Inclusion Complexation Behaviors of 3-Tigloyl-Azadirachtol with β-Cyclodextrin Derivatives

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Abstract: A series of β-cyclodextrin/3-tigloyl-azdirachtol inclusion complexes were prepared from β-cyclodextrin, heptakis(2,3,6-tri-O-methyl)-β-cyclodextrin, mono(6-ethylene-diamino-6-deoxy)-β-cyclodextrin and mono(6-diethylene-triamino-6-deoxy)-β-cyclodextrin with 3-tigloyl-azdirachtol (azadirachtin B, AZ-B) in *ca*. 90%, and their inclusion complexation behaviors were investigated by means of UV/Vis, ^{1}H NMR and 2D NMR spectroscopy. The results showed that the AZ-B could be efficiently encapsulated in the cyclodextrin cavity in aqueous solution to produce complexes that were more stabilized than free AZ-B. Furthermore, the water solubility of AZ-B was obviously increased to high levels up to 4-6.3 mg/ml (calculated as AZ-B) after inclusion complexation with cyclodextrins. This satisfactory water solubility and high stability of the cyclodextrin–AZ-B complexes will be potentially useful, for their application as biopesticide and herbal medicine or healthcare products. The enhanced binding ability of cyclodextrins toward AZ-B was discussed from the viewpoint of the size/shape–fit concept and multiple recognition mechanism between host and guest.

Keywords: 3-Tigloyl-azadirachtol, Azadirachtin B, β-Cyclodextrin, Inclusion complexation, Supra-molecular chemistry.

1. INTRODUCTION

Extracts of the Neem tree (*Azadirachta indica*), as biopesticide have been explored over the past three decades, which have been found to be not only a very potential insect antifeedant but also an insect growth controlling agent [1-6]. The Advantages of neem extracts are fast and complete degradation when applied to plants or soil, low risk to human and non-target organisms and so far, no selection of resistant target organisms [7]. Furthermore, the neem extracts also have great application as herbal medicine for skin diseases, anti-malarial, anti-tuberculosis, anti-worms, anti-clotting, blood detoxifier, anti-viral, anti-periodontitic, anti-bacterial, and anti-fungal, etc. [8-10]. Among the tetranortriterpenoid extracts in so-called limonoids, azadirachtin A (AZ-A) and 3-tigloyl-azadirachtol (AZ-B, Chart 1) are the most mainly and important active ingredients [11]. However, commercially available neem trees formulations show some disadvantages such as the short time persistence of active ingredients, caused by sensitivity to high temperatures and UV light, and its poor solubility in water, which usually prepared as microemulsion with some additives [12-16]. On the other hand, the complex structure of the limonoid has made it difficult to judge which parts of the molecule are responsible for the biological effect to explore the further application of limonoids materials [17-18].

Cyclodextrins (CDs, Chart 2), a kinds of truncated-cone polysaccharides mainly made-up of six to eight D-glucose monomers linked by α-1,4-glucose bonds with hydrophobic central cavity and hydrophilic outer surface, are known to be able to encapsulate model substrates to form host-guest complexes or superamolecular species as results to usually enhance drug solubility in aqueous solution and affect the chemical characterization of drugs [19-26]. Furthermore, it is demonstrated that some modified cyclodextrins can greatly enhance the original binding ability and molecular selectivity of parent CD cavities, which further expands the research and application of the complexes [27].

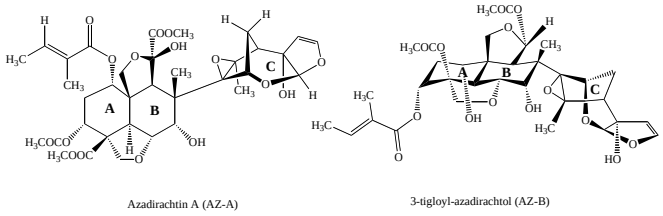

Chart 1. 3-tigloyl-azadirachtol (AZ-B).

