Effect of Sodium Perchlorate on the Binding of 2-(4′-Aminophenyl)- and 2-(4′-(*N*,*N*′-Dimethylamino)phenyl)benzothiazole with β-Cyclodextrin in Aqueous Solution

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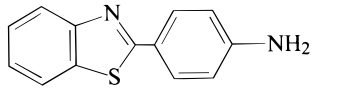
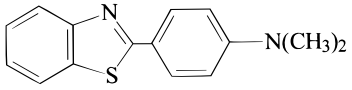
The host−guest complexation of 2-(4′-aminophenyl)- and 2-(4′-(*N*,*N*-dimethylamino)phenyl)benzothiazole (APB and DAPB, respectively) with β-cyclodextrin (β-CDx) were studied in aqueous solution by use of fluorescence spectroscopy. The induced circular dichroic (ICD) spectra of the inclusion complexes were also measured to confirm complexation and to estimate the orientation of the guest molecule in the cyclodextrin cavity. The association constants for binding of the molecules with β-CDx were estimated in phosphate buffer containing varying concentrations of $NaClO_4$. The association constant for binding of DAPB is almost 4 times as high as that of APB. In the case of DAPB, the association constant was found to decrease with an increase in salt concentration. However, the association constant of APB initially increases and then decreases with an increase in salt concentration. The optical rotation data of β-CDx in the absence and presence of $NaClO_4$ suggest complexation of ClO_4^- with β-cyclodextrin.

Introduction

Substances such as urea, guanidinium chloride (GnCl), and $LiClO_4$ are often referred to as salting-in agents for their ability to enhance water solubility of hydrocarbons, e.g., butane and benzene.[1] In contrast to the salting-in effects of $LiClO_4$, LiCl causes benzene and benzaldehyde[2] to salt out of water and is referred to as a salting-out reagent. Breslow et al.[3] have investigated the effects of salting-in and salting-out agents on chemical reactions in aqueous as well as nonaqueous media. They observed that salting-out reagents dramatically increase the rate of bimolecular organic reactions while salting-in agents retard reaction rates. They also noted that GnCl which is a salting-in agent in water acts as a salting-out agent in ethylene glycol and formamide solutions.[4] The increase of reaction rates has been attributed to increased hydrophobic interaction between reactants.[5] Recently, Sarkar et al. reported salting effects on the hydrophobic binding of sodium 2-(*p*-toluidino)naphthalene-6-sulfonate (TNS) with cyclodextrins (CDx).[6] They proposed that the decrease of fluorescence intensity of TNS in aqueous cyclodextrin solutions in the presence of $LiClO_4$, GnCl, and CsBr is due to a direct interaction of the ions with the hydrophobic probe molecule bound to the CDx cavity. In contrast, the increase in fluorescence intensity of TNS in the presence of LiCl and tetra-*n*-butylammonium bromide (TBBr) ion has been interpreted as due to the reduction of local solvent polarity. While the salting-out effect is well understood,[7,8] the reason for salting-in effects is not very clear. The increase or decrease of hydrocarbon solubility due to the presence of salting-in or salting-out agents, respectively, has been correlated with the size and thus the polarizability of the ions.[9] For example, ions with small size and high polarizability (e.g., Li^+ and Cl^-) decrease aqueous solubility of hydrocarbons, while large ions which have low polarizability (e.g., ClO_4^- and I^-) increase the solubility of hydrocarbons in water. This has been discussed nicely in an article by Breslow.[10]



(APB)



(DAPB)

Figure 1. Molecular structures of APB and DAPB.

Most studies on the effects of salting-in and salting-out reagents on solubility and chemical reactions, including those mentioned above, are often performed in the presence of a high concentration (>1 M) of the salt. However, the effects in the presence of low concentration of the salting-in and salting-out agents have not been vigorously studied. Therefore, to understand the phenomenon, we have investigated the influence of NaCl and $NaClO_4$ on the complexation equilibria of two structurally similar, charge neutral compounds, APB and DAPB (see Figure 1 for structures), with β-CDx in aqueous solutions by exploiting the changes in their fluorescence properties. It is the aim of this work to characterize the mechanism of the effect of ClO_4^- on the binding of the substrates with the β-CDx in aqueous medium.

