Selective binding and highly sensitive fluorescent sensor of palmatine and dehydrocorydaline alkaloids by cucurbit[7]uril†

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The complexation behavior of palmatine (P) and dehydrocorydaline (DHC) alkaloid guest molecules by cucurbit[7]uril (CB7) host have been investigated by means of fluorescence spectra in aqueous phosphate buffer solution (pH 7.2). It is found that each alkaloid exhibits dramatic fluorescence enhancement upon complexation with CB7, and the intensity of the emittance is strong enough to be readily distinguished by the naked eye. Although the two guests possess similar structure, the complex stability constant of P with CB7 is 5.4 times larger than that of DHC. ^{1}H NMR studies show that the binding modes differ much, *i.e.*, deep encapsulation for P-CB7 and shallow encapsulation for DHC-CB7. Furthermore, the solvent effects and salt effects during the course of complexation have also been investigated, showing they significantly influence the binding ability and selectivity of CB7 with the alkaloid guests. Particularly, addition of a small amount (4 vol%) of ethanol increases the P/DHC selectivity to 17.2.

Introduction

Much attention is currently being devoted to pharmaceutical molecule recognition by synthetic receptors. The resulting host-guest complexes could be successfully utilized to develop fluorescent probes and sensors, to decrease toxicity and to increase bioactivity and water solubility of the pharmaceutical molecule. Cucurbiturils (CBs)[1] are macrocyclic container molecules composed of glycoluril monomers joined by pairs of methylene bridges. They are well-known to form stable host-guest complexes or supramolecular species with positively charged molecules such as bipyridinium, ammonium, imidazolium and dye molecules through ion-dipole, hydrogen-bonding as well as hydrophobic interactions.[2–6] However, the binding behavior of biological and pharmaceutical molecules by CBs have rarely been reported.[6c–e,7,8] Kim *et al.*[7] have demonstrated that CB7 could form a stable 1:1 complex with the anticancer drug oxaliplatin by encapsulating the cyclohexyl ring of the guest inside the cavity. The high stability of the complex suggests the potential use of such complexes in controlled release of drugs. Inoue and Kim *et al.*[6c] reported the sequence recognition and self-sorting of a dipeptide by CB6 and CB7. More recently, Collins *et al.*[8] reported the solubilisation and cytotoxicity of albendazole encapsulated in CBs.

In the present paper, we wish to report the binding behavior and sensitive fluorescent sensor of palmatine (P) and dehydrocorydaline (DHC) alkaloids by CB7, a CB homologue with good water-solubility ($2–3 \times 10^{-2}$ M[2a,b,9,10]) and medium-sized cavity (internal cavity diameter: *ca.* 7.3 Å, portal diameter: *ca.* 5.4 Å[2a,b,10]) (Chart 1). P and DHC are clinically important natural isoquinoline alkaloids, and they have attracted much attention due to their various biochemical and pharmacological effects.[11] For example, P possesses antimicrobial, antimalarial, anti-inflammatory, antipyretic, hepatoprotective and antitumor activities.[12,13] And DHC shows inhibition of acetylcholinesterase.[14] Herein, our interest is not only to elucidate the molecular recognition of P and DHC alkaloids with CB7 host, but also to examine the highly effective complexation-induced fluorescence enhancement. Moreover, the salt effects and solvent effects have also been investigated to see how they affect the molecular recognition abilities and binding

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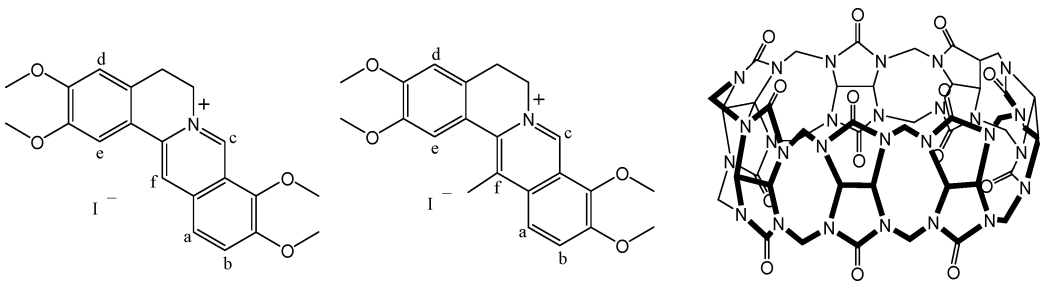

Chart 1 Structural formulae of CB7 host and alkaloid guest molecules.

selectivity during the course of host-guest complexation. It is well known that isoquinoline alkaloids are promising small molecules in photodynamic therapy (PDT),[15] because they can produce singlet oxygen ($^1O_2$) and the oxidation of biological substrates. The very low quantum yield of isoquinoline alkaloids; however, limits their utilities. The present fluorescent sensor behaviors are very interesting because the marked increase in fluorescence could restore efficacy to isoquinoline alkaloids' use as PDT therapeutics. Through the present studies, the applications of P and DHC alkaloids will be broadened, and even be given new characteristics and significance.