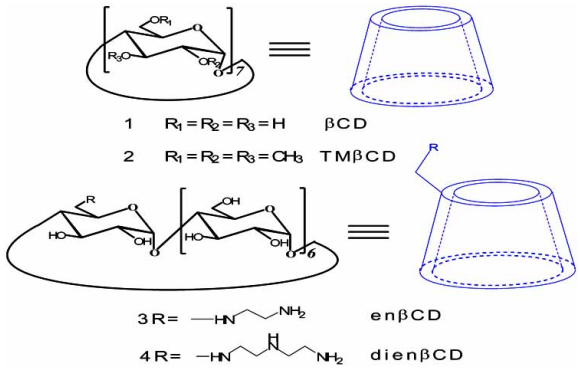

Chart 2. βCD, TMβCD, enβCD, dienβCD.

We have reported recently, the inclusion complexation behavior of AZ-A with native CDs and their methylated derivatives in both solution and the solid state [28]. The results

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show that the water solubility of AZ-A was obviously increased after inclusion complexation with CDs. Herein, as a part of our ongoing investigation; concerning the complexation behavior of neem active ingredients with CDs, we have investigated the interaction of AZ-B with a series of CDs such as βCD, heptakis (2,3,6-tri-O-methyl)-βCD (TMβCD), mono(6-ethylene-diamino-6-deoxy)-βCD (enβCD) and mono (6-diethylene-triamino-6-deoxy)-βCD (dienβCD) in aqueous solution. It is our special interest to explore the binding behaviors of native CDs and modified CDs with AZ-B, the solubility and the stability effect of CDs towards AZ-B, which will provide a useful approach to achieve novel AZ-B based biopesticide and herbal medicine or healthcare products with higher water solubility, stability and bioactivity.

2. EXPERIMENTAL

2.1. Materials

AZ-B (80%) was obtained by our laboratory from neem seeds in Yunnan Province, P. R. China. βCD of reagent grade was recrystallized twice from water. Heptakis (2,3, 6-tri-O-methyl)-β-cyclodextrin [29], mono(6-ethylene- diamino-6-deoxy)-β-cyclodextrin and mono (6-diethylene-triamino-6-deoxy)-β-cyclodextrin [30,31] were prepared according to the reported procedures, respectively.

2.2. Measurements

UV/vis spectra were performed on a Shimadzu UV 3600 spectrophotometer. Considering the poor water solubility of AZ-B, a mixed solvent of water-ethanol (v:v = 5:1) was used in the spectral measurements. ^{1}H NMR experiments were performed on a Varian Mercury VX400 spectrometer at 298 K in a deuterium oxide solution. Rotating-frame Overhauser effect spectroscopy (ROESY) experiments were run on a Varian Mercury VX300 instrument. The Samples were kept at least 24 hrs before measurement for equilibration. All 2D NMR experiments were carried out in D_2O.

2.3. Molecular Modeling

The molecular modeling was performed by the molecular simulation method with the Windows-based software Materials Studio v.4.1 (Accelrys Inc., San Diego, CA, USA). The geometry optimization was performed by using the Universal Force Field procedure available in this package. Algorithm: smart, Energy: 0.001 kcal/mol, Force: 0.5 kcal/mol/Å, max. interations: 5000.

2.4. Preparation of βCD/AZ-B Complex

AZ-B (0.03 mm, 19.8 mg) and βCD (0.01 mM, 12.6 mg) were completely dissolved in a mixed solution of ethanol and water (ca. 7 ml, v:v = 1:5) respectively, the mixture was stirred for 4 days at room temperature. After evaporated the ethanol from the reaction mixture, the uncomplexed AZ-B was removed by filtration. The filtrate was evaporated under on reduced pressure to remove the solvent and dried in vacuum to give βCD/AZ-B complex (yield 93%). ^{1}H NMR (400 MHz, D_2O, TMS): δ 0.6–2.42 (m, 20H, AZ-B protons), 3.36–3.80 (m, >50H, H-2–6 of βCD and some protons of AZ-B), 4.87–4.89 (s, 7H, H-1 of βCD), 5.00–7.00 (6H, AZ-B protons).

