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Molecular Binding Ability and Selectivity of Natural α-, β-, γ-Cyclodextrins and Oligo(ethylenediamino) Modified β-Cyclodextrins with Chinese Traditional Medicines

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Key words: Chinese traditional medicine, cyclodextrin derivative, molecular recognition, inclusion complexation, binding ability

Abstract

The binding ability and inclusion complexation behavior of natural α-, β-, γ-cyclodextrins (**1–3**) and two mono[6-oligo(ethylenediamino)-6-deoxy]-β-cyclodextrins (**4**, **5**) with four Chinese traditional medicines, that is, α-asarone (AS), ferulic acid (FA), magnolol (MA) and honokiol (HO), have been investigated in aqueous phosphate buffer solutions(pH = 7.20). The spectral titrations have been performed at 25 °C by using fluorescence spectroscopy to calculate the complex stability constants (K_S) and Gibbs free energy changes (ΔG°) for the stoichiometric 1 : 1 inclusion complexation of hosts **1–5** with guest medicines. The results obtained indicate that the different guest medicines fit in with hydrophobic cavities of different sizes and the appended substitutes of hosts **4** and **5** change the hydrophobic microenvironment of β-cylcodextrin **2**, influencing the original binding ability and molecular selectivity of host **2** consequently. The binding ability and inclusion complexation behavior of these hosts **1–5** are discussed according to the size/shape fit concept and hydrogen bonding interaction between host cyclodextrins and guest medicine molecules.

Introduction

α-Asarone (AS), ferulic acid (FA), magnolol (MA) and honokiol (HO), acting as the main active components of some Chinese traditional medicines, possess the pharmacological effects of antioxidation and free radical scavenging, exhibiting heart muscle relaxing, platelet aggregation inhibition, and anxiolytic-like activity, etc. [1–2]. Unfortunately, they are all practically insoluble in aqueous media, and this limits their use in certain cases where organic solvents need to be avoided. Therefore, the increase of their solubility in aqueous solution seems to be important. On the other hand, cyclodextrins are cyclic oligosaccharides consisting of six (α), seven (β) or eight (γ) linked D-glucopyranose units. Possessing well-defined hydrophobic cavities, cyclodextrins can selectively bind various organic, inorganic and biological molecules to form stable host–guest inclusion complexes, which have been extensively used as molecular recognition for amino acids [3–5], alcohols [6–7], and dyes [8], etc. Recently, the use of cyclodextrins for solubilization and drug targeting have attracted growing interest in science and technology [9–11].

In the present paper, we wish to report our investigation results on the binding ability and molecular selectivity of four medicine molecules (AS, FA, MA and HO) with α-, β-, γ-cyclodextrins (**1–3**) and two β-cyclodextrin derivatives, mono[6-(2-aminoethyleneamino)-6-deoxy]-β-cyclodextrin (**4**) and mono[6-(5-amino-3-azapentylamino)-6-deoxy]-β-cyclodextrin (**5**). The two modified β-cyclodextrins were chosen to investigate their inclusion complexation behaviors with guest medicines for possessing better aqueous solubility than the parent cyclodextrin and improved the microenvironment of hydrophobic cavity. The spectral titrations have been performed in phosphate buffer solution (pH 7.20) at 25 °C by fluorescence spectroscopy to calculate the complex stability constants (K_S) and Gibbs free energy changes ($-\Delta G^\circ$) for different host cyclodextrins, which are discussed according to the size/shape-fitting concept between host cyclodextrins and guest medicines and the effects to the inclusion complexation behaviors by introducing functional substituent.

