatives HP-α-CD, HP-β-CD and HP-γ-CD.



$R_1 = R_2 = H$ (compound I)

$R_1 = R_2 = CH_2CH_2P(O)(OEt)_2$ (compound II)

Scheme 1. Structures of the 4-amino-1,8-naphthalimides employed in this study.

Experimental details

Materials

4-Amino-1,8-naphthalimide (**I**) was purchased from Aldrich and used as received. α-Cyclodextrin hydrate, β-cyclodextrin hydrate, γ-cyclodextrin hydrate, sulfated β-cyclodextrin (sodium salt), hydroxypropyl-α-cyclodextrin (molar substitution = 0.6, $M_W \sim 1,180$) and hydroxypropyl-γ-cyclodextrin (molar substitution = 0.6, $M_W \sim 1,580$) were purchased from Aldrich and used as received. Hydroxypropyl-β-cyclodextrin was a gift from Cerestar and was used as received.

4-(2-Phosphonoethylamino)-N-(2-phosphonoethyl)-1,8-naphthalimide, tetraethylester (**II**) was synthesized by

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reacting 4-nitro-1,8-naphthalic anhydride with two equivalents of diethyl 2-aminoethylphosphonate [16,17], as described below.

Diethyl 2-aminoethylphosphonate

2-Bromoethylphthalimide (Aldrich) was reacted with triethylphosphite (Aldrich), as described in reference 16. The phosphonate ester obtained from this reaction was then treated with hydrazine, following the method in reference 17, giving diethyl 2-aminoethylphosphonate in good yields.

Compound **II**

4-nitro-1,8-naphthalic anhydride (Aldrich) (293 mg, 1.2 mmol) and diethyl 2-aminoethylphosphonate (442 mg, 2.4 mmol) were dissolved in 10 mL 1-methyl-2-pyrrolidone (Merck), and the mixture was heated at 115 °C for 2 h. After cooling, the reaction mixture was diluted with 200 mL of methylene chloride (Merck), and chromatographed in silica gel, giving 246 mg of **II** (0.46 mmol, 38% yield). ^{1}H-NMR ($CDCl_3$ δ: 1.37 (m, 12H, —CH_3), 2.30 (m, 4H, —CH_2—P), 3.70 (m, 2H, —NH—CH_2), 4.17 (m, 8H, O—CH_2), 4.42 (t, 2H, N—CH_2), 6.67 (d, 1H, Ar), 6.78 (t, 1H, —NH), 7.63 (t, 1H, Ar), 8.24 (d, 1H, Ar), 8.46 (d, 1H, Ar), 8.57 (d, 1H, Ar). The product was recrystallized from ethyl acetate before using for complexation studies.

Equipment

Absorption spectra were taken with a Shimadzu MultiSpec 1501 spectrophotometer. Fluorescence spectra were registered either with a Spex DM3000F (frontal face mode) or with a Hitachi F-2500 fluorescence spectrophotometer.

Methods

Aqueous solutions with varying concentrations of CDs for binding studies were prepared as follows: concentrated stock solutions (*ca* 0.1 M) of the CDs were prepared in either 0.01 M phosphate buffer (pH = 7.0) or in 0.01 M HCl (pH = 2.0). Aliquots from these stock solutions were then mixed with aliquots from the buffer (or HCl) solution in test tubes to give the desired CD concentration (total volume of 2.0 mL). Compound **I** or **II** (2 μL from a 1 mM stock solution in N,N-dimethylformamide) was then added to each tube, giving [ANI] = 1.0×10^{-6} M. The tubes were mixed in a shaker for 24 h at 25 °C for equilibration before measuring the fluorescence spectra.

Results and discussion

The absorption spectra of 4-amino-1,8-naphthalimides were not quite sensitive to the polarity of the medium (not shown). The absorption maximum of compound **I**, for example, was found at 432 nm in water and ethanol solutions. The same value was found in concentrated solutions of α-CD, β-CD, γ-CD, HP-β-CD and sulfated β-CD (sodium salt). The absorption spectra, therefore, were not very useful in studying the complex formation between compounds **I** and **II** and cyclodextrins.