2.5. Preparation of TMβCD/AZ-B Complex

TMβCD/AZ-B complex was similarly prepared in ca. 88% yieds from TMβCD and AZ-B. ^{1}H NMR (400 MHz, D_2O, TMS): δ 0.6–2.42 (m, 20H, AZ-B protons), 3.36–3.80 (m, >110H, H-2–6 of TMβCD and OCH_3-2, 3, 6 of TMβCD and some protons of AZ-B), 4.87–4.89 (s, 14H, H-1 of βCD), 5.00–7.00 (6H, AZ-B protons).

2.6. Preparation of enβCD/AZ-B Complex

enβCD/AZ-B complex was similarly prepared in ca. 90% yieds from enβCD and AZ-B. ^{1}H NMR (400 MHz, D_2O, TMS): δ 0.6–2.42 (m, 20H, AZ-B protons), 2.66–3.10 (m, 4H, -NCH_2- of enβCD), 3.36–3.80 (m, >50H, H-2–6 of enβCD and some protons of AZ-B), 4.87–4.89 (s, 7H, H-1 of enβCD), 5.00–7.00 (6H, AZ-B protons).

2.7. Preparation of Dienβcd/AZ-B Complex

dienβCD/AZ-B complex was similarly prepared in ca. 91% yieds from dienβCD and AZ-B. ^{1}H NMR (400 MHz, D_2O, TMS): δ 0.6–2.42 (m, 20H, AZ-B protons), 2.66–3.10 (m, 8H, -NCH_2- of dienβCD), 3.36–3.80 (m, >50H, H-2–6 of dienβCD and some protons of AZ-B), 4.87–4.89 (s, 7H, H-1 of dienβCD), 5.00–7.00 (6H, AZ-B protons).

3. RESULTS AND DISCUSSION

3.1. Spectral Titration

As AZ-B shows a characteristic absorbance at 215 nm, where both nature CDs and modified CDs show some absorbance to disturb the results, general spectral titration can not be used to examine their inclusion complex, and calorimetric titration is also difficult to perform due to the very small enthalpy change of the inclusion complex process. Therefore, phenolphthalein in pH 10.5 was used as a kind of spectral molecular probe to determine the binding constants of host-guest inclusion complexes by mean of complete spectral titration method [32], and quantitative investigation of the inclusion complex behavior of all kinds of CDs with AZ-B are examined in a water/ethanol (v:v=5:1) mixed solution owing to the rather poor water solubility of AZ-B. As reported [33], phenolphthalein forms 1:1 inclusion complexes with CDs, so the binding constant (K_s') can be accessed with equation:

$$K_s' = 1/K_d = \frac{[CD][PP]}{[CD \cdot PP]} = \frac{([CD]_0 - \Delta A/\Delta\varepsilon)([PP]_0 - \Delta A/\Delta\varepsilon)}{\Delta A/\Delta\varepsilon} \quad (1)$$

Where $[CD]_0$ and $[PP]_0$ were the initial concentrations of CDs and phenolphthalein respectively, equation (1) was achieved by equation (2):

$$\Delta A = \frac{\Delta\varepsilon([CD]_0 + [PP]_0 + K_s') - \sqrt{(\Delta\varepsilon)^2([CD]_0 + [PP]_0 + K_s')^2 - 4(\Delta\varepsilon)^2[CD]_0[PP]_0}}{2} \quad (2)$$

Then experimental data was applied into equation (2), using a nonlinear least squares curve-fitting method, we obtained the separating constant (K_d) and molar absorbance constant ($\Delta\varepsilon$) [34]. Fig. (**1**) (inset) illustrated a typical curve-fitting plot for the titration of phenolphthalein with enβCD, which showed the excellent fits between the experimental and calculated data [35]. In the repeated measurements, the K_d values were reproducible within an error of

±5%. The K_d and $\Delta\varepsilon$ for the inclusion complexes of CDs with phenolphthalein were listed in Table **1**.