Experimental

Materials

α-Asarone (AS), ferulic acid (FA), magnolol (MA) and honokiol (HO) as standard (National Institute for the Control of Pharmaceutical and Biological Products, Beijing, China), α-, β- and γ-cyclodextrins purchased from Nakalai Tesque (Kyoto, Japan) were used without further purification. Mono[6-(2-aminoethyleneamino)-6-deoxy]-β-cyclodextrin (**4**) and mono[6-(5-amino-3-azapentylamino)-6-deoxy]-β-cyclodextrin (**5**) were prepared according to the

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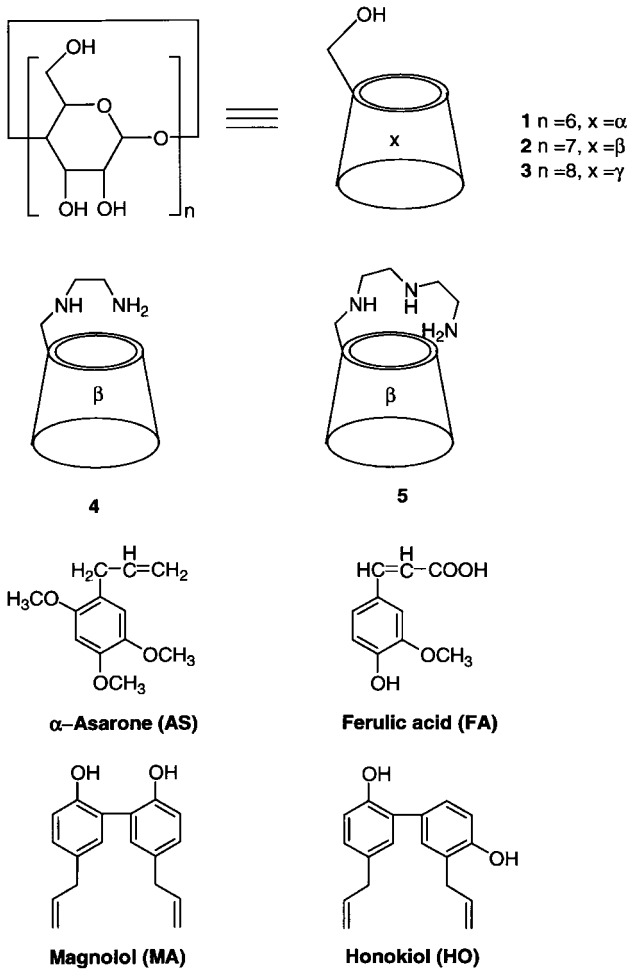

Chart 1.

procedures reported by Harada *et al.* [12]. Disodium hydrogen phosphate and sodium dihydrogen phosphate were dissolved in distilled, deionized water to make a 0.1 M phosphate buffer solution of pH 7.2 for spectral titration.

Solution preparation

Four medicines were dissolved in methanol to make a solution of 0.002 mol·dm^{-3}, then 50 μl of the above solution was diluted with phosphate buffer solution to 10 ml and a solution (methanol/water = 0.5/99.5) of 0.01 mmol·dm^{-3} was obtained to use for spectral titrations.

Spectral measurements

The stability constants and Gibbs free energy changes of four Chinese traditional medicines with cyclodextrins **1–5** were determined by using fluorescence spectrometry in phosphate buffer solution (pH 7.20) at 25 °C.

Fluorescence spectra were performed in a conventional quartz cell (10 × 10 × 45 mm) on a JASCO FP-750 spectrophotometer equipped with a temperature controller and with excitation and emission slits of 5 nm width.