Experimental section

General

Fluorescence spectra were measured in a conventional rectangular quartz cell (10 × 10 × 45 mm^3) at 25 °C on a Shimadzu spectrofluorometer model FP-5301PC, with the excitation and emission slits at a width of 5 nm. The fluorescence experiments were kept at a constant temperature of 25 °C through a Shimadzu TB-85 Thermo Bath unit. The excitation wavelengths for P and DHC were 347 and 350 nm.

Materials

The two guest isoquinoline alkaloids, *i.e.*, palmatine (P) and dehydrocorydaline (DHC), were commercially available and used without further purification. CB7 was synthesized and purified according to the literature reports.[2d,16] β-cyclodextrin (β-CD) was obtained from Tokyo Kasei and dried under reduced pressure before use. All other chemicals were commercially available and used without further purification, except otherwise noted. The phosphate buffer solution of pH 7.2 was prepared by dissolving disodium hydrogen phosphate (25.79 g) and sodium dihydrogen phosphate (4.37 g) in distilled, deionized water (1000 ml) to make a 0.1 mol dm^{-3} solution.

Results and discussion

Fluorescence sensing

It is well known that molecular sensing based on host-guest complexation is a significant topic in organic chemistry. Fluorescence spectra of P and DHC alkaloids with CB7 host were performed at 298.15 K in a phosphate buffer solution of pH 7.2. As shown in Fig. 1, fluorescence characteristics of P and DHC showed dramatic changes upon addition of CB7, namely steep increases in fluorescence intensity. The fluorescence quantum yield (Φ_F) increased by factors of 8.2 and 5.8 for P-CB7 and DHC-CB7 complexes from that of the corresponding value of P and DHC guests. Significantly, the intensity of the emittance is strong enough to be readily distinguished by the naked eye with a UV lamp. As can be seen from Fig. 2, P-CB7 and DHC-CB7 complexes show yellow and cyan fluorescence, respectively.

An equimolar mixture of P and DHC in pH 7.2 phosphate buffer solution barely fluoresces. Addition of an equivalent amount of CB7 to the above solution leads to yellow fluorescence, and the fluorescence spectrum of the three-component mixture is more consistent with that of P-CB7 complex (Fig. S5). When an excess of CB7 is added, the emission peak appears at 488 nm (Fig. S6), which is between that of the P-CB7 (497 nm) and the DHC-CB7 (476 nm) complex. These observations indicate that the P-CB7 complex is mainly formed in the case of the presence of 1 mol equivalent of CB7, while both P-CB7 and DHC-CB7 complexes are constructed in the case of the presence of an excess of CB7. The above results suggest that CB7 shows good molecular selectivity for P/DHC pairs, which will be further proved by the spectral titration experiments.