Experimental Section

Materials. The APB and DAPB were synthesized and purified as described in the literature.[11] The β-CDx was obtained from Sigma Chemicals and was used as received. Analytical grade NaCl, $NaClO_4$, NaH_2PO_4, and Na_2HPO_4 were procured from Sigma Chemicals and were used without further purification.

Solution Preparation. For solubility measurements, purified crystals of APB (or DAPB) were added to buffered solutions containing various amount of $NaClO_4$. The resulting mixtures

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were sonicated for 0.5 h to obtain saturated solutions and then filtered through Nalgene filter (0.2 mm) after equilibration at room temperature. Absorbance values were measured at 320 nm for APB and at 350 nm for DAPB in reference to the respective solvent.

To prepare samples for binding studies, saturated solutions of APB and DAPB in 50 mM phosphate buffer (pH 7.0) were used as stock solutions. An aliquot (1.0 mL in the case of APB and 0.5 mL in the case of DAPB) of the stock solution was diluted to 10 mL containing the appropriate amount of β-CDx and $NaClO_4$. The solutions were allowed to equilibrate at room temperature for at least 6 h before measurement. Fluorescence spectra of APB and DAPB were measured by exciting the solutions at 320 and 350 nm, respectively. All spectra were blank subtracted. The peak area in the spectral range was used as a measure of fluorescence intensity.

Determination of Binding Constants. The binding constants and stoichiometric ratios of the inclusion complexes of APB and DAPB were estimated from the Benesi−Hildebrand (BH)[12] plots using fluorescence data. The derivation of the BH relationships has been discussed elsewhere[13,14] and therefore will not be detailed here. The following equations were used for 1:1 and 2:1 β-CDx−substrate association:

$$\frac{1}{F - F_0} = \frac{1}{F_m - F_0} + \frac{1}{K[CD]_0(F_m - F_0)} \quad (1)$$

$$\frac{1}{F - F_0} = \frac{1}{F_m - F_0} + \frac{1}{K'[CD]_0^2(F_m - F_0)} \quad (2)$$

where $[CD]_0$ represents the analytical concentration of β-CDx, F_0 and F are the fluorescence intensities in the absence and presence of β-CDx, respectively, F_m is the limiting intensity of fluorescence, and K represents association constant. The apparent K values were obtained from the slope and intercept of the BH plots.

Apparatus. The absorption spectra were measured on a Shimadzu UV-3101PC UV−vis−NIR scanning spectrophotometer equipped with a thermostated cell holder. Fluorescence measurements were performed on a SPEX Fluorolog Model P2T 211 spectrofluorometer. The circular dichroic spectra were recorded on a Jasco 710 spectropolarimeter. Optical rotations of β-CDx solutions were measured on a Jasco DIP-370 digital polarimeter. All measurements were done at room temperature (~25 °C).

Results

The absorption spectra of the aqueous solutions of APB and DAPB do not show any change upon addition of β-CDx in the concentration range 1−8 mM. In addition, the absorption spectra of both compounds remain unaffected when $NaClO_4$ was added to the solution. In saturated aqueous solution containing 8 mM β-CDx, both compounds exhibited a positive band corresponding to the long-wavelength absorption band in their respective induced circular dichroic (ICD) spectrum (Figure 2). It is interesting to note that although the molar absorptivities of the compounds at the respective absorption maximum are very similar,[15,16] the molar ellipticity of APB is much higher as compared to that of DAPB. At room temperature, the aqueous solubilities of both APB and DAPB are very low. However, the solubility in both cases increases with an increase in concentration of $NaClO_4$ as shown in Figure 3. The addition of an equivalent amount of NaCl, however, did not show any significant change of solubility.