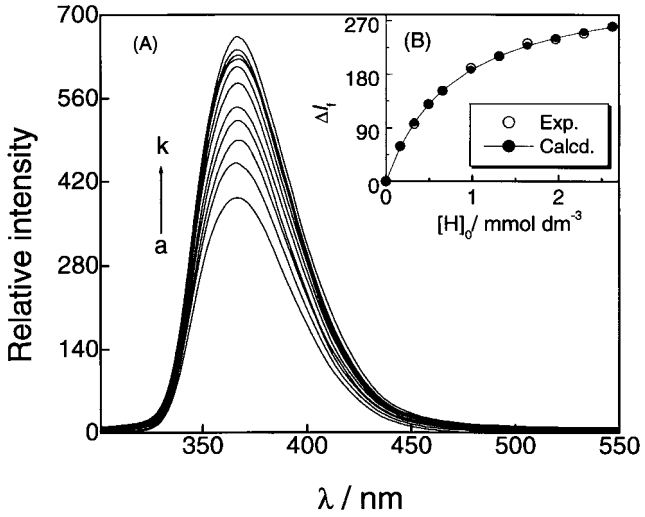

Figure 1. (A) Fluorescence spectral changes of α-asarone (9.7 μM) upon addition of β-cyclodextrin **2** in phosphate buffer solution (pH 7.20) at 25 °C; the concentration of **2** (from a to k): 0, 0.16, 0.33, 0.49, 0.66, 0.98, 1.31, 1.64, 1.97, 2.30 and 2.62 mM, respectively; excitation at 280 nm. (B) Least-squares curve-fitting analyses for the above inclusion complexation.

Results and discussion

Spectral titrations

In the titration experiments using fluorescence spectrometry, the fluorescence intensity of medicines (0.01 mM) gradually increased upon the addition of varying concentrations (from 0 to 2.0 mM) of hosts **1–5**. The typical fluorescence spectral changes upon addition of cyclodextrin **2** to α-asarone (AS) solution are shown in Figure 1A. The changes of fluorescence intensity induced by host **2** indicated that the reaction of the cyclodextrin **2** and AS has formed the host-guest inclusion complex.

Assuming 1 : 1 stoichiometry for the inclusion complexation of guest medicines (G) with cyclodextrins (H), the complexation can be expressed by Equation (1).

$$H + G \overset{K_S}{\rightleftharpoons} H \cdot G \tag{1}$$

The stability constants (K_S) of the inclusion complex formed can be calculated from the analysis of the sequential changes in fluorescence intensity (ΔI_f) at varying host concentrations by using a non-linear least squares curve-fitting method according to the Equation (2) [13].

$$\Delta I_f = \frac{\{\alpha([H]_0+[G]_0+1/K_S) \pm \sqrt{\alpha^2([H]_0+[G]_0+1/K_S)^2 - 4\alpha^2[H]_0[G]_0}\}}{2} \tag{2}$$

Here $[G]_0$ and $[H]_0$ refer to the total concentration of the guest medicines and host cyclodextrins, respectively; α is the proportionality coefficient, which may be taken as a sensitivity factor for the fluorescence change upon complexation.

For all host compounds examined, the ΔI_f values as a function of $[H]_0$ give excellent fits, verifying the validity of the 1 : 1 complex stoichiometry as assumed above. The typical curve-fitting analyses result for the inclusion complexation of host **2** with AS is shown in Figure 1B, where

Table 1. Stability constant (K_S) and Gibbs free energy change ($-\Delta G^\circ$) for the inclusion complexation of cyclodextrins (**1–5**) with four guest medicines at 25 °C in phosphate buffer solution (pH 7.20)

Guest	Host	K_S	logK_S	ΔG° (−kJ/mol)
AS	**1**	763	2.88	16.45
	2	1280	3.11	17.73
	3	2050	3.31	18.90
	4	889	2.95	16.83
	5	751	2.88	16.41
FA	**1**	1113	3.05	17.39
	2	4090	3.61	20.62
	3	707	2.85	16.25
	4	1580	3.20	18.26
	5	356	2.55	14.56
MA	**1**	4200	3.62	20.68
	2	5170	3.71	21.19
	3	1180	3.07	17.53
	4	21400	4.33	24.72
	5	625	2.80	15.96
HO	**1**	9130	3.96	22.60
	2	2360	3.37	19.25
	3	859	2.93	16.75
	4	5660	3.75	21.42
	5	2560	3.41	19.45

no serious deviations are found. When repeated measurements were made, the K_S value was reproducible within an error of ±5%, which corresponds to an estimated error of 0.15 kJ/mol in the free energy change of complexation (ΔG°). The complex stability constants and the Gibbs free energy changes (-ΔG°) obtained are listed in Table 1.