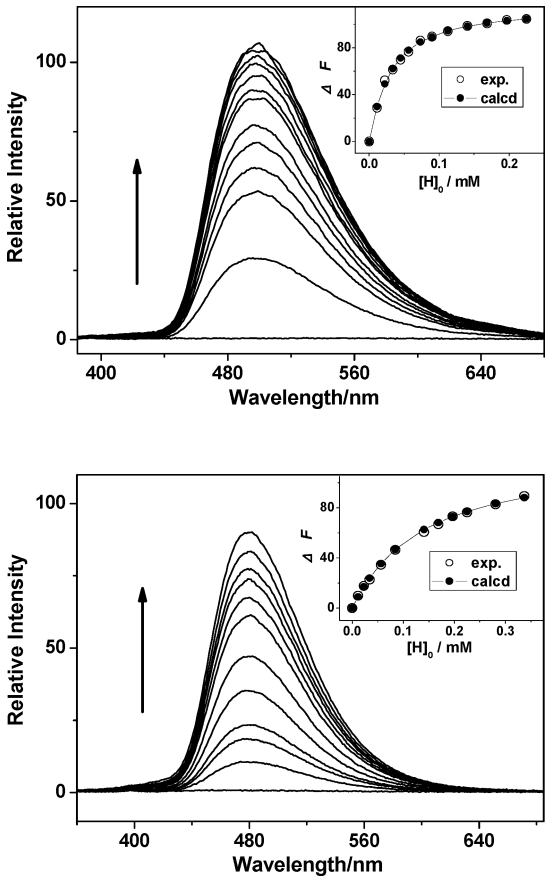

Fig. 1 Fluorescence spectra of P (upper) and DHC (lower) in the presence and absence of CB7 in aqueous phosphate buffer solution (pH 7.2) at 298.15 K. Inset: the nonlinear least-squares analysis of the differential intensity (ΔF) to calculate the complex stability constant (K_S). The concentrations of P and DHC are 1.25×10^{-5} and 1.42×10^{-5} mol dm^{-3}, respectively.

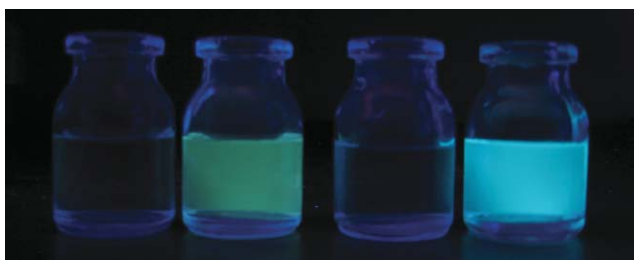

Fig. 2 Visible emission observed from samples of P and DHC with CB7. Left to right: P, P + CB7, DHC, DHC + CB7.

In the control experiments, under identical conditions the fluorescence intensities of the guests were not appreciably affected by the addition of glycoluril, *i.e.*, the monomeric unit of CB7. Generally, the fluorescence intensity of P and DHC are sensitive to changes in their microenvironment. That is, they barely fluoresce in a hydrophilic microenvironment but emit strong fluorescence in a highly hydrophobic one. These phenomena indicate that the P and DHC alkaloid molecules must insert into the hydrophobic cavity of CB7, and the resulting decrease of polar microenvironment led to the fluorescence enhancement.

To quantitatively assess the inclusion complexation behavior of these compounds, spectral titrations of CB7 with P and DHC were performed at 298.15 K in a phosphate buffer solution of pH 7.2. Assuming 1:1 inclusion complexation stoichiometry between CB7 and the two alkaloids, the complex stability constants (K_S) could be calculated by analyzing the sequential changes in fluorescence intensity (ΔF) of guest that occurred with changes in host concentration. This analysis was carried out by using a nonlinear least-squares curve-fitting method. For each alkaloid guest examined, the plot of ΔF as a function of $[H]_0$ gave an excellent fit ($R > 0.99$), verifying the validity of the 1:1 inclusion complexation stoichiometry assumed. Additionally, the 1:1 inclusion complexation stoichiometry have also been proved by job's experiments (see Electronic Supplementary Information).

It is seen from the data in Table 2 that CB7 could form stable complexes with the two positively charged alkaloids. In such cases, both hydrophobic interactions and ion-dipole interactions are crucial forces. In addition, although the two alkaloids' structures are very similar, their binding abilities with CB7 differ so much. The substitution of hydrogen for methyl in DHC, affording P, dramatically increases the K_S value 5.4 times. This observation indicates that maybe different binding modes are carried out when the two guests interact with CB7, which will be discussed in a later part of this paper.

For comparison purposes, we investigated the interaction of the two alkaloids with β-CD. β-CD has an internal cavity with a diameter of 6–7 Å,[17] and it is comparable in diameter to the CB7 cavity (internal cavity diameter: *ca.* 7.3 Å, portal diameter: *ca.* 5.4 Å[2a,b,10]). As shown in Fig. S1 & 2 and Table 1, the fluorescence enhancement and Φ_F increases were negligible when using β-CD as host. During the course of molecular recognition, hydrophobic interactions are the main dominating forces for the β-CD system.[18] The middle portion of P and DHC guests are high-polarity, and the resulting weak binding abilities of them with β-CD (K_S = 629 & 588 M^{-1}) led to the very small fluorescence enhancement. Furthermore, it is seen from the data in Table 2 that both CB7 and β-CD give the same K_S order, *i.e.*, P > DHC upon interaction with P and DHC. When CB7 shows a high molecular selectivity of 5.4 for P/DHC pairs, no significant selectivity is found for β-CD. Guests P and DHC give the same host selectivity of CB7 > β-CD. Typically, the K_S value for CB7 with P is enhanced by factor of 68 compared with β-CD.