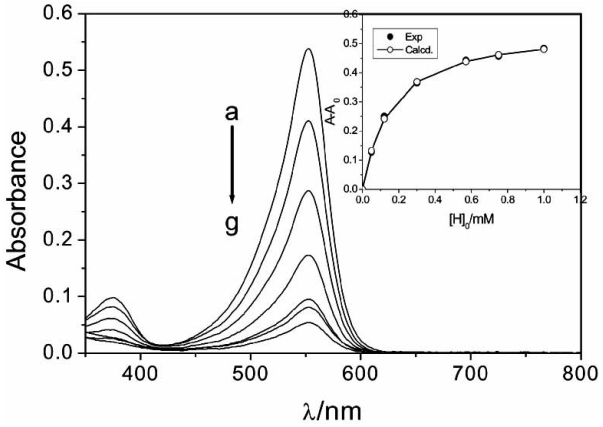

Fig. (1). Absorption spectral changes of phenolphthalein (3×10^{-5}M) upon addition of host enβCD ($0\sim1\times10^{-3}$M from *a* to *g*) in a pH 10.5 aqueous buffer water/ethanol (v:v = 5:1) mixed solution and the nonlinear least squares analysis (inset) of the differential intensity (ΔA at 553 nm) to calculate the separate constant (K_d) and molar absortanse constant ($\Delta\varepsilon$).

Table 1. The K_d and $\Delta\varepsilon$ Values for the Inclusion Complexation of Hosts βCD, enβCD and dienβCD with Guest Phenolphthalein in pH 10.5 Aqueous Buffer Water/Ethanol (v:v = 5:1) Mixed Solution at 25 °C

Guest	Host	K_d / 10^{-4} M	$\Delta\varepsilon$ / dm^3·mol^{-1}·cm^{-1}
Phenolphthalein	βCD	8.46	7680
	enβCD	1.56	16820
	dienβCD	1.92	18080

To keep the stable concentrations of the host CDs and guest phenolphthalein, AZ-B was join into the system, which appeared in the other equation. The complexation stability constants (K_s) of AZ-B with CDs can be calculated according to the following equation (3):

$$K_s = \frac{c_0 - c - (a_0 - a)}{c[b_0 - (c_0 - c) + (a_0 - a)]} \quad (3)$$

$$a = a_0 - \frac{\Delta A}{\Delta\varepsilon} = \frac{(\Delta A_\infty - \Delta A)}{\Delta\varepsilon} \quad (4)$$

$$c = \frac{K_d(a_0 - a)}{a} \quad (5)$$

Where a_0, b_0 and c_0 were initial concentrations of phenolphthalein, AZ-B, and CDs respectively, *a*, *b* and *c* were balance concentrations of them [33]. Fig. (**2**) illustrates a typical plot for complete binding phenolphthalein and AZ-B with enβCD, in which the absorbance intensity of phenolphthalein increased gradually with the step-wise addition of AZ-B. The stability constant (K_s) values were accessed with the average value of five concentrations of AZ-B. The K_s and Gibbs free energy change ($-\Delta G°$) for the inclusion complex of CDs with AZ-B were listed in Table **2**.