Molecular binding ability and selectivity

Although several weak interactions are known to be involved in the molecular recognition by cyclodextrins and their derivatives, the most important are the van der Waals and hydrophobic interactions, both of which are related to the size/shape-fit relationship between host and guest. Otherwise, hydrogen bonding and electrostatic interaction can also contribute to the inclusion complexation behaviors to some extent. In the present study of medicine molecules with a series of cyclodextrins, the size/shape-fitted relationship and hydrogen bonding are considered to be the important factors determining the inclusion complex stability. Significantly, the complex stability constants clearly reflect the binding abilities of native and modified cyclodextrins toward guests, which are in direct relation to their solubilization to these medicines.

Effect of the size of hydrophobic cavity

As can be seen from Table 1, the three native α-, β-, and γ-cyclodextrins possessing hydrophobic cavities of different diameters (0.45 nm, 0.7 nm and 0.85 nm) display obvious distinctions in binding ability toward four medicine molecules. We can easily deduce that the inclusion complexation behaviors of **1–3** with four medicines are mostly related to the size/shape-fit relationship between the host and guest. Since there are many substituent groups on benzene ring of AS, it can only be partially included into the smallest hydrophobic cavity of α-cyclodextrin and the lowest complex stability constants of AS with α-cyclodextrin is reasonable. Possessing larger cavities than α-cyclodextrin **1**, β- and γ-cyclodextrins (**2**, **3**) can form much more stable inclusion complexes with AS, giving the highest stability constant for inclusion complexation of **3** with AS. This may be ascribed to the well size/shape-fit between the larger hydrophobic cavity of **3** and AS. It is interesting that **1–3** give the different and opposite selectivity toward FA and AS, such as $3.2(K_{2/FA}/K_{2/AS})$ for **2** toward FA/AS and $2.9(K_{3/AS}/K_{3/FA})$ for **3** toward AS/FA. As compared with AS, FA possesses the relative small molecular size and the carboxyl on its substituent may form hydrogen bond with the hydroxyl of cyclodextrin. Therefore, it is deduced that FA may not include deeply into the cavity of γ-cyclodextrin **3**, leading to the weak interaction of FA with **3** than **1** and **2**. On the other hand, MA and HO possess the most similar structures except for the different location of a hydroxyl group, but the K_S values of their inclusion complexation with hosts **1–3** are obviously different. α-Cyclodextrin gives the higher K_S value for HO rather than MA, but β- and γ-cyclodextrins give opposite results. This may be attributed to the hydrogen bonding interaction between phenol hydroxyl of guest and the secondary hydroxyl groups of cyclodextrin.

Effect of the appended substituent

In order to investigate in detail how the modified residues of cyclodextrin affect binding ability upon the inclusion complexation with guest molecules, the stability constants of hosts **4** and **5** with four guest molecules were also determined by spectral titrations. As can be seen from Table 1, the introduction of substituents strongly affected the binding ability and molecular selectivity of host **2**. As compared with the case of native β-cyclodextrin **2**, the inclusion complexation of host **4** and MA gives the highest K_S value as 21400, and the molecular selectivity changes to 3.78 for host **4** toward HO/MA pair from 2.19 for **2** toward MA/HO pair. The above results indicated that hosts **4** and **5** offered the different hydrophobic microenvironment upon guest addition. The ethylene diamine group appended to **2** gives the host **4**, which improved the hydrophobic cavity and contributed a new recognition site for forming hydrogen bonding interaction with the hydroxyl groups on guests MA and HO molecules, so host **4** displays the higher binding abilities toward MA and HO than host **2**. On the other hand, host **5** possessing the diethylene triamine substituent group gives the lower K_S values than host **2** for all guests except for HO. One possible explanation for above results is that the longer side chain of host **5** partially includes into its own hydrophobic cavity [14], decreasing the binding ability of host **5** toward guests to some extent.

