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Preparation and Characterization of the Solid Inclusion Compounds of α-, β-Cyclodextrin with Phenylalanine (D-, L- and DL-Phe) and Tryptophan (D-, L- and DL-Trp)

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Abstract

The solid cyclodextrin (α-, β-CD) inclusion compounds of phenylalanine (D-, L- and DL-Phe) and tryptophan (D-, L- and DL-Trp) were prepared and the stoichiometry of host and guest in the supermolecules was determined to be 1:1 based on elemental analyses. β-CD formed inclusion compounds with α-aromatic amino acid (α-AAA) in higher yield in contradistinction to α-CD. The yields of the α- or β-CD inclusion compounds of a pair of optical isomers of chiral aromatic amino acids and their racemic modification decreased in the order L- > DL- > D-form. The complexation between CD and α-AAA caused a change in shape, location and diffracted intensity of the X-ray diffraction peaks of both host and guest. The decomposition temperature of the inclusion compounds was not only slightly higher than that of a pure host but also remarkably higher than that of a pure guest. Upon inclusion the signals of CD protons inside the cavity shifted to upfield while those of the protons outside the cavity had only smaller changes, and the proton signals of the aromatic ring of guest shifted to a certain extent. The chemical shift changes of 4-H and 5-H located in small end side of cavity were a bit bigger than those of 2-H and 3-H located in large one, suggesting that aromatic ring of a guest molecule within a host cavity might be kept near small end side of cavity. An ESI-MS experiment has proved the formation and stability of the 1:1 CD inclusion compounds of α-AAA in aqueous solution.

Introduction

Cyclodextrins (CDs) are cyclic oligomers that have α-1,4 linked D-glucose units and consist of six, seven, and eight glucose units, which named as α-, β- and γ-CD respectively [1]. Each of the chiral glucose units is in the rigid 4*C*1-chair conformation, giving the macrocycle the shape of a hollow truncated cone. A schematic drawing of α- and β-CD is shown in Figure 1. The primary hydroxyls of the glucose units in a CD molecule are located at the narrow face of the cone and the secondary hydroxyls at the wide face. CDs have a remarkable property of including various molecules in aqueous solution [2, 3]. Many aspects of cyclodextrin chemistry were reviewed in Volume 3 of Comprehensive Supramolecular Chemistry [4] and in several articles in a special issue of Chemical Reviews [5]. The formation of CD inclusion compounds can effectively improve solubility and stability of guest molecules so they have been widely used in recognition field of model enzyme [6, 7], molecular devices [8, 9] and so on. So far a large number of papers on the formation and stability of CD inclusion compounds in aqueous solution have been published [10–14]. Due to significant solubility differences between complexes of CD and optical isomers of guest, they were also usually used with analytical purposes [15, 16]. In the recent decade, crystal structure studies or physical and chemical characterization of CD inclusion compounds has also become an important aspect of CD chemistry [17–21]. The preparation and characterization of the solid supermolecules of CD with biphenyl derivatives, vanillin and pyridine derivatives as well as the interaction between CDs and the guests in aqueous solution had been investigated in our previous papers. An aromatic ring of the guest molecules preferred to penetrate into CD cavity from small end side according to nuclear magnetic resonance data [3].

A proteinogenic α-amino acid is an important organic molecule and most of them have higher solubility in water. By far, there are many papers dealing with inclusion phenomena and chiral recognition of the 22 proteinogenic α-amino acids, on the basis of CD complexation with them in aqueous solution, using microcalorimetry, fluorescence polarization, UV–Vis and circular dichroism methods [22–24].

Several years ago Stezowski and his co-workers reported a single crystal study on supramolecular inclusion

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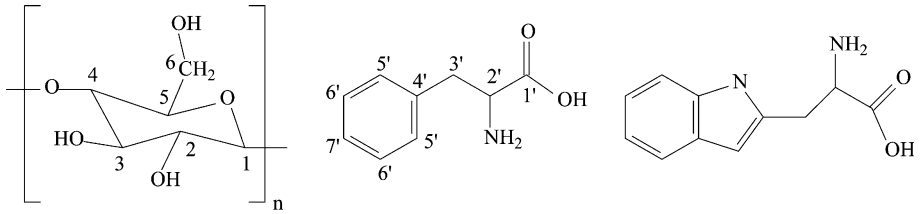

n=6, α-CD
n=7, β-CD

Phe

Trp

Figure 1. Schematic drawings of the hosts (α-, *β*-CD) and guests (Phe, Trp).

compounds, in which *β*-CD served as the host and guest molecules were the derivatives of Phe, indicating that for the different guest molecules, similar modes of penetration are observed in the CD torus [25]. In our earlier work the formation and stability of CD inclusion compounds of some α-amino acids and peptides with low molecular weight were investigated in aqueous solution [26–28]. The complexation stoichiometry of CD and amino acids or their simple derivatives in aqueous solution was found to be one to one in most cases [22, 26].

Theoretically there should be strong interaction between a proteinogenic α-aromatic amino acid (α-AAA) and a parent CD on the basis of CD cavity property with a good matching to structure, size and aromatic hydrophobic character of the guest molecules [29–31]. However, the values of association constant (K_a) for the complexes, α-AAA–CDs, are all very small and only with a range of 10^1–10^3 mol^{-1} dm^3 [22]. And a binding difference in complexation of a parent CD to a L-α-AAA and to a D-α-AAA in aqueous solution is usually so trifling that chemical resolution of two enantiomers could be very difficult by forming their CD inclusion compounds. It should be anticipated that in a solid CD inclusion compound of a racemic mixture (DL-α-AAA) in equal parts of two optical opposites, the relative mole percent content of two inclusion compounds: D-α-AAA–CD and L-α-AAA–CD ought to be close agreement. Therefore in the interest of improving the efficiency of resolution and identification between two enantiomers of a chiral compound besides using modified derivatives of CD as a host, more studies are also needed to reveal some fine differences, including preparation, properties and spectral discrimination, between two CD inclusion compounds of a pair of optical active isomers of an amino acid. To our knowledge, there was so little data on historical efforts in analytical characterization of complexes of solid complexes of CDs with amino acids. To have a further realization on the inclusion reaction of CDs with D-, L-, DL-α-AAA and on the spectral differences among D-α-AAA–CD, L-α-AAA–CD and DL-α-AAA–CD, α- and *β*-CD were selected as hosts and chiral phenylalanine (D-, L- and DL-Phe) and tryptophan (D-, L- and DL-Trp) were selected as guests in the work (see Figure 1).

This work deals with characterization of the solid complexes of CDs with amino acids, which is related to our previous paper published in Chin. J. Inorg. Chem., in which inclusion phenomena on the supramolecular system of CDs with D-, L- and DL-Phe were studied in aqueous solution [32]. The 12 solid inclusion compounds: D-Phe–α-CD, L-Phe–α-CD, DL-Phe–α-CD, D-Trp–α-CD, L-Trp–α-CD, DL-Trp–α-CD, D-Phe–*β*-CD, L-Phe–*β*-CD, DL-Phe–*β*-CD, D-Trp–*β*-CD, L-Trp–*β*-CD and DL-Trp–*β*-CD were prepared and characterized by using thermogravimetric (TG), differential thermal analysis (DTA), X-ray powder diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), electrospray ionization mass spectrometry (ESI-MS) and ^1H nuclear magnetic resonance (NMR) methods.