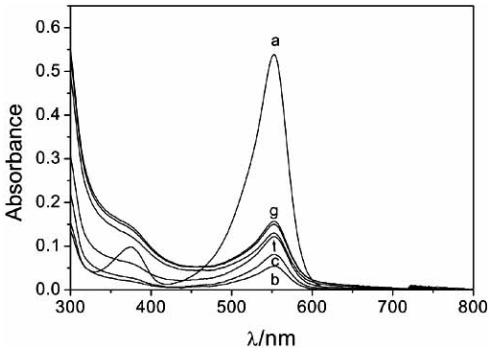

Fig. (2). Effect of enβCD and AZ-B on the UV/vis spectrophotometric of phenolphthalein in a pH 10.5 aqueous buffer water/ethanol (v:v = 5:1) mixed solution. *a*. 3×10^{-5} M phenolphthalein; *b*. *a* + 5×10^{-4} M enβCD; *c-g*. *b* + AZ-B (5×10^{-4} M~5×10^{-3}M).

Table 2. Complex Stability Constant (K_s) and Gibbs Free Energy Change ($-\Delta G°$) for Inclusion Complexation of AZ-B Guest with Various Hosts βCD, enβCD and dienβCD in Aqueous Buffer Water/Ethanol (V:V = 5:1) Mixed Solution (pH 10.5) At 25 °C

Host	Guest	K_s / M^{-1}	log K_s	$-\Delta G°$ / kJ·mol^{-1}
βCD	AZ-B	310	2.49	14.2
enβCD		1080	3.03	17.3
dienβCD		1010	3.00	17.1

3.2. Enhanced Binding Ability

Extensive studies have revealed that the size/shape–fit concept plays a crucial role in the inclusion complexation of CD with guest molecules of various structures. On the basis of the size/shape–fit concept, weak intermolecular forces such as ion–dipole, dipole–dipole, van der Waals, electrostatic, hydrogen bonding and hydrophobic interactions are known to co-operatively contribute to the inclusion complex. From Table **2**, we can see the binding constants for the complexation of AZ-B by βCD, enβCD and dienβCD in the order:

enβCD > dienβCD > βCD

By comparing the enhancement effect of all kinds of βCD for AZ-B, the enβCD and dienβCD gave the higher *K*s enhancement for AZ-B than, that of native βCD. It was demonstrated that monoamino βCD can enhance binding ability to guest due to several weak intermolecular forces cooperatively [35]. From these factors, we may conclude that the guest AZ-B was better bound by the monoamino βCD than native βCD. Considering the structural features of the hosts and guests, we deduce that, upon inclusion complexation, the hydrogen bond between the ethylenediamino arm of enβCD or dienβCD, which was located close to the accommodated AZ-B molecule, and the hydroxyl group or the oxygen atom in AZ-B may strengthen the host-guest association. Therefore, enβCD and dienβCD displayed the obviously enhanced binding abilities for guest AZ-B. Although the present hypothesis is drawn from a rather limited variation of

tion of modified CD, this concept should be extended more generally to a wide variety of synthetic CD-based species and subsequently open a new channel on the design of novel formulation of AZ-B.