Different spectral behavior

It is noteworthy that the fluorescence intensity of HO at 348 nm decreases with the addition of varying concentrations

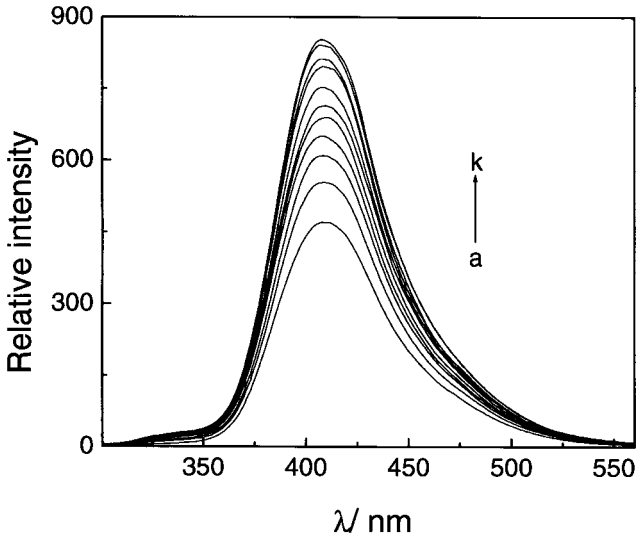

Figure 2. Fluorescence spectral changes of magnolol (7.5 μM) upon addition of **5** in phosphate buffer solution (pH 7.20) at 25 °C; the concentration of **5** was from 0 to 1.9 mM (from a to k); excitation at 290 nm.

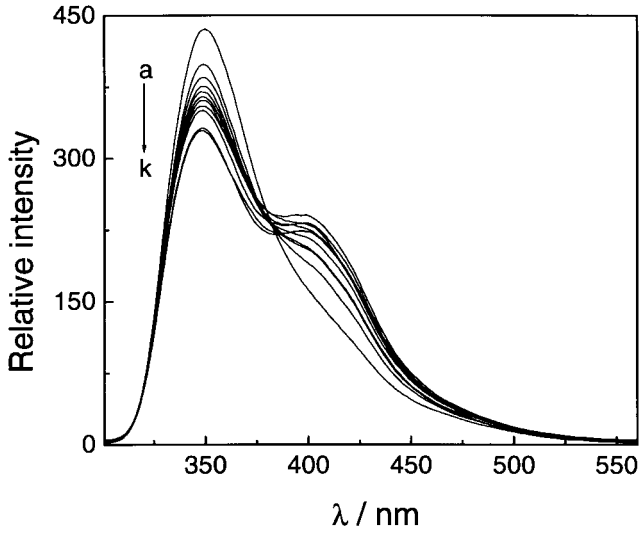

Figure 3. Fluorescence spectral changes of honokiol (10.7 μM) upon addition of **5** in phosphate buffer solution (pH 7.20) at 25 °C; the concentration of **5** was from 0 to 1.9 mM (from a to k); excitation at 290 nm.

of host **5**, which is different from the fluorescence intensity increasing at 348 nm upon the addition of all other hosts. Otherwise, the fluorescence intensity also increased upon addition of host **5** into another guest medicines, such as MA. The comparative fluorescence spectra of host **5** with MA and HO were shown in Figures 2 and 3, respectively. Although MA has the most similar structure to HO, its fluorescence intensity shows the changes as a usual rule, which demonstrates that, in most cases, the fluorescence intensity increases with the fluorescent moiety enter into the apolar hydrophobic cavity from the polar aqueous solution [15]. More interestingly, along with the fluorescence intensity decreases at 348 nm, a new fluorescence emission peak appears at ~400 nm and its intensity increases with the addition of host **5**. This probably means that the complex formed by HO and host **5** changed the fluorescence behavior of HO itself and gave the different fluorescence emission. This interesting abnormal fact may be explained as the conjugated system constructed by chromophoric groups in HO molecule was disturbed by HO including into the cavity of host **5**.

Conclusion

The molecular recognition behaviors of host cyclodextrins **1–5** with four Chinese traditional medicines were studied by fluorescence spectrometry. The results indicate that the size/shape-fit relationship gives the largest contribution to the binding ability of host cyclodextrins. The hydrophobic and hydrogen bonding interactions are the main interactions between hosts and guests, and the substitute on the side arm attached to the edge of β-cyclodextrin plays a crucial role in guest inclusion.

Acknowledgements

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