Experimental

Materials

All α-aromatic amino acids are chromatographic pure and used without further purification. *β*-CD was purchased from Shanghai Chemical Reagent Company and recrystallized twice from deionized water. α-CD was purchased from Nihon Toshin Chemical Company. All the other materials are analytically pure. Li_2CO_3 and Ni_2SO_4 were recrystallized from deionized water and gadolinium oxide was dissolved by concentrated hydrochloric acid.

Preparation of the solid inclusion compounds

Inclusion compounds of α-aromatic amino acids with CDs
The phenylalanine (D-, L-, DL-Phe) or tryptophan (D-, L-, DL -Trp) was mixed with α- and *β*-CD respectively in 60 ml of deionized water and stirred for 24 h at room temperature. The original molar ratio of α-aromatic amino acids and CD was 1:1. After the solvent was drawn out from the reaction system below 35 °C, the crude product was washed with a small amount of deionized water repeatedly and dried *in vacuo*. The solid inclusion compounds were obtained after adequately dried. They were all white powder.

Complexes of α-aromatic amino acids with CDs in the presence of inorganic ions
Inorganic ions as one part of buffer solution are usually present in CD–guest reaction systems in aqueous

solution. In order to estimate the influence of inorganic ions on preparation (formation and composition) of CD solid complexes of organic guests, an α-aromatic amino acid and $NiSO_4$ or $GdCl_3$ were mixed in 2:1 molar ratio in 60 ml of deionized water. The mixture was refluxed for 3 h at 60 °C and cooled to room temperature. After adding CD aqueous solution of 30 ml to the above mixed solution, the reaction mixture was stirred for 24 h at room temperature. The original molar ratio of α-AAA and CD was 1:1. After solvent was drawn out from the reaction system below 35 °C, the crude product was washed with a small amount of deionized water repeatedly and dried *in vacuo*.

Characterization of the solid inclusion compounds

X-Ray powder diffraction (XRD) of the samples was reached on a Philips X′Pert Pro X-ray diffractometer. The samples were irradiated with monochromatized CuK_α and analyzed with $5° \le 2\theta \le 40°$. The voltage and current are 40 kV and 40 mA, respectively.

Fourier transform infrared (FTIR) spectra were recorded on Bruker EQUINOX55 spectrometer and obtained from KBr pellets in the 4000–400 cm^{-1} regions.

Electrospray ionization mass spectrometry (ESI-MS) was recorded on Finigan MS LCQ electrospray ionization mass spectrometer. All samples before use were kept for 3 h under ultrasonic vibration at room temperature. The highest measured concentration of samples is 1 mg cm^{-3}, using deionized water as a solvent.

Thermogravimetric (TG) and differential thermal analysis (DTA) was recorded on ZRY-1 TG-DTA integration thermal analyzer of Shanghai Balance Instrument Company at a heating rate of 10.00 °C min^{-1} with a temperature range of 0–500 °C under a nitrogen atmosphere.

Nuclear magnetic resonance (^1H NMR) spectra were obtained on Bruker-Am-500 NMR spectrometer at 500 M Hz at 25 °C, using D_2O as a solvent and 2,2-Dimethyl-2-silapentane- 5-sulfonate sodium salt (DSS) as an external reference.

Results and discussion

Initially, the 1:1 stoichiometry of the inclusion compounds of α-AAA with CD was suggested through elemental analyses of the solid samples.

Preparation analysis

The 12 inclusion compounds prepared are listed in Table 1 along with their composition, yields estimated based on the original concentration of host, melting points (decomposition temperature) and results of elemental analyses.

β-CD formed solid inclusion compounds with the aromatic amino acids in higher yields (above 64%) in contradistinction to α-CD (below 47%). This could be because the α-CD inclusion compounds of α-AAA might also so highly soluble in water as α-CD that they had suffered more mass loss than α-AAA–β-CD when washed with a small amount of deionized water repeatedly. Moreover, upon complexation between an α-AAA and

Table 1. The melting points (decomposition temperature) of all free components and preparation of the solid inclusion compounds of CDs with aromatic amino acids

Compound	Yield	Decomp. Temp. (°C)	Composition	Anal. Calcd. (%)			Found (%)		
	(%)			C	H	N	C	H	N
α-CD	–	345.1	$C_{36}H_{60}O_{30}\cdot 3H_2O$	42.11	6.43	–	41.99	6.56	–
β-CD	–	346.0	$C_{42}H_{70}O_{35}\cdot 4H_2O$	41.79	6.47	–	41.94	6.39	–
D-Phe	–	285.0	$C_9H_{12}O_2N$	–	–	–	–	–	–
L-Phe	–	283.5	$C_9H_{12}O_2N$	–	–	–	–	–	–
DL-Phe	–	272.7	$C_9H_{12}O_2N$	–	–	–	–	–	–
D-Trp	–	310.0	$C_{11}H_{12}O_2N_2$	–	–	–	–	–	–
L-Trp	–	305.0	$C_{11}H_{12}O_2N_2$	–	–	–	–	–	–
DL-Trp	–	307.3	$C_{11}H_{12}O_2N_2$	–	–	–	–	–	–
D-Phe–α-CD	32.5	346.6	$C_{45}H_{71}O_{32}N\cdot 6H_2O$	43.27	6.67	1.12	43.01	6.72	1.29
L-Phe–α-CD	46.3	345.5	$C_{45}H_{71}O_{32}N\cdot 5H_2O$	44.01	6.60	1.14	43.80	6.78	1.33
DL-Phe–α-CD	42.2	345.8	$C_{45}H_{71}O_{32}N\cdot 5H_2O$	44.01	6.60	1.14	43.77	6.91	1.27
D-Trp–α-CD	37.2	346.8	$C_{47}H_{72}O_{32}N_2\cdot 5H_2O$	44.55	6.48	2.21	44.16	6.69	2.25
L-Trp–α-CD	44.8	346.0	$C_{47}H_{72}O_{32}N_2\cdot 4H_2O$	45.19	6.41	2.24	44.85	6.48	2.44
DL-Trp–α-CD	40.9	346.2	$C_{47}H_{72}O_{32}N_2\cdot 4H_2O$	45.19	6.41	2.24	44.93	6.50	2.36
D-Phe–β-CD	64.4	348.3	$C_{51}H_{81}O_{37}N\cdot 8H_2O$	42.41	6.72	0.97	42.32	6.77	1.10
L-Phe–β-CD	76.7	347.4	$C_{51}H_{81}O_{37}N\cdot 7H_2O$	42.95	6.67	0.98	43.10	6.59	1.21
DL-Phe–β-CD	73.5	347.9	$C_{51}H_{81}O_{37}N\cdot 7H_2O$	42.95	6.67	0.98	43.26	6.44	1.07
D-Trp–β-CD	69.1	351.7	$C_{53}H_{82}O_{37}N_2\cdot 7H_2O$	43.44	6.56	1.91	43.51	6.36	1.99
L-Trp–β-CD	78.0	347.6	$C_{53}H_{82}O_{37}N_2\cdot 6H_2O$	43.98	6.50	1.94	44.09	6.43	2.15
DL-Trp–β-CD	69.6	350.5	$C_{53}H_{82}O_{37}N_2\cdot 6H_2O$	43.98	6.50	1.94	44.25	6.22	2.08

the same CD, the selectivity only reflected in the yields of the CD inclusion compounds decreases in the order L- > DL- > D-form based on the data listed in Table 1. This order basically corresponds to an order of the values of the association constants (see Table 2) of L-α-AAA with a parent CD under the same pH and temperature condition in aqueous solution [22], suggesting that the yields of CD complexes of L-, DL - and D-form may be related to their ability to associate with the columnar CDs in aqueous solution.