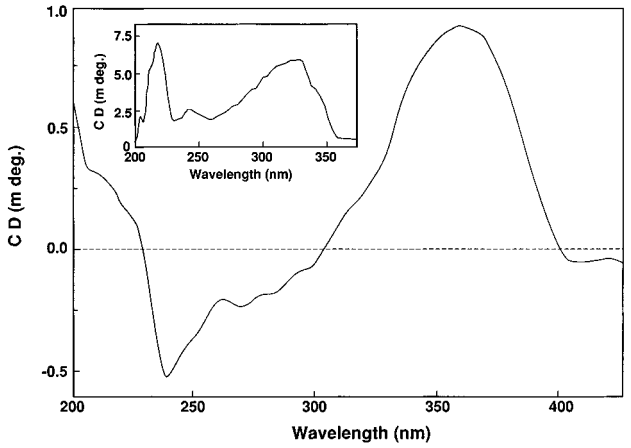

Figure 2. Induced circular dichroic spectra of APB (insert) and DAPB (saturated solutions in 8 mM β-CDx).

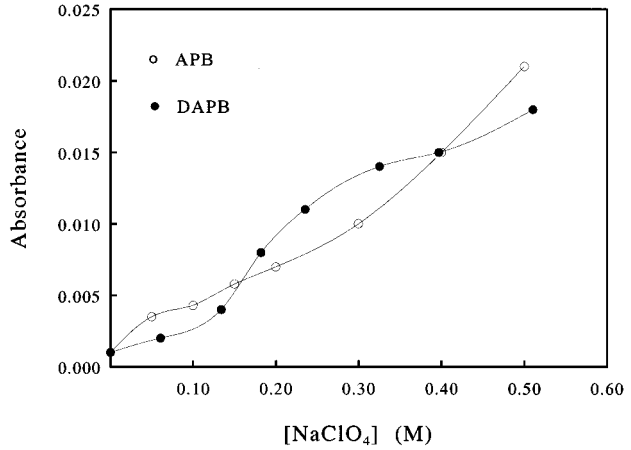

Figure 3. Plots of absorbance of APB and DAPB in water as a function of concentration of $NaClO_4$.

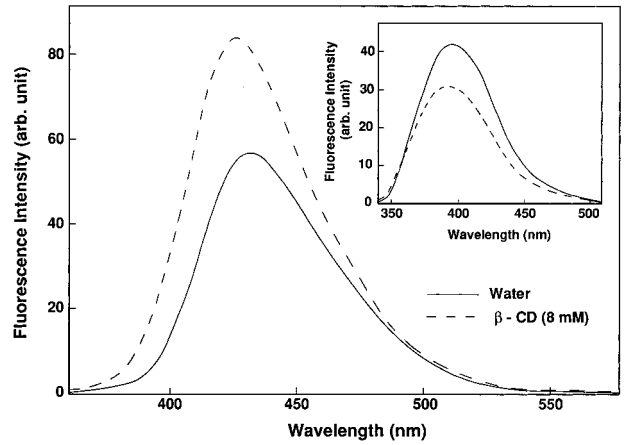

Figure 4. Fluorescence spectra of APB (insert) and DAPB in the absence and presence of 8 mM β-CDx.

The fluorescence spectra (Figure 4) of APB and DAPB show respectively a decrease and increase of intensity in the presence of β-CDx. The intensity change is much higher in the case of the latter than in the former with the same concentration of β-CDx. The association constants (K) for the binding of both compounds in deionized water as well as buffered solution have been estimated from the plot of the data by use of eq 1. The linearity of the plots (not shown here) suggests a 1:1 stoichiometry for the inclusion complexes of both APB and DAPB. Use of eq 2 failed to produce good fit to the experimental data. In the absence of salt, the K value (see Table 1) for DAPB is

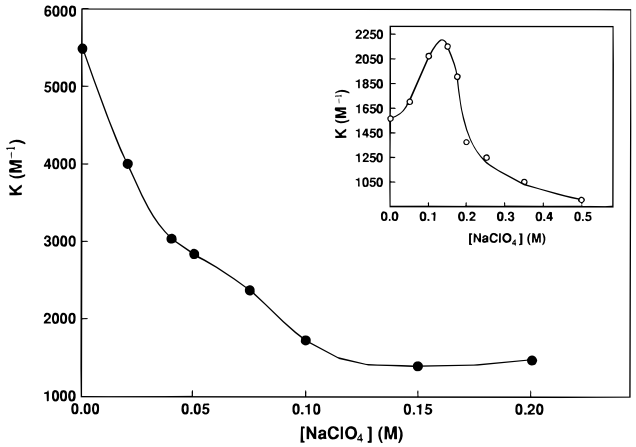

Figure 5. Plots of association constants (K) of APB (insert) and DAPB as a function of $NaClO_4$ concentration.