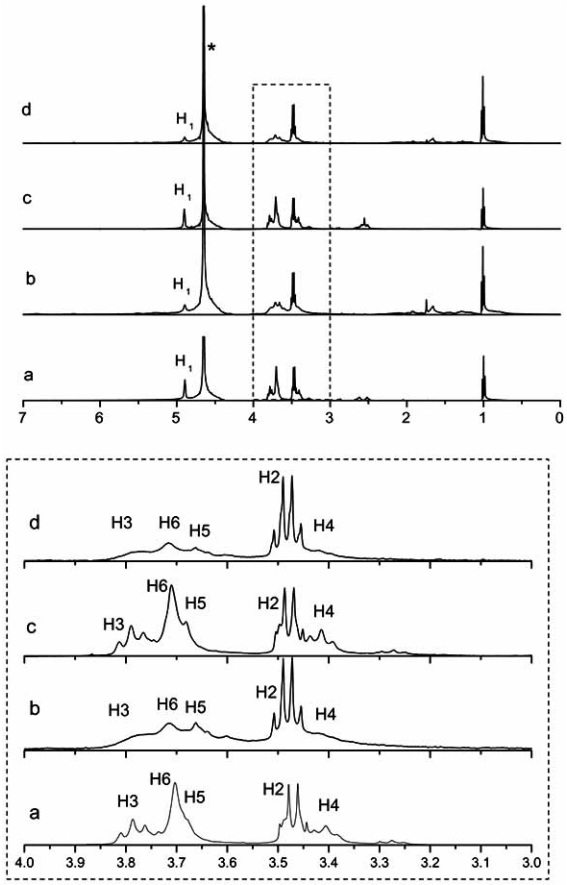

Fig. (3). ^{1}H NMR spectra of dienβCD and enβCD in the absence and presence of AZ-B in D_2O at 25 ºC, respectively. (a) enβCD, (b) enβCD/AZ-B complex, (c) dienβCD, (d) dienβCD/AZ-B complex (asterisk highlights the water peak).

3.3. Inclusion Mode

In order to explore the possible inclusion mode of CD-AZ-B complexes, we compared the ^{1}H NMR spectra of AZ-B in the presence of host CDs (Fig. **3**), where the ^{1}H resonances of enβCD and dienβCD were assigned according to the reported method [36]. Owing to the poor water solubility, solid AZ-B was transparent to ^{1}H NMR under most conditions when D_2O was used as solvent. As illustrated in Fig. (**4**), a majority of AZ-B (20H) display the chemical shifts at δ 1.0-2.5 ppm, which are distinct from the CD protons. By comparing the integration area of these protons with that of the CDs H-1 protons, we can calculate the inclusion stoichiometry of CD-AZ-B complexes, that is, 1:1 for βCD-AZ-B complexes, enβCD-AZ-B complexes and dienβCD-AZ-B complexes. In addition, the 1:1 inclusion stoichiometry is also observed in the cases of TMβCD/AZ-B complex. In a preliminary work, we have reported the 2:1 inclusion between TMβCD and AZ-A [28], although AZ-A and AZ-B have the similar structure. A possible reason may be the small difference on the geometry of AZ-A and AZ-B. For example, the steric hindrance around the ethylenylcarboxyl group in AZ-B is smaller than that in AZ-A. Therefore, TMβCD cavity can include this moiety of AZ-B deeper than the case of AZ-A, which will lead to the different stoichiometry between AZ-A and AZ-B.

Table 3. The Chemical Shifts (d) of βCD, TMβCD, en-βCD and Dienβcd in the Absence and Presence of AZ-B in D_2O at 25 ºC

Species	δ (ppm)					
	H-1	H-2	H-3	H-4	H-5	H-6
βCD	4.88	3.47	3.77	3.39	3.69	3.69
βCD complex	4.90	3.49	3.77	3.43	3.72	3.72
TMβCD	5.15	3.20	3.55	3.53	3.61	3.71
TMβCD complex	5.13	3.18	3.55	3.45	3.59	3.70
enβCD	4.89	3.47	3.79	3.41	3.69	3.70
enβCD complex	4.89	3.48	3.77	3.45	3.66	3.72
dienβCD	4.90	3.48	3.79	3.41	3.68	3.71
dienβCD complex	4.90	3.47	3.77	3.42	3.66	3.72

As can be seen from Table **3**, after inclusion complex with AZ-B, the H-5 proton of βCD shift ca. 0.03 ppm and that of TMβCD shift ca. 0.02 ppm. In contrast, a weak effect is observed on the δ values of H-3 protons of βCD and TMβCD. Because both H-3 and H-5 protons are located in the interior of CD cavity, and H-3 protons are near at the wide side of cavity while H-5 protons near the narrow side. This phenomenon may indicate that AZ-B should penetrate into the CD cavity of βCD and TMβCD from the narrow side. It is fairly noteworthy that H-3 (ca. 0.02, 0.02 ppm) and H-5 protons (ca. 0.03, 0.02 ppm) of enβCD and dienβCD show appreciable shifts after forming inclusion complex, revealing that AZ-B may enter the cavity of enβCD and dienβCD from not only the narrow side but also the wide side.