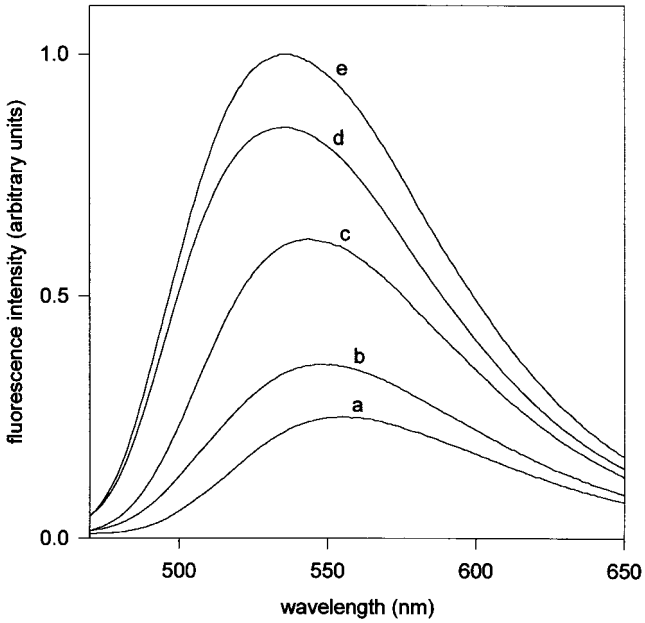


Figure 1. Fluorescence emission spectra of 4-amino-1,8-naphthalimide (**I**) in water (a), 0.05 M HP-α-CD (b), 0.10 M HP-γ-CD (c), 0.15 M HP-β-CD (d) and ethanol (e). Excitation wavelength = 430 nm. [**I**] = 1×10^{-6} M.

In contrast to the absorption spectra, the fluorescence emission spectra of **I** and **II** were quite sensitive to changes in the environment of the molecules. As seen in Table 1 and Figure 1, the emission maxima of the dyes were blue-shifted and the emission intensities increased as going from water to lower polarity solvents. The emission maximum of **I**, for instance, changes from 555 nm in water to 536 nm in ethanol and 497 nm in methylene chloride, followed by an increase in intensity of about four-fold in the organic solvents. The emission data in solutions of cyclodextrins (Figure 1, Table 1) show that the dyes are in a less polar environment than in water, indicating inclusion of the naphthalimides within the CD cavities. The emission spectra of **I** and **II**, on the other hand, were not affected by the presence of concentrated maltose in a control experiment (Table 1), showing that the effect observed with CD solutions was indeed due to inclusion, rather than an external effect due to the presence of concentrated carbohydrate.

It can be seen in Table 1 that α-CD had almost no effect on the emission spectra of **I** and **II**. A moderate effect was observed with HP-α-CD, β-CD, sulfated β-CD and γ-CD. The most striking changes, however, were observed with HP-β-CD and HP-γ-CD (Figure 1), which gave emission spectra for **I** and **II** similar to those obtained in ethanol solution. The polarity inside the cavity of CDs has been regarded as similar to that of ethanol, and therefore the results obtained indicate that the chromophores are completely included in HP-β-CD and HP-γ-CD. For the other CDs, inclusion was apparently not complete, with a portion of the molecule being exposed to the aqueous environment. In the particular case of α-CD, there was no evidence for com-

Table 1. Fluorescence emission data for compounds **I** and **II** in different solvents and in cyclodextrin solutions

Solution	Compound **I**[a]		Compound **II**[a]	
	λ_{max}^{em} (nm)[b]	I/I_w[c]	λ_{max}^{em} (nm)[b]	I/I_w[c]
H_2O	555	1	550	1
EtOH	536	3.94	535	1.83
CH_2Cl_2[d]	498	4.69	513	1.53
HCl 0.1 M	556	0.48	550	0.79
Maltose 0.4 M	553	1.23	549	1.07
α-CD 0.05 M	555	1.07	550	1.15
HP-α-CD 0.05 M	547	1.43	548	1.35
β-CD 0.01 M	547	1.30	543	1.21
HP-β-CD 0.01 M (0.15 M)	537 (535)	2.41 (3.49)	536 (534)	1.59 (1.74)
Sulfated β-CD[e] 0.06 g/mL	544	1.39	547	1.07
γ-CD 0.036 M	547	1.59	543	1.58
HP-γ-CD 0.033 M (0.1 M)	544 (543)	2.12 (2.33)	542 (541)	1.66 (1.64)

[a] [**I**] = [**II**] = 1.0×10^{-6} M.
[b] $\lambda_{ex} = 430$ nm.
[c] Intensity ratio (solution/water).
[d] $\lambda_{ex} = 410$ nm.
[e] Sodium salt.

plex formation, since the emission spectra were not changed relative to the spectra in water.