As shown in Table 1, thermal decomposition temperature of the CD inclusion compounds of α-AAA is not only slightly higher than that of the parent CD but also significantly higher than that of α-AAA. The decomposition temperature of the CD inclusion compounds decreases in the order: D- > DL- > L-α-AAA, possibly reflecting the side chain effects of different spatial arrangement (D- or L-configuration) of atoms in an aromatic amino acid molecule [26].

Although the association ability of aromatic amino acids with CDs is usually very weak (see Table 2), α- or β-CD inclusion compounds of aromatic amino acids can still exist steadily in aqueous solution at room temperature even during ESI-MS measurement.

Kahle and Holzgrabe determined the binding constants of the complexes between α- or β-CD and the enantiomers of a series of aliphatic and aromatic amino acids, and dipeptides, using a potentiometric titration method [39]. Nishijo and Tsuchitani investigated the interaction of L-Trp with α-CD in a 0.1 M phosphate buffer at pH 7.4 with calorimetry, suggesting the driving force for inclusion complex formation might be mainly van der Waals-London dispersion force, and the contribution of hydrogen bonding was secondary in importance [40].

From the composition of the CD inclusion compounds of α-AAA shown in Table 1, the stoichiometry of α-AAA:CD is all one to one based on elemental analyses, and there have always been some water molecules of crystallization in CDs or their solid inclusion compounds. The numbers of hydration waters are not directly related to the sizes of CD cavity and guest molecule. The solubility of CD and guest molecules in water, the hydrophobicity of guest molecule and the preparation/crystallization conditions are the key factors in determining the number of hydration waters in the crystal lattice. As for the CD–AAA reaction systems, the number of crystal water molecules may depend on the large and small sizes of the host and guest. It decreases in the order: β-CD complex > α-CD complex for the same guest and increases in the order: Trp complex < Phe complex for the same host, only according to the data listed in Table 1. These results indicate that the complexation between a CD with larger cavity and a guest with smaller volume might be more favorable for the formation of crystal water molecules included in the crystal structures for free water molecules in aqueous solution.

The reaction mixture of L-Phe and $GdCl_3$ was treated with β-CD. The crude product was dried *in vacuo* and washed with a small amount of deionized water repeatedly. In the solid product obtained, main component is the 1:1 CD inclusion compound: L-Phe–β-CD (*m*/*e*, mass to charge ratios, 1298.5; relative abundance, 100%), the β-CD inclusion compound of the coordination complex of L-Phe with $GdCl_3$: β-CD–$(L\text{-Phe})_2Gd(H_2O)$ (*m*/*e*, 1664.1; relative abundance, 46%) is only one minor component by ESI-MS. However after $NiSO_4$ was substituted for $GdCl_3$, L-Phe–β-CD is only

Table 2. Association constant (K_a) and standard free energy ($-\Delta G^\circ$) for 1:1 inclusion complexation of various guests with CDs at 298 K in aqueous solution

Host	Guest	pH	K_a/mol^{-1} · dm^3	Log K_a	$-\Delta G^0$/kJ · mol^{-1}	$-\Delta\Delta G^0$/kJ · mol^{-1}	K_L/K_D	Ref
α-CD	L-Phe	5.01	7.9 ± 1.7	0.90 ± 0.24	5.2 ± 3.4			33
	L-Phe	7.4	42.7	1.63	9.3			34
	L-Phe	7.6	25.5	1.41	8.0			35
	L-Phe	11.0	15.8	1.20	6.8			36
	L-Phe	11.3	25.1 ± 1.0	1.40 ± 0.02	8.0 ± 0.1			37
	L-Phe	13.6	7.9 ± 1.4	0.90 ± 0.14	5.0 ± 1.0			38
	L-Phe	6.84	125.0	2.10	12.0	11.4	1.78	32
	D-Phe	6.84	70.4	1.85	10.6			32
	D-Phe	5.01	19.5	1.29	7.4			33
	D-Phe	11.0	7.9 ± 1.7	0.90 ± 0.24	5.2 ± 3.4			36
	D L-Phe	6.84	99.8	2.00	11.4			32
	L-Trp	7.4	21.4	1.33	7.6			34
	L-Trp	11.3	28.2 ± 1.0	1.45 ± 0.02	8.3 ± 0.1			36
β-CD	L-Phe	5.01	3.0 ± 3.3	0.48 ± 0.52	3 ± 3			33
	L-Phe	11.3	107.2 ± 1.1	2.03 ± 0.05	11.6 ± 0.3			36
	L-Phe	6.84	26.5	1.42	8.1	2.8	3.01	32
	D-Phe	6.84	8.80	0.94	5.3			32
	DL-Phe	6.84	17.0	1.23	7.0			32
	L-Trp	7.4	213.8	2.33	13.3			34
	D-Trp	8.9	12.9 ± 1.6	1.11 ± 0.21	6.3 ± 1.3			38

one minor product (*m*/*e*, 1298.5; relative abundance, 73%) and two principal molecular ion peaks appear at *m*/*e*, 1133.5 (relative abundance, 100%) and 1169.6 (relative abundance, 92%), respectively corresponding to *β*-CD and *β*-CD·2H_2O by ESI mass spectra.

X-ray analysis

X-ray powder diffraction analysis is one of the most important methods to characterize solid inclusion compounds of CD. The three typical diffraction patterns of α-CD, the physical mixture of α-CD and L-Trp (1:1, w/w) and the α-CD inclusion compound of L-Trp are shown in Figure 2.

To go by appearances, the inclusion compounds' XRD curve shows more amorphous when compared with those of the free components and physical mixture. This result indicates that a disorder phenomenon could occur upon inclusion [41].

In Figures 2 and 3, the X-ray curve of pure α-CD has no strong peaks when $2\theta < 11°$. The strongest diffraction peak of α-CD and *β*-CD is respectively located at 2θ values of 11.8° and 18.2°, and the second is at 2θ values of 21.7° for α-CD and 13.4° for *β*-CD. The observed locational differences of diffraction peaks could be due to difference between number of glucose units of α-CD and that of *β*-CD, which results in making their macrocyclic conformation different [42] and bearing up on the X-ray diffraction figure of them.