Table 4. The Partial Chemical Shifts (d) of 3-tigloyl-azadirachtol in D_2O

Proton	H-2	H-16	H-3'	H-4'	H-5'
d (ppm)	1.99	1.16	6.93	1.77	1.76
peak	m	m	m	s	d

2D NMR spectroscopy has recently become an important method to obtain information about the spatial proximity between the atoms of host and guest by observing the intermolecular dipolar cross-correlations. Two protons, which are closely located in space, can produce a NOE cross-correlation between the relevant protons in NOESY or ROESY spectrum [37]. In contrast with the reports [38], the partial chemical shifts of AZ-B in D_2O were accessed, which were

were listed in Table **4**. As can be seen from the ROESY spectrum of the AZ-B–TMβCD complex and AZ-B–enβCD complex, Fig. (**4**) exhibited clear NOE cross-correlations (peaks A) between the methyl protons of tigloyl and the H-5 protons of TMβCD, and NOE cross-correlations (peaks B) between the H-16 of C ring and the H-5 protons of TMβCD. These cross-correlations demonstrated that the tigloyl and C ring unit of AZ-B were, at least, partly accommodated in the CD cavity from narrow side. It is fairly noteworthy that the complexe of AZ-B and enβCD were not only revealed NOE cross-correlations (peaks C) between the H-16 of C ring and NOE cross-correlations (peaks D) between the methyl of tigloyl with the H-3 protons of enβCD, but also NOE cross-correlations (peaks E) between the H-2 protons of A ring with the H-3 and H-5 protons of enβCD, indicating that the tigloyl and A ring unit of AZ-B was deeply accommodated in the CD cavity from narrow side and C ring unit of AZ-B was also accommodated in the CD cavity from wide side. Based on the observation, we deduced that the CD cavity of TMβCD might respectively include the tigloyl unit and C ring unit of AZ-B from the narrow side to form the inclusion complex. In contrast, the CD cavity of enβCD might deeply include the tigloyl and A ring unit of AZ-B from the narrow side and C ring unit of AZ-B from the wide side to form the inclusion complex. No NOE cross-correlations between the ethylenediamino arm in enβCD or dienβCD and the H-3/H-5 protons of CD could be observed, indicating that the ethylenediamino arm was not included in the CD cavity. Moreover, the molecular simulation study was also performed to explore the possible binding mode between host and guest. By combining the results of molecular simulation study and ROESY experiments, the host-guest binding mode were illustrated in Fig. (**5**), where two modes coexisted with equilibrium. This kind of inclusion mode can also be deduced for dienβCD/AZ-B complex. As it can be seen in Fig. (**5**), possessing a distorted macrocyclic ring, TMβCD cavity showed the similar inclusion depth to that of βCD cavity.

3.4. Water Solubility

The water solubility of CD–AZ-B complex is assessed by preparation of its saturated solution [39]. An excess amount of complex was put into 5 ml of water (pH ca. 7) and the mixture was stirred for 1h. After removing the insoluble substance by filtration solution, the filtrate is evaporated under reduced pressure to dryness and the residue is dosed by weighing method. The results show that the water solubility of βCD/AZ-B, TMβCD/AZ-B, enβCD/AZ-B, and dienβCD/AZ-B complexes, comparing with that of AZ-B (ca. 100 μg/ml), is increased dramatically approximate 4.0, 4.4, 5.7, and 6.3 mg/mL (calculated as AZ-B residue), respectively. In the controlled experiment, a clear solution is obtained after dissolving βCD/AZ-B (10.9 mg), TMβCD/AZ-B (12.7 mg), enβCD/AZ-B (16 mg), or dienβCD/AZ-B (18 mg) complex, respectively, which is equivalent to 4.0, 4.4, 5.7, and 6.3 mg of AZ-B, in 1 ml of water at room temperature. This subsequently confirms the reliability of the obtained satisfactory water solubility of CD–AZ-B complexes, which will be beneficial for the utilization of this compound as biopesticide and medicine or healthcare products.