The above results are consistent with the dimensions of the CD cavities [15]. The cavity of α-CD (internal diameter = 5.7 Å) is too small to host a naphthalene ring. β-CD and γ-CD (internal diameter = 7.8 and 9.5Å, respectively), on the other hand, have larger cavities and do form inclusion complexes with other naphthalimide derivatives, as shown in a previous report from our group [18]. The presence of the flexible hydroxypropyl substituents in HP-α-CD, HP-β-CD and HP-γ-CD causes an extra increase in the cavity size, allowing a better fit of the host molecules.

The changes in fluorescence spectra of CD-incorporated dyes can be used to study the binding process in more detail, in order to obtain the stoichiometries and stability constants for complex formation. In the present work, we chose to study in more detail the complexes of HP-β-CD and HP-γ-CD, since these CDs caused the most pronounced changes on the spectroscopic behaviour of the naphthalimide dyes.

Figure 2 shows the effect of increasing the concentration of HP-β-CD on the emission spectrum of compound **II** in aqueous solution (0.01 M phosphate buffer, pH = 7). The spectral changes observed indicate the gradual incorporation of compound **II** in the CD cavity. No further changes were observed as the CD concentration was increased above 0.1 M, indicating that at this point all the dye was in the complexed form.

The data in Figure 2 were treated assuming a 1:1 binding model (Equation (1)). If this model is correct, a Benesi–Hildebrand plot of the data according to Equation (2) should be linear, and the stability constant can be obtained by dividing the intercept by the slope of the plot [19, 20]. In these equations, S is the fluorescent guest (**I** or **II** in this case), S-CD the inclusion complex, K_{eq} the stability constant for complex formation, I/I_0 the fluorescence intensity ratio between the dye in the presence (I) and in the absence

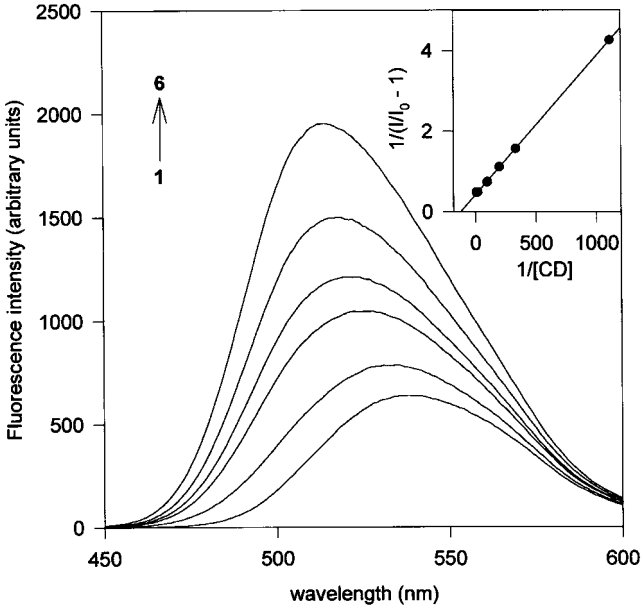


Figure 2. Effect of the addition of HP-β-CD on the emission spectra of compound **II** (in water containing 0.01 M phosphate buffer, pH = 7.0). [HP-β-CD] (M): 0 (**1**), 9×10^{-4} (**2**), 3×10^{-3} (**3**), 5×10^{-3} (**4**), 1×10^{-2} (**5**), 1×10^{-1} (**6**). Excitation wavelength = 430 nm. [**II**] = 1×10^{-6} M. Inset: plot of the emission data according to Equation (2) (the solid line represents the linear regression of the experimental points).