The four major diffraction peaks at 2θ values of 4.9° (strongest), 14.8° (secondly), 9.7° and 19.8° are observed in the XRD pattern of L-Trp as guest molecule. By comparing the data from diffraction patterns of host, guest and their mixture, it was easily found that the six top peaks at 2θ values of around 4.9°, 9.7°, 12.2°, 14.8°, 19.8° and 22.0° in the mixture have such a close similarity or resemblance as to be essentially equal or interchangeable with those of the pure α-CD and L-Trp. Since the X-ray diffraction figure of the physical mixture is only simple stacking of peaks of both host and guest, this suggests that there is no chemical reaction between host and guest in solid state during physical mixture. As for α-CD before and after mixed, the two peaks lying at 2θ values of 12.2° and 22.0° have a visible intensity change possibly derived from their overlapping with some small peaks of L-Trp having higher relative molar content in the physical mixture.

The curve of the L-Trp–α-CD shown in Figure 2 distincts from that of L-Trp–*β*-CD shown in Figure 3. The strongest peak at 19.6°, the second at 22.4° and the other major peaks at 7.2°, 7.9°, 12.9° and 21.0° for L-Trp–α-CD are observed, which are all different from those of the physical mixture of α-CD and L-Trp. *β*-CD is a very crystalline molecule with the strongest peak at 18.2°, the second at 13.4°, and the other peaks at 2θ values of 4.75°, 12.7°, 19.7°, 21.1°, 22.8°, 23.9°, 24.3°, and 35.9° [43]. However, the major peaks at 10.5°, 12.6° (strongest), 17.1°, 18.9°, 19.6° (secondly), 21.0° and 22.8° in the XRD pattern of L-Trp–*β*-CD are observed. For the two peaks corresponding to 12.6° and 19.6° in *β*-CD, intensity of the former increases predominatingly and that of the latter decreases very obviously in the case of L-Trp–*β*-CD or DL-Trp–*β*-CD. New peaks at 10.5°, 17.1° and 18.9° are observed in diffractograms of both L-Trp–*β*-CD and DL-Trp–*β*-CD, indicative of interaction between a CD and an α-AAA.

The solid inclusion compound of racemic modification DL-Trp with *β*-CD should be regarded as a mixture

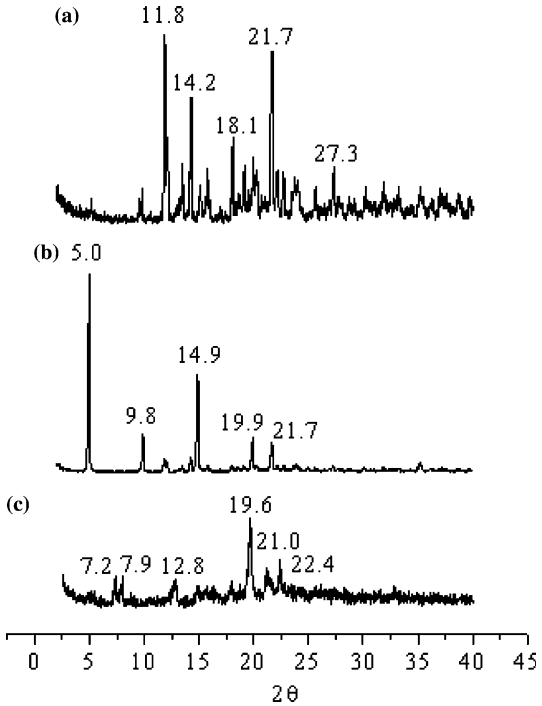

Figure 2. XRD spectra of α-CD and *L*-Trp system: (a) α-CD, (b) L-Trp–α-CD mixture, (c) L-Trp–α-CD complex.

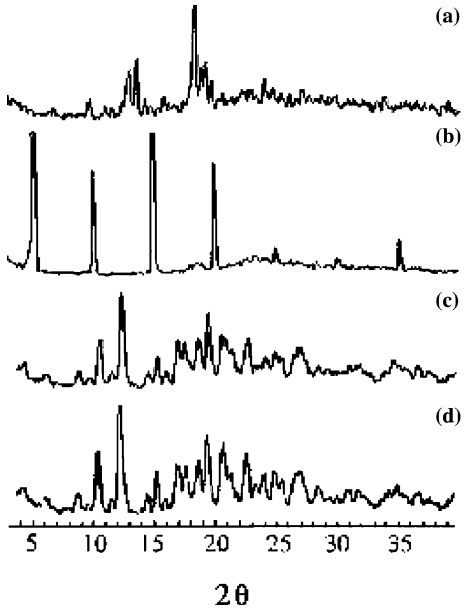

Figure 3. XRD spectra of β-CD and Trp system: (a) β-CD, (b) L-Trp, (c) L -Trp–β-CD complex (d) D-Trp–β-CD complex.

of the two inclusion compounds: D-Trp–β-CD and L-Trp–β-CD, and the percent content of L-Trp–β-CD in the mixture could be slightly more than that of D-Trp–β-CD since the association constant of L-Trp with β-CD is a bit bigger than that of D-Trp with β-CD in aqueous solution [22]. It has been found that the diffractogram of the inclusion compounds of DL-Trp is very similar to that of the inclusion compound of D-Trp or L-Trp. In other words, X-ray diffraction curves of the three inclusion compounds: D-Trp–β-CD, L-Trp–β-CD and DL-Trp–β-CD are so alike that it is quite difficult for only using a XRD method to investigate the relativity between the structural differences among D-Trp, L-Trp and DL-Trp and their inclusion phenomena with the same CD. In this case of the XRD pattern of α-AAA–CD, the number of peaks is the minimum, indicating some degree of amorphization [41, 43].

The XRD results for Phe–α-CD, D-Trp–α-CD and α-AAA–β-CD system are very similar to those for Trp–α-CD and Trp–β-CD system as above [44]. Genarally speaking, for the same host such as β-CD but different guests such as D- and L-α-Trp, there are too little differences in their XRD spectra of complexes (see Figure 3c and d). For the same guest such as L-α-Trp but different hosts such as α-CD and β-CD (see Figures 2c and 3c), the XRD spectra of two complexes show highly exceptional differences, including location and intensity of major diffraction peaks, which are very different from those of L-α-Trp, α-CD and β-CD. The complexation could cause a change (see Table 3) of shape, location and diffracted intensity of the peaks formerly attributed to host or guest in diffractograms as a result of modification or transformation of conformation characteristic of host or guest upon inclusion. All these results open out the possibility of formation of a solid inclusion compound between α-AAA and CD.

TG and DTA analysis

Thermal analysis is also one of the most important methods to characterize solid inclusion compounds of CD [45]. In this paper, the representative TG and DTA curves of pure β-CD, L-Trp, D-Trp, the physical mixture and the inclusion compounds are illustrated in Figure 4.