TABLE 1: Association Constants (K) of APB and DAPB in Deionized Water and 50 mM Phosphate Buffer Containing Varying Concentrations of $NaClO_4$

$[NaClO_4]$ (M)	$K \pm 50$ (M^{-1})		$[NaClO_4]$ (M)	$K \pm 50$ (M^{-1})	
	APB	DAPB		APB	DAPB
deionized water	1542	5664	0.15	2150	1428
0.00	1553	5476	0.175	1903	
0.02		4005	0.20	1375	1505
0.04		3054	0.25	1252	
0.05	1703	2858	0.35	1050	
0.075		2391	0.50	905	
0.10	2072	1747			

almost 4 times as high as that of APB. Apparently, phosphate buffer has no significant effect on the inclusion of the fluorophores into the CDx cavity. Also, no significant effect on the fluorescence intensity of the molecules was observed upon addition of equivalent amount of NaCl in the presence and absence of β-CDx.

When $NaClO_4$ was added to the solution of DAPB containing 8 mM β-CDx, the fluorescence intensity decreased. In the case of APB, the fluorescence intensity increased upon addition of $NaClO_4$. However, in the absence of β-CDx, $NaClO_4$ has no effect on the fluorescence spectra of the compounds. The association constants of APB as well as DAPB with β-CDx were estimated in the presence of $NaClO_4$. Table 1 summarizes the values of the association constants of both molecules. As noted, the K value for DAPB decreases with an increase in $NaClO_4$ concentration (see Figure 5). In contrast, in the case of APB, the association constant initially increases, reaching a maximum at around 0.15 M, and then decreases with a further increase in concentration of $NaClO_4$. At higher concentrations, the association constants of both molecules seem to reach a plateau. This is consistent with the solubility data as shown in Figure 3. The addition of 0.42 M $NaClO_4$ in β-CDx solution decreases the optical rotation ($[\alpha]^{26}_D$) from $+139.5°$ to $+134.8°$. The optical rotation of the β-CDx solution is, however, slightly less than the reported value ($+162°$).[17] The association constants of both molecules remain unaffected in the presence of NaCl in the concentration range 0−0.5 M.

Discussion

The fluorescence properties of APB and DAPB in various solvents have been reported in the literature.[15,16] It has been observed that the fluorescence quantum yields of the former molecule in hydrocarbon solvents are lower than those in polar solvents.[15] However, in the case of DAPB, the fluorescence quantum yield increased in going from polar to hydrocarbon solvents.[16] Since the cyclodextrin cavity is hydrophobic, the decrease and increase of fluorescence intensity of APB and DAPB, respectively, suggest formation of inclusion complexes. The formation of inclusion complexes with β-CDx is indicated by the increase of aqueous solubility of both compounds in the presence of β-CDx. This is further confirmed by the appearance of the ICD spectra of the molecules in the presence of β-CDx. The appearance of a positive ICD band at the position of the lowest energy absorption band suggests that the molecules are oriented axially in the β-CDx cavity.[18] The stronger ICD band in the case of APB suggests that the inclusion of the phenyl ring is deeper than in the case of DAPB. Since the length of the molecules (∼11 Å) is greater than the height of the cyclodextrin cavity (∼7.8 Å), the phenyl ring should remain exposed to the bulk water above the upper rim of the β-CDx molecule. However, if this was true, then one would expect a weak ICD band in the case of APB. The strong ICD signal is a result of the strong coupling of the sum moments of the oscillating dipoles induced by the electric field of the light in the σ-bonds of the β-CDx with the electric transition moment of the aromatic guest.[18c] Since the lowest energy transition of the molecules is localized in the phenyl ring,[15] the phenyl ring of APB must be completely included in the β-CDx cavity for stronger interaction. Thus, it appears that, in the case of APB, the inclusion occurs with the phenyl ring in the head first position as shown in Figure 6 (a) although the possibility of inclusion of the benzothiazole moiety in the CDx cavity cannot be ruled out. In the case of DAPB, such inclusion is sterically hindered by the $-N(CH_3)_2$ group. As a result, the phenyl ring is protruding into the bulk solvent (Figure 6b). Due to the capping effect of the $-N(CH_3)_2$ group, the molecule remains locked inside the cavity as reflected by the higher association constant value as compared to that of APB. However, in the absence of the $-N(CH_3)_2$ group, APB can easily pass through the CDx cavity and thus experiences a weaker binding which results in a smaller association constant.