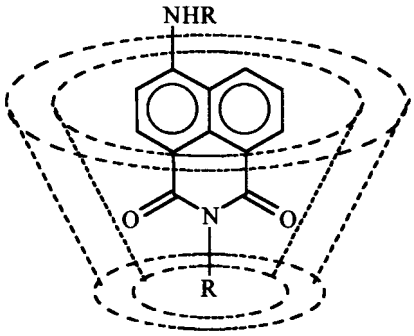
(I_0) of CD, and Φ_{CD}/Φ_0 is the quantum yield ratio between complexed (Φ_{CD}) and free (Φ_0) naphthalimide. Equation (2) has been used by several authors to determine stability constants between cyclodextrins and fluorescent guests [19, 20].

$$S + CD \rightleftarrows S\text{-}CD \quad K_{eq} = [S\text{-}CD]/[S][CD] \qquad (1)$$

Table 2. Stability constants for naphthalimide:cyclodextrin complexes determined using Equation (2)

CD	K_{eq} (M^{-1})	
	Compound **I**	Compound **II**
HP-β-CD (pH = 2.0)[a]	106	155
HP-β-CD (pH = 7.0)[b]	193	121
HP-γ-CD (pH = 7.0)[b]	113	301

[a] 0.01 M HCl.
[b] 0.01 M phosphate buffer.



Scheme 2. Proposed structure for the complexes between 4-amino-1,8-naphthalimides and hydroxypropyl-cyclodextrins.

$$\frac{1}{(I/I_0 - 1)} = \frac{1}{(\Phi_{CD}/\Phi_0 - 1)} + \frac{1}{(\Phi_{CD}/\Phi_0 - 1)K_{eq}} \frac{1}{[CD]} \quad (2)$$

A plot of the data in Figure 2 according to Equation (2) is shown in the inset of the figure. The graphic is linear, indicating the validity of the 1:1 binding model for the complex **II**:HP-β-CD. The treatment was also employed for the complexes **II**:HP-γ-CD, **I**:HP-β-CD and **I**:HP-γ-CD. The plots were all linear, indicating the formation of 1:1 complexes in all cases. The effect of pH on the inclusion process was also studied. The results of the binding studies are summarized in Table 2.

Although the differences between the values in Table 2 were not very large in comparison to the accuracy of the method, some conclusions can be drawn. It can be observed from the table that, at pH = 7, compound **I** forms more stable complexes with HP-β-CD than with HP-γ-CD. This result is consistent with a tighter fit of the guest in the smaller cavity of HP-β-CD, and a looser fit in the larger cavity of HP-γ-CD. The same trends have been observed for non-substituted 1,8-naphthalimides [18].

In the case of compound **II**, a different behaviour was observed. This naphthalimide has more affinity for HP-γ-CD than for HP-γ-CD, in contrast to the case of compound **I**. The difference can be explained by taking in account the large size of the substituent groups in **II**, increasing the volume of the whole molecule. This would cause a hindrance for the inclusion of **II** in the smaller cavity of HP-β-CD, but it could, on the other hand, improve the fit within the larger cavity of HP-γ-CD.

The effect of the pH on the inclusion behaviour of **I** and **II** is not very pronounced (Table 2). The stability of the complex **I**:HP-β-CD is increased in pH = 7, relative to pH = 2, what is consistent with the protonation of the dye in acidic pH, leaving it more hydrophilic. In the case of compound **II**, the effect of the pH on the inclusion behaviour is the opposite of that found for **I**, although the difference in K_{eq} between the two pHs is rather small.

The small effect of the pH on the affinities of naphthalimides for the CD cavities could be due to inclusion of the dyes with the imide side towards the interior of the cavity, leaving the 4-amino group exposed to the water, as suggested in Scheme 2. If the inclusion complexes had the amino group inside the cavity (an orientation opposite to that in Scheme 2), a pronounced effect of protonation would be expected, what was not the case. Nevertheless, a more detailed study is necessary in order to understand better these pH effects.

Conclusions

4-amino-1,8-naphthalimides form 1:1 inclusion complexes with a variety of cyclodextrins. The inclusion in the CD cavity causes blue shifts in the emission maxima of the dyes, as well as increases in the quantum efficiency. The most pronounced effects were found with hydroxypropyl-β-CD and hydroxypropyl-γ-CD. The binding data suggests that the guests are included with the imide side towards the interior of the cavity, leaving the amino group exposed to the aqueous environment.

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