From the TG curves shown in Figure 4, β-CD releases about 4 water molecules (calc. 3.89), namely

Table 3. The characteristic peaks of hosts, guests and their complexes in the XRD spectra

Compound	Major characteristic peaks (2θ)
α-CD	11.8° (strongest), 14.2°, 18.1°, 21.7° (secondly), 27.3°
β-CD	12.7°, 13.4° (secondly), 18.2°(strongest), 19.0°, 19.7°, 23.9°
L-Phe	5.5° (secondly), 16.9°, 22.8° (strongest), 28.7°, 34.6°
D-Phe	5.6° (secondly), 17.0°, 22.7° (strongest), 28.8°, 34.5°
DL-Phe	5.4° (secondly), 17.1°, 22.8° (strongest), 28.7°, 34.4°
L-Trp	4.9° (strongest), 9.7°, 14.8° (secondly), 19.8°, 35.1°
D-Trp	4.8° (strongest), 9.7°, 14.9° (secondly), 19.7°, 35.2°
DL-Trp	4.9° (strongest), 9.6°, 14.8° (secondly), 19.8°, 35.0°
L-Phe–α-CD	5.8 (strongest), 17.5, 22.8 (secondly), 28.5, 34.5
D-Phe–α-CD	5.9 (strongest), 17.4, 22.7 (secondly), 28.6, 34.6
DL-Phe–α-CD	5.8 (strongest), 17.5, 22.7 (secondly), 28.6, 34.6
L-Trp–α-CD-	7.2, 7.9, 12.9, 19.6 (strongest), 21.0, 22.4 (secondly)
D-Trp–α-CD	7.3, 7.9, 12.8, 19.6 (strongest), 21.1, 22.4 (secondly)
DL-Trp–α-CD	7.2, 8.0, 12.7, 19.5 (strongest), 21.1, 22.3 (secondly)
L-Phe–β-CD	5.7 (strongest), 17.4, 22.7 (secondly), 28.6, 34.5
D-Phe–β-CD	5.8 (strongest), 17.3, 22.7 (secondly), 28.6, 34.6
DL-Phe–β-CD	5.6 (strongest), 17.3, 22.7 (secondly), 28.6, 34.5
L-Trp–β-CD	10.5°, 12.6° (strongest), 17.1°, 18.9°, 19.6° (secondly), 21.0°, 22.8°
D-Trp–β-CD	10.4°, 12.4° (strongest), 17.0°, 18.8°, 19.4° (secondly), 21.1°, 22.7°
DL-Trp–β-CD	10.5°, 12.4° (strongest), 17.2°, 18.9°, 19.3° (secondly), 21.0°, 22.8°

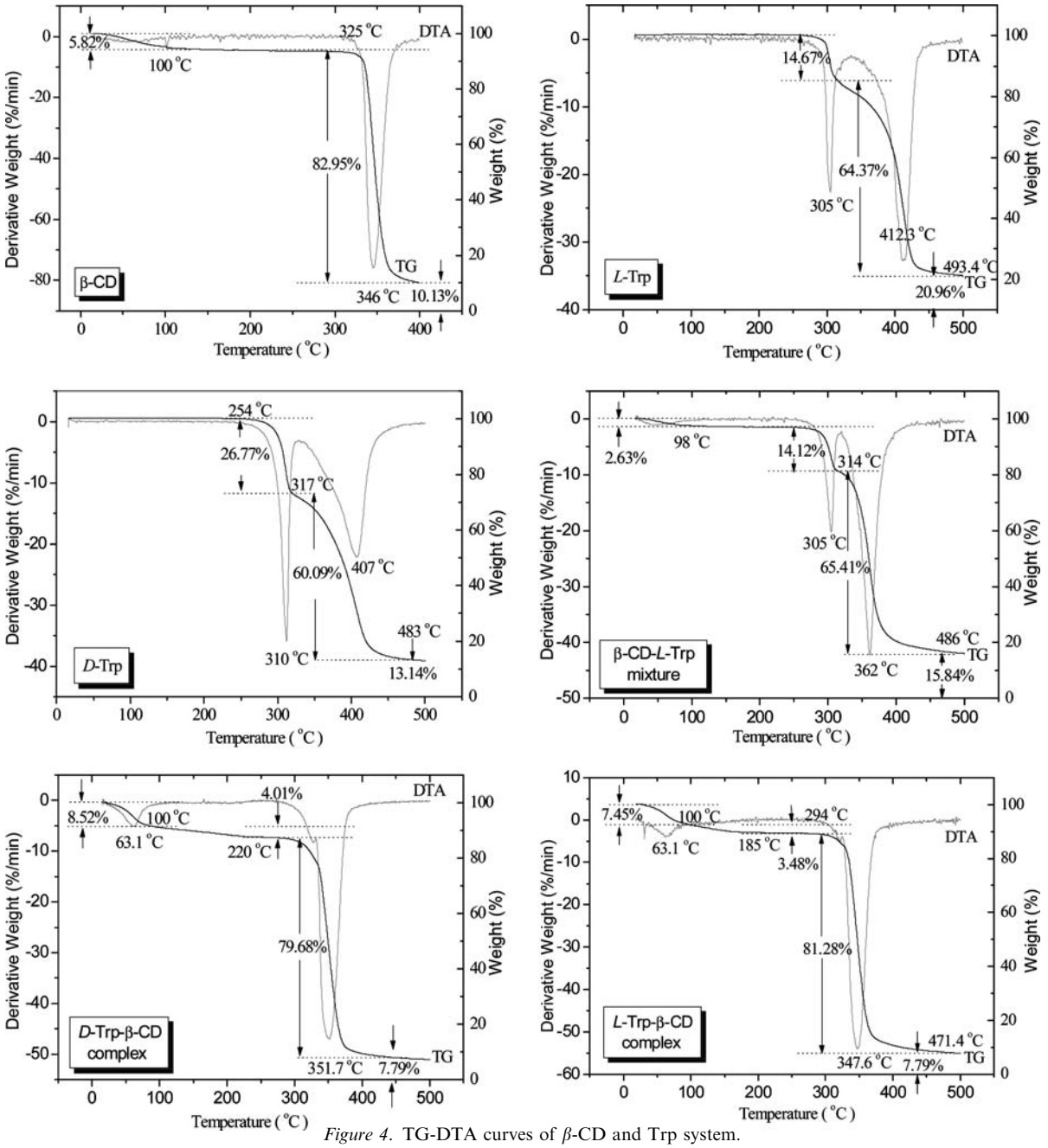

Figure 4. TG-DTA curves of β-CD and Trp system.

lattice water or crystal water inside or outside the cavity of CD below 100 °C with accompanying a 5.82% loss of mass between 28–100 °C. The water contents determined from the TG curves correspond to those from elemental analyses (Table 1). When temperature rises above 325 °C, β-CD begins to melt and the DTA curve shows a sharp endotherm at 346 °C with accompanying an 82.95% loss of mass between 325–395 °C.

The DTA curves of D-Trp and L-Trp show a sharp melting endothermic peak at 310 and 305 °C and a sharp decomposition endothermic peak at 407 and 412.3 °C respectively. Their TG curves show a 26.77 and 14.67% loss of mass corresponding to a melting-decomposition endotherm for D-Trp between 254–317°C and for L-Trp between 270–316 °C, a 60.09 and 64.37% loss of mass corresponding to a decomposition endotherm for D-Trp between 317–483 °C and for L-Trp between 316–493.4 °C respectively. And the TG curves of D-Trp and L-Trp show an 86.86 and 79.04% loss of mass when heated above 483 and 493.4 °C, respectively.

The TG-DTA curve of the physical mixture of β-CD and L-Trp shows an endotherm at 98 °C corresponding to pure β-CD because of losing some water molecules, a sharp endothermic peak at 305 °C corresponding to pure L-Trp due to melting of L-Trp and a sharp decomposition endotherm at 362 °C which is quite different from that of host (346 °C) or guest (412.3 °C) in free components' TG-DTA curves. Although it has been reported that a host-guest interaction in a physical mixture could occur [41], the new peak observed at 362 °C might be reasonably regarded as overlapping between melting-decomposition endothermic peak of

β-CD (346 °C) and the decomposition endothermic peak of L-Trp (412.3 °C) in this work.