Table 1 Fluorescence quantum yields (Φ_F) of P, DHC and their complexes in phosphate buffer solution (pH 7.2) at 298.15 K

		Complex	
	Guest	CB7	β-CD
P	0.0147	0.1207	0.0157
DHC	0.0320	0.1854	0.0328

Table 2 Complex stability constants (K_S/M^{-1}) for 1:1 intermolecular complexation of P and DHC with CB7 and β-CD in phosphate buffer solution (pH 7.2) at 298.15 K

	P	DHC
CB7	42600 ± 300	7860 ± 100
β-CD	629 ± 15	588 ± 22

^{1}H NMR spectra

It is very important to investigate the binding modes between CB7 and alkaloid guests for elucidation of the mechanism of molecular recognition. Fig. S9 illustrates some typical ^{1}H NMR spectra for inclusion complexation of CB7 with P and DHC. The P-CB7 complex shows significant upfield shifts for the isoquinoline aromatic protons H_a, H_b, H_c and H_f ($\Delta\delta = 0.12$–0.75 ppm), indicating P binds with CB7 *via* deep encapsulation of the isoquinoline group. In addition, slight downfield shifts are observed for the aromatic protons from the benzodioxole moiety (H_d and H_e), which is due to the deshielding effect of the carbonyl groups of CB7 on this portion of the guest.[1b] For the DHC-CB7 complex, H_b and H_c from the isoquinoline moiety shift to higher field, however it is different for P-CB7, H_a which shifts to lower field. These observations suggest that DHC shallowly include in the CB7 cavity. Furthermore, we also carried out a molecular dynamics simulation[19] on the system, and two snapshots are shown in Fig. S10, which are consistent with our experimental results. As can be seen from the compound structures (Chart 1), alkaloids P and DHC have similar frameworks, but with a small difference in the C-f substituent: a hydrogen atom in P and a methyl group in DHC. However, the results from ^{1}H NMR experiments and molecular dynamics simulation show that the binding modes of P and DHC alkaloids with CB7 are quite different.

Environment effects

It is well known that the environment effects, including salt effects and solvent effects, should significantly affect the K_S values and molecular selectivity upon complexation of CB7 with a guest molecule. Kaifer and coworkers[20] have demonstrated that the K_S values of CB7-methyl viologen complex decrease with increasing ionic strength, with more pronounced effects for solutions containing divalent Ca^{2+} ions than for solutions containing monovalent Na^+ ions. The salt-induced transfer of Neutral Red from CB7 to bovine serum albumin has also been studied with respect to its potential in drug delivery.[21] Our previous work[5d] reported the environment effects of complexation of β-CD, calix[4]arenesulfonate and CB7 with dye guests. In the present alkaloid-CB7 complexation system, we also examine the effects of salt and solvent in the host-guest binding behaviors.

To examine the influence of salts and solvents on the binding abilities of CB7 toward alkaloid guests, we performed the fluorescence titration experiments in different environments. And the assessed binding constants are listed in Table 3.

From Table 3, it can be seen that when the distilled, deionized water was replaced by phosphate buffer solution (0.1 mol dm^{-3}, pH 7.2), the binding constants significantly decreased 56.8 and

Table 3 Complex stability constants (K_S/M^{-1}) for 1:1 intermolecular complexation of P and DHC with CB7 in different environments

Addition	P	DHC	$K_P:K_{DHC}$
None[a]	2420000 ± 30000	324000 ± 2000	7.5
Buffer[b]	42600 ± 300	7860 ± 100	5.4
Buffer & NaCl[c]	4070 ± 50	1450 ± 40	2.8
LiCl[d]	210000 ± 4000	30400 ± 600	6.9
NaCl[d]	6980 ± 40	2240 ± 40	3.1
KCl[d]	4270 ± 60	1650 ± 50	2.6
RbCl[d]	6400 ± 60	2050 ± 40	3.1
CsCl[d]	6210 ± 70	2040 ± 50	3.0
EtOH[e]	910000 ± 8000	53000 ± 600	17.2

[a] Distilled, deionized water. [b] Phosphate buffer solution (0.1 mol dm^{-3}, pH 7.2). [c] Addition of NaCl (0.4 mol dm^{-3}) in phosphate buffer solution (0.1 mol dm^{-3}, pH 7.2). [d] Addition of LiCl, NaCl, KCl, RbCl or CsCl (0.4 mol dm^{-3}) in distilled, deionized water. [e] Addition of ethanol (4 vol%) in distilled, deionized water.