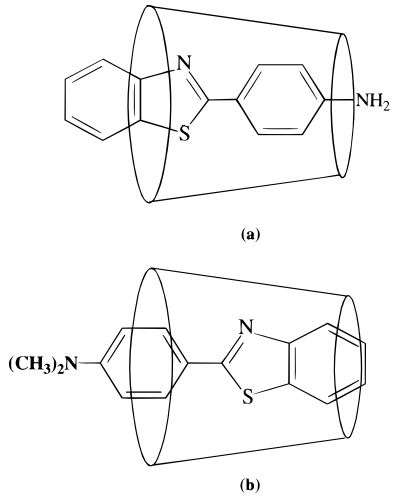

Figure 6. Proposed structures of the inclusion complexes (a) β-CDx−APB and (b) β-CDx−DAPB.

In addition to structural requirements, there could also be other factors that influence the binding of the molecules with the β-CDx. In fact, the mechanism of the formation of an inclusion complex consists of several steps. The first step is the approach of substrate to the β-CDx molecule. In the second step, the breakdown of the water structure around the part of the molecule that is going to be included into the β-CDx cavity is required. Two types of water structure, however, must be

considered: (a) tightly bound water solvating the polar or charged groups (OH, NH_2, $N(CH_3)_2$) which are capable of hydrogen bonding and (b) water around hydrophobic groups (such as aromatic ring). The third step involves breakdown of the water structure inside the β-CDx cavity and transfer of some water molecules out of the cavity. In the fourth step, the mechanism involves interactions of the substituents of the substrate molecule with groups on the rim or on the inside of the β-CDx cavity. The subsequent step is the formation of hydrogen bonds between substrate and β-CDx. Finally, the water structure around the exposed parts of the substrate after the inclusion process is reconstituted. According to the above discussion, the differences in the association constants of APB and DAPB may also be linked to differences in the solvation of the guest molecules. Since the molecules are structurally similar, it seems that APB necessitates the removal of more tightly bound water molecules from the polar group ($-NH_2$) in order for it to enter the β-CDx cavity.

The effect of ClO_4^- could probably be explained in light of the binding process of the solute molecules with β-CDx. Two explanations for the effect of ClO_4^- ion on the binding of APB and DAPB can be offered: (i) The ClO_4^- anion has an influence on the structure of water; if hydrophobic interactions are essential for the binding of the substrate to the β-CDx, the water structure must have a decisive influence on the equilibrium constant.[19] (ii) The ClO_4^- ion competes with the substrate in a manner similar to that described for the I^- ion.[17] At high salt concentrations, the water structure being broken by the ClO_4^- ions, the substrate becomes more soluble in water. An increase in solubility of β-CDx in water in the presence of $NaClO_4$ has also been reported in the literature.[20] The increased aqueous solubility of both substrate and β-CDx reduces the probability of hydrophobic interactions between them, thus decreasing the association constant. The decrease of the binding of APB and DAPB with β-CDx seems to be mainly due to the well-known salting-in effect of $NaClO_4$ as indicated by the solubility data (Figure 3). However, the competition of the ClO_4^- ions for binding with the β-CDx molecule cannot be overlooked. Indeed, such interaction between ClO_4^- and β-CDx is indicated by the decrease in optical rotation of the latter in the presence of ClO_4^- ion. The association constant for the system α-CDx$-ClO_4^-$ has been reported to be 29.4 M^{-1}.[21] Although no such data are available for the β-CDx$-ClO_4^-$ system, a similar value for the association constant can be assumed. X-ray crystallographic studies[22] suggest that the ClO_4^- ion is probably located in the hydrophobic plane roughly defined by the primary hydroxyl groups.