The TG-DTA curves of two inclusion compounds: L-Trp–β-CD and D-Trp–β-CD were different from that of β-CD, especially obviously from those of guest and the host–guest physical mixture. The DTA curves of both L-Trp–β-CD and D-Trp–β-CD show a small clear dehydration endothermic peak at 63.1 °C. From the comparison of these TG curves, the 5.82% loss of water in pure β-CD has increased to 7.45% in L-Trp–β-CD or to 8.52% in D-Trp–β-CD between 28–100 °C. Hereby in L-Trp–β-CD and D-Trp–β-CD the number of water molecules determined from the TG curves is about 6 (calc. 5.98) and 7 (calc. 6.92), respectively, which nicely corresponds to those according to the results of elemental analyses (Table 1) and is more than that in pure β-CD under the same dry condition. These indicate that the CD inclusion compounds of α-AAA could have accommodated some extra water molecules included in the cavity of host or penetrated into the fit clearance of the supermolecules in comparison with pure CD. Moreover, the DTA curve of L-Trp–β-CD or D-Trp–β-CD shows disappearance of the melting-decomposition point peaks of L-Trp at 305 °C or D-Trp at 310 °C when compared with the physical mixture and pure guest. Since the elemental analyses and XRD experimental results have indicated the formation of CD inclusion compound of Trp, the phenomenon described above further suggests that some groups of Trp molecule as guest may have been so tightly bound in the hydrophobic cavity of β-CD as host that they still stay on the solid supramolecular inclusion compound even heated above melting-decomposition temperature of guest. However, a new loss of mass between 100–220 °C has also been observed in the TG curve of the β-CD inclusion compound of Trp. D-Trp–β-CD shows a 4.01% loss of mass between 100–220 °C and that of L-Trp–β-CD shows a 3.48% loss of mass between 100–185 °C. It is notable that these do not happen in the TG curve of free components or their mixture. Some studies on the kinetic of oxidation of amino acids have shown that amino acid oxidation is inhibited by the presence of CDs [46]. Therefore, the new loss of mass is supposed to be attributed to melting-decomposition or volatilization of a small amount of indole, a component of tryptophan, which could be formed when tryptophan or its complex was heated in the presence of a definite amount of water.

Furthermore, the melting-decomposition endothermic peaks of L-Trp–β-CD and D-Trp–β-CD upshifted to 347.6 °C and 351.7 °C respectively when compared with that of pure β-CD (346 °C) according to their DTA curves. Hence, the melting-decomposition temperature of the CD inclusion compounds is not only slightly higher than the pure β-CD but also remarkably higher than the pure L-Trp and D-Trp. The TG-DTA analyses for α-AAA–α-CD and Phe–β-CD system are very similar to those for Trp–β-CD system as above. The similarities between the two DTA curves of α- and β-CD inclusion complexes for the same guest, include a very minor change (location, shape and intensity) to all corresponding peaks and the same number of the peaks in two curves. For the same host such as β-CD but different guests such as D- and L-Trp, there are a little differences in the TG-DTA spectra of two complexes (see Figure 3), such as location (347.6°C for L-Trp–β-CD and 351.7 °C for D-Trp–β-CD) of the melting-decomposition peak, which is very different from that of L-α-Trp (305 °C and 412.3 °C) but is close to that of β-CD (346 °C). Upon inclusion, thermal stability of the parent CDs as host and chiral α-aromatic amino acids as guest can be improved to a certain degree. The comparison of these TG-DTA curves could suggest the formation of the host-guest inclusion compounds of α-AAA and CD. The slight differences of decomposition temperature among L-AAA–CD, D-AAA–CD and DL-AAA–CD have been observed (see Table 1), indicating TG-DTA analysis could play some role in identifying the solid CD inclusion compounds of a single pair of amino acid enantiomers, and of mixed amino acids.

FTIR spectra analysis

FTIR spectra technique can play an important role in characterizing formation of solid CD inclusion compounds [47]. Only a few spectral differences among D-Phe–α-CD, α-CD and the physical mixture were found in this experiment (see Table 4).

The FTIR spectrum of pure α-CD or its mixture with D-Phe shows several principal absorption peaks at 1027.9, 1078.4 and 1157.9 cm^{-1} respectively, which could be due to υC-O of cyclic ether groups in CD. But in the α-CD inclusion compound of D-Phe, the first of these absorption peaks clearly shifted to higher wave numbers: 1035.1, the other two somewhat shifted to lower wave numbers: 1074.9 and 1154.6 cm^{-1} respectively (see Figure 5). The FTIR spectral pattern of D-Phe or its mixture of α-CD, shows some absorption peaks at 1501.4, 1586.6 and 1620.1 cm^{-1} respectively, which could be ascribed to the $\upsilon_{C=C}$ stretching of the aromatic moiety. In contrast to that in the FTIR spectra patterns of the D-Phe inclusion compound of α-CD the first two of three peaks have slightly moved to higher

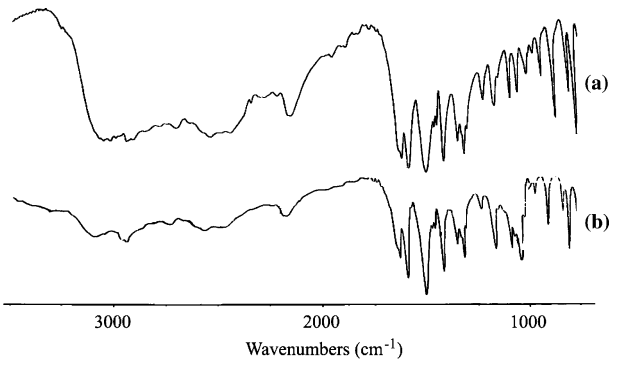

Figure 5. IR spectra of α-CD and Phe system: (a) D-Phe, (b) D-Phe–α-CD complex.

wave numbers: 1502.8, 1588.5 and the other one slightly moved to lower wave numbers: 1619.0 cm^{-1}.

The FTIR spectra analyses for L-Phe–α-CD, Trp–α-CD and α-AAA–β-CD system are similar to those for D-Phe–α-CD system [44]. These phenomena as above could be due to some host-guest interaction between the phenyl group or indole group of α-AAA and CD in these complexes. The spectral patterns of α-AAA–CD show that a location shift of those peaks around 1080 cm^{-1} corresponding to the CD characteristic absorption peaks is bigger than that of those peaks around 1560 cm^{-1}. Moreover, in FTIR spectra patterns there are few significant differences among L-AAA–CD, D-AAA–CD and DL-AAA–CD, suggesting FTIR spectra analysis might not play a very important role in distinguishing solid CD inclusion compound of L- or D-form amino acid from that of their racemic mixture.

^{1}H NMR spectroscopy analysis

^{1}H NMR is a very important technique in studying CDs inclusion compounds because the chemical shift changes (Δδ) of the CD and guest protons before and after complexation, are closely related to the magnitude of CD-guest interaction [48, 49].