41.2 times for P-CB7 and DHC-CB7, respectively. Further addition of 0.4 mol dm^{-3} NaCl to the buffer solution dramatically decreased the K_S values to 4070 and 1450 M^{-1}. The salt-induced decrease of binding ability is mainly caused by the competing ion-dipole interactions between Na^+ and alkaloid molecules. The dependence of the binding constants of P and DHC with CB7 on the cation type was also studied for the alkali series (as chlorides). As can be seen from Table 3, the effects of alkali cations are very pronounced on the formation of CB7-alkaloid inclusion complexes, since the binding constants of P and DHC by CB7 in the presence of a moderate concentration (0.4 mol dm^{-3}) of alkali chloride are 1~4 orders of magnitude lower than those determined in pure water. Potassium-induced effects are more significant than other alkali cations. When the pure water was replaced by K^+ solution, the binding constants significantly decreased by a factor of 570 and 180 for P-CB7 and DHC-CB7, respectively. So the binding strength of the two alkaloids with CB7 can be effectively modulated by the adjustment of the salt concentration and the type of salt. Recently, Nau *et al.*[10] reported that sodium-induced decreases of binding abilities of organic ammonium ions by CB6 were stronger than other alkali cations. That is to say, the K_S values of CB6 and CB7 complexes are decreased with more pronounced effects for solutions containing different alkali ions, Na^+ for CB6 and K^+ for CB7.

Upon addition of a small amount of ethanol (4 vol%) to the water solution, the binding constants of the two complexes also decreased (2420000→910000 M^{-1} for CB7-P; 324000→53000 M^{-1} for CB7-DHC). However, the solvent effects are much weaker than salt effects, since relatively small decreases of K_S values (2.7 and 6.1 times) are found. It has been well documented that the addition of alcohols could extrude water from the cavity and make the cavity more hydrophobic, and thus strengthen the binding of guests. On the other hand, some main noncovalent interactions working between host and guest, such as electrostatic and hydrogen bond interactions, would be weakened to some extent when some water molecules were replaced by alcohols. The ion-dipole interactions play a great role in the complexation of CB7 with P and DHC. Therefore, the addition of ethanol weakened the binding ability of CB7 with alkaloid guests. Additionally, CB can form stable complexes with alcohol guests,[22] so some ethanol molecules may enter into the cavity of CB7, possibly taking some disadvantage of the complexation between host and guest.

As can be seen from Table 3, the salt and solvent effects also significantly affect the molecular selectivity upon complexation of CB7 with P and DHC guest molecule. The addition of Na^+ decreases the molecular selectivity for P/DHC pairs from 7.5 (water) to 5.4 (buffer), 3.1 (NaCl) and 2.8 (buffer & NaCl). In contrast, the addition of a small amount of ethanol to water drastically increases the selectivity from 7.5 to 17.2. The controllable molecular selectivity by salt and solvent can make CB7 possess potential applications in purification and separation of alkaloids from herbs.

Conclusion

In summary, we have presented the molecular recognition and fluorescence sensor behavior of P and DHC alkaloids by CB7. Upon complexation with CB7, each alkaloid guest exhibits a steep increase in fluorescence intensity and the intensity of the emittance is strong enough to be readily distinguished by the naked eye. Although the two alkaloids' structures are very similar, their binding abilities and binding modes with CB7 differ much from each other. The complex stability constant of P with CB7 is 5.4 times larger than that of DHC and the large increase in binding ability is due to the different binding modes, *i.e.*, deep encapsulation for P-CB7 and shallow encapsulation for DHC-CB7. In addition, the solvent effects and salt effects also exert an extraordinary influence over the binding ability and selectivity of CB7 with the alkaloid guests. Addition of a small amount of ethanol reduced the binding affinity but improved the molecular selectivity of P/DHC. The binding affinity and molecular selectivity were both decreased with increasing ionic strength.

The present studies of selective binding and drastic fluorescence enhancement can help improve CB7's applications in bioorganic and medical chemistry, including determination, purification and separation of alkaloids and drug delivery. In particular, the large fluorescence enhancement of P and DHC in the presence of CB7 could make them possess the potential to serve as PDT therapeutics. Endeavors to explore the applications of alkaloid-CB7 complexes are currently in progress.

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