The factors discussed in the preceding paragraph, however, do not explain the rise of K value in the case of APB in the low concentration range of $NaClO_4$. Therefore, there must be some other binding process involved in the case of APB. The possibility that the ClO_4^- ion forms a ternary complex thus stabilizing the β-CDx$-$substrate inclusion complex cannot be ruled out. In fact, the formation of ternary complexes in the presence of small organic molecules such as alcohols and hydrocarbons are reported in the literature.[23] Clearly, as DAPB failed to show the above effect, the $-NH_2$ group of APB must be involved in such complex formation through specific interactions, e.g., hydrogen bonding. As discussed above, the break down of the water structure around the $-NH_2$ group of the substrate is critical for binding to occur. In the low concentration range (0.0$-$0.15 M), the ClO_4^- ions (probably through specific interactions with the functional group(s) of the substrate) break the water structure around it, thus facilitating inclusion of the molecule (the phenyl ring) into the CDx cavity. After inclusion, the ClO_4^- ion through specific interaction with the primary hydroxyl group(s) of β-CDx stabilizes the ternary complex. The effect of ClO_4^- ion on the binding of APB can therefore be explained in terms of interactions with the hydroxyl groups as well as the hydrophobic cavity of β-CDx. However, at high concentrations, this effect is overshadowed by the salting-in effect, resulting in a decrease of K value. Since the $-N(CH_3)_2$ group of DAPB is less polar and is protruded into the bulk solvent, the stabilization through ternary complex formation is insignificant as compared to the salting-in effect even at low salt concentration. As a result, a continuous decrease of K with the rise of $[ClO_4^-]$ is observed.

Similar effects have also been observed in our laboratory in a different study[24] involving the binding of *N*-phenyl-1-naphthylamine (NPN) with β-CDx. We have observed that the fluorescence intensity of NPN increases upon addition of increasing amounts of GnCl to the β-CDx solution. Since GnCl is also known to act as a salting-in reagent in aqueous solution, one would expect a decrease of fluorescence intensity of NPN as it is released from the β-CDx cavity as a result of salting-in effect. This suggests that either GnCl acts as a salting-out agent in aqueous solution containing β-CDx or there must be some kind of interaction (the nature of which is probably decided by the functional group(s) of the substrate) between the salt and the β-CDx$-$NPN inclusion complex.

Conclusions

The binding of APB and DAPB with β-cyclodextrin has been studied by fluorescence spectroscopic methods. The high association constant for DAPB compared to that of APB is due to its more hydrophobic nature and the presence of the $-N(CH_3)_2$ capping group. Both molecules are axially oriented in the cyclodextrin cavity. However, in contrast to DAPB, APB enters the cavity with the phenyl ring in the head first position. The decrease of the binding constant for both molecules in the presence of $NaClO_4$ has been attributed to the salting-in effect and to the competition of the ClO_4^- ion for complexation with the cyclodextrin molecule. The increase of association constant of APB in the low concentration range of the salt is most probably due to the formation of a ternary complex through specific interaction with the $-NH_2$ group located near the lower rim of the cyclodextrin molecule formed by the primary hydroxyl groups which stabilizes the β-CDx$-ClO_4^-$ inclusion complex. Interactions of the ClO_4^- ion with the inclusion complexes of organic substrates with cyclodextrins should be the subject of thorough studies before any generalization is made. Further studies on the effects of salting-in agents on the binding equilibria of organic compounds with different functional groups are in progress in this laboratory.

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Supporting Information Available: Benesi$-$Hildebrand plots of APB$-\beta$-CDx and DAPB$-\beta$-CDx in deionized water and with $NaClO_4$ (4 pages). Ordering information is given on any current masthead page.

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