The 3-H chemical shifts (δ) of β-CD in free component and in its inclusion compound of L-Phe are 3.877 and 3.963 ppm (Δδ, 0.086 ppm) respectively, and the δ values of 5-H in pure β-CD and in L-Phe–β-CD are 3.776 and 3.873 ppm (Δδ, 0.097 ppm) respectively (see Figure 6). Upon complexation the chemical shift changes of 1-H, 2-H and 4-H outside CD cavity are 0.076, 0.062 and 0.084 ppm respectively, which are somewhat smaller than that of 3-H and 5-H inside CD cavity. The δ values of 6′-H, 5′-H, and 7′-H for aromatic protons of L-Phe have obviously shifted from 7.333, 7.280 and 7.239 ppm to 7.413, 7.374 and 7.320 ppm (Δδ, 0.080, 0.094 and 0.081 ppm), respectively.

The ^{1}H NMR spectroscopy data listed in Table 4 for α-AAA–α-CD, Trp–β-CD and D-Phe–β-CD system are almost similar to those as above for L-Phe–α-CD system [44]. The proton signals inside β-CD cavity shift to upfield as a result of the magnetic field of the aromatic π cloud and the proton signals of the aromatic ring of guest shift to a certain extent, suggesting that interaction of significant magnitude between phenyl group or indole group protons of α-AAA and the protons of carbon atoms lying on the inner surface of CD cavity [49]. Lipkowitz and his coworkers examined the enantioselective binding of tryptophan to α-CD and by NMR spectroscopy and molecular dynamics simulations, and found both enantiomers of Trp to be highly localized on the interior of the cavity effectively behaving like a tight fit and to have similar modes of binding to α-CD [50].

Grigera and his cowokers reported molecular dynamics simulations of Phe–CD system *in vacuo* and in aqueous solution and given detailed information of the dynamics of the complexes by describing the relative movement of the aromatic ring with respect to the polar region [51]. They found that the complexs in water are not very stable, in agreement with experimental data, while in all other situations studied the complexes are stable within the computational limits.

The perceptible chemical shift changes (Δδ) of both CD and guest protons especially those in the host cavity have clearly demonstrated the formation of the CD inclusion compounds of α-AAA. Furthermore, the chemical shift changes (Δδ) of 5-H and 4-H located in small end side of cavity is distinctly bigger than that of 3-H, 1-H and 2-H located in large side, possibly suggesting that a phenyl ring or indole ring of the guest molecules might take up its residence in the small end side of the host cavity.

ESI-MS analysis

Electrospray ionization mass spectrometry technique is one of important methods to investigate the formation and composition of inclusion compound. It has been

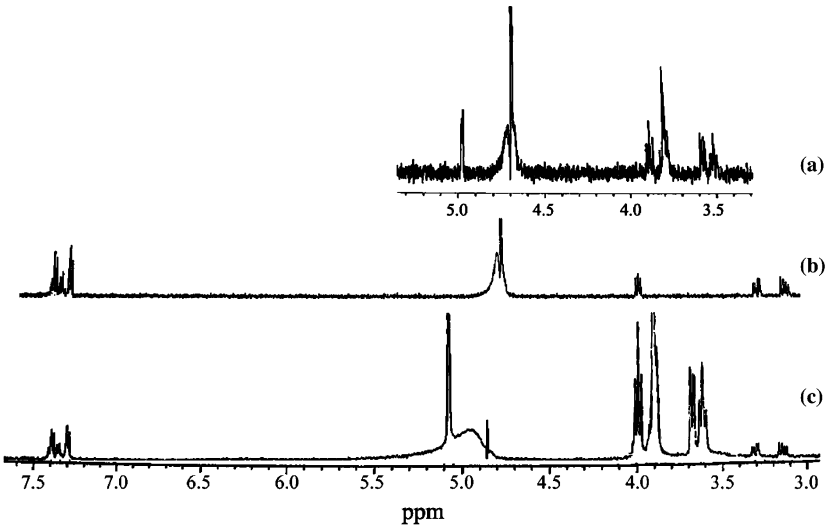

Figure 6. ^{1}H NMR spectra of β-CD and Phe system: (a) β-CD, (b) L-Phe, (c) L-Phe–β-CD complex.

Table 4. Representative FTIR, ^{1}H NMR and ESI-MS data of CD-α-AAA system

Compound	FTIR	^{1}H NMR (Δδ, ppm)[a]				MS (*m*/*e*)	
	(υ_{C-O} of CD, cm^{-1})	2-H	3-H	4-H	5-H	Found	Composition
α-CD	1027.9, 1078.4, 1157.9	–	–	–	–	972.2	$C_{36}H_{60}O_{30}$
D-Phe–α-CD	1035.1, 1074.9, 1154.6	0.049	0.080	0.070	0.091	1136.9	$C_{45}H_{71}O_{32}N$
L-Phe–α-CD	1034.7, 1075.0, 1154.8	0.058	0.084	0.073	0.095	1136.9	$C_{45}H_{71}O_{32}N$
DL-Phe–α-CD	1034.7, 1076.4, 1157.5	0.056	0.083	0.072	0.092	1136.9	$C_{45}H_{71}O_{32}N$
D-Trp–α-CD	1035.4, 1075.1. 1155.2	0.054	0.079	0.068	0.090	1176	$C_{47}H_{72}O_{32}N_2$
L-Trp–α-CD	1034.2, 1075.5, 1155.3	0.062	0.085	0.077	0.099	1176	$C_{47}H_{72}O_{32}N_2$
DL-Trp–α-CD	1034.9, 1075.8, 1156.0	0.060	0.085	0.078	0.092	1176	$C_{47}H_{72}O_{32}N_2$
β-CD	1029.4, 1080.8, 1157.3	–	–	–	–	1169.4	$C_{42}H_{70}O_{35}{\cdot}2H_2O$
D-Phe–β-CD	1035.2, 1075.9, 1156.2	0.052	0.082	0.075	0.094	1298.3	$C_{51}H_{81}O_{37}N$
L-Phe–β-CD	1034.5, 1076.0, 1156.4	0.062	0.086	0.084	0.097	1298.3	$C_{51}H_{81}O_{37}N$
DL-Phe–β-CD	1034.6, 1076.5, 1156.7	0.059	0.087	0.079	0.096	1298.3	$C_{51}H_{81}O_{37}N$
D-Trp–β-CD	1035.6, 1077.1, 1155.5	0.061	0.088	0.077	0.099	1337.8	$C_{53}H_{82}O_{37}N_2$
L-Trp–β-CD	1035.1, 1077.4, 1155.9	0.067	0.091	0.085	0.104	1337.8	$C_{53}H_{82}O_{37}N_2$
DL-Trp–β-CD	1035.3, 1078.2, 1156.4	0.064	0.089	0.085	0.099	1337.8	$C_{53}H_{82}O_{37}N_2$

[a] Δδ is the chemical shift change of the protons of host before and after inclusion.

shown that the CD cavity only with very weak polarity was preferred to accommodate an anion of inorganic salt by estimating data from electrospray ionization mass spectra of the mixed solution of an inorganic salt with a CD, in which the original molar ratio of inorganic salt and CD was 1:1.

The electrospray ionization mass spectrographic data of the mixture solution of α-CD and $CsNO_3$ show a minor molecular ion peak at *m*/*e*, 972.2 (relative abundance, 24%) and a principal molecular ion peak *m*/*e*, 1035.2 (relative abundance, 100%) respectively corresponding to α-CD and the one to one supramolecular anion α-CD–NO_3^- (see Figure 7).

The ESI-MS data of the mixture solution of β-CD and Li_2CO_3 show a principal molecular ion peak at *m*/*e*, 1133.6 (relative abundance, 100%) and a minor molecular ion peak at *m*/*e*, 1195.2 (relative abundance, 29%) respectively corresponding to β-CD and the 1:1 supramolecular anion β-CD–CO_3^{2-}. These results indicate that the host–guest interaction between α-CD and NO_3^- might be different from that between β-CD and CO_3^{2-}. In other words, α-CD may be more willing than β-CD to accommodate an anion guest to form a supramolecular anion. There usually exists very weak association ability between a CD and an inorganic ion [49]. This may be because the inorganic ions, especially cations only with small volume and very strong hydrophilicity, could not fit enough for the CD's conformation or structure such as the large size and hydrophobic inner surface of cavity [26, 49].

The ESI-MS data of β-CD show a principal molecular ion peak at *m*/*e*, 1169.4 (relative abundance, 100%) corresponding to β-CD?2H_2O and a minor molecular ion peak at *m*/*e*, 1133.5 (relative abundance, 68%) corresponding to β-CD (see Figure 8).

The ESI-MS data of the solid β-CD inclusion compound of L-Phe show a principal peak at *m*/*e*, 1298.3 (relative abundance, 100%) corresponding to L-Phe–β-CD, two minor peaks at *m*/*e*, 1169.3 (relative abundance, 34%) and 1133.5 (relative abundance, 14%), respectively corresponding to β-CD?2H_2O and β-CD (see Figure 9). The ESI-MS analyses listed in Table 4 for α-AAA–α-CD, Trp–β-CD and D-Phe–β-CD system are by and large similar to those for L-Phe–β-CD system. These results reveal the formation and highly stability of the CD inclusion compounds of α-AAA, because α-AAA–CD has only less partially disassociated in aqueous solution even during ESI-MS measurement.

Electrospray ionization mass spectrographic data of the mixed solution of L-Phe with β-CD after adding

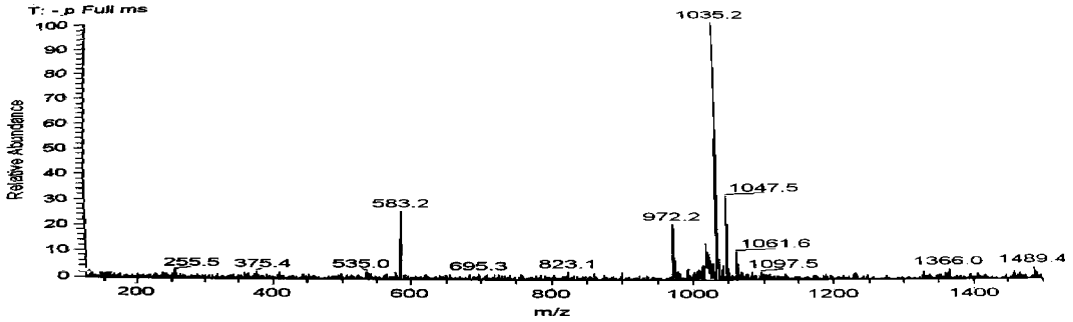

Figure 7. ESI mass spectra of the mixture solution of α-CD and $CsNO_3^-$.

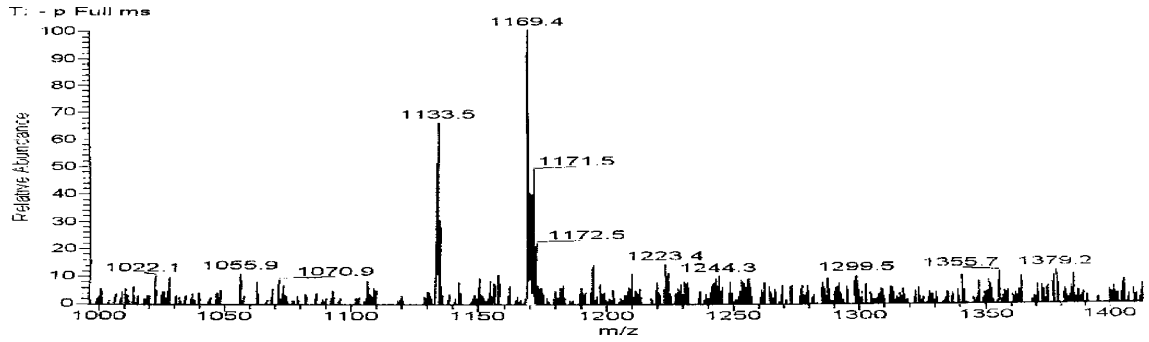

Figure 8. ESI mass spectra of β-CD in deionized water.

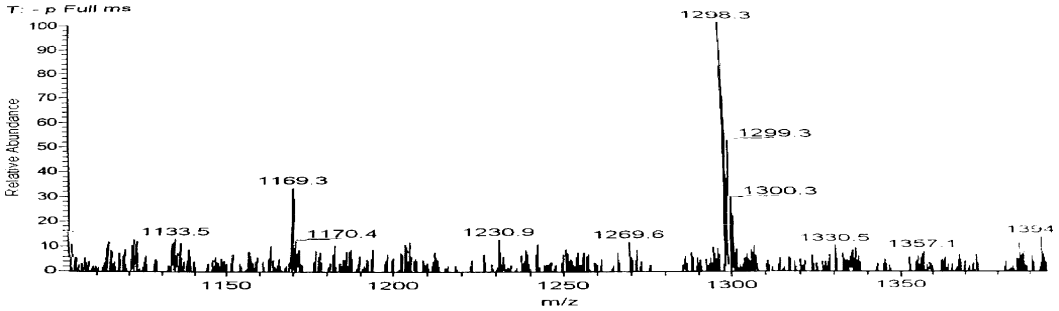

Figure 9. ESI mass spectra of the solid β-CD inclusion compound of L-Phe in deionized water.

$GdCl_3$ show a principal molecular ion peaks at *m*/*e*, 1298.5 (relative abundance, 100%) corresponding to the β-CD inclusion compound of L-Phe: L-Phe–β-CD and a minor molecular ion peaks at *m*/*e*, 1664.1 (relative abundance, 46%) corresponding to the β-CD inclusion compound of the coordination complex of L-Phe with $GdCl_3$: β-CD–(L-Phe)$_2$Gd(H_2O). These are very similar to those described in preparation analysis section for the solid complex obtained. When $NiSO_4$ is substituted for $GaCl_3$, results similar to those described in preparation analysis section have been also obtained. The findings suggest that inorganic ionic have a very important effect on the complexation of α-AAA with α- or β-CD, although the mechanism of interaction among CD, aromatic amino acid and inorganic salt is to be realized by further experiments. The influence of inorganic salts such as the nickel and gadolinium salts in aqueous solution on compleaxtion of CDs to amino acids is being investigated.

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