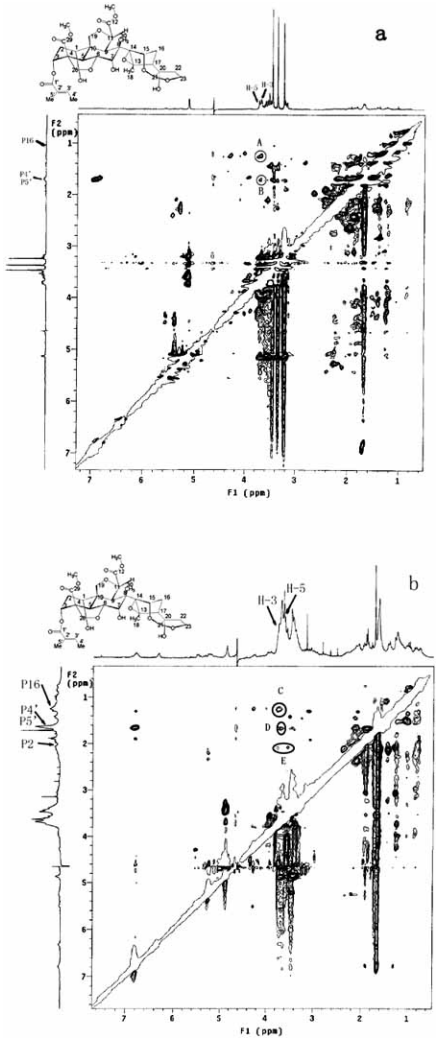

Fig. (4). ROESY spectrum of βCD/AZ-B complexes in a D_2O with a mixing time of 24 hours. (a) TMβCD/AZ-B complex; (b) enβCD/AZ-B complex.

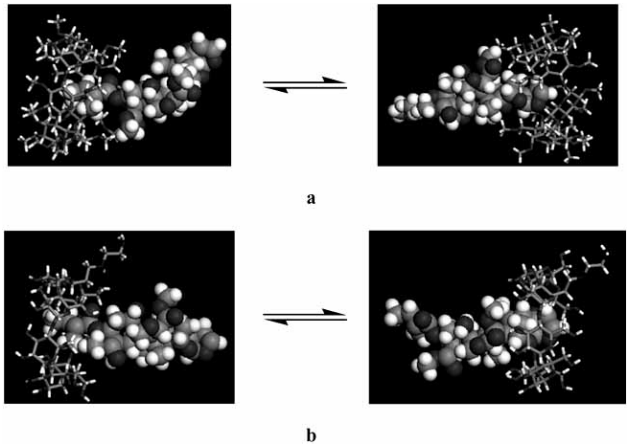

Fig. (5). Possible inclusion modes of CD/AZ-B complexes. (a) TMβCD/AZ-B complex: inclusion of tigloyl unit (left) and C ring unit (right) of AZ-B from the narrow side of TMβCD (b) enβCD/AZ-B complex: inclusion of tigloyl and A ring unit of AZ-B from the narrow side (left) and C ring unit of AZ-B from the wide side (right) of enβCD.

3.5. Stability in Aqueous Solvent

In order to evaluate the capability of CDs as carriers for AZ-B, we tracked the decomposition of the encapsulated AZ-B. AZ-B (ca. 1 mg) was dissolved thoroughly, with 10 ml water to produce the concentrations of AZ-B, which UV absorbance (215 nm) was the scope of ca. 1. The solid complexes were similarly prepared with 10 mL water to keep the UV absorbance (215 nm) of AZ-B at the scope of 0.9 to 1.1, which include βCD/AZ-B (2.7 mg), TMβCD/AZ-B (2.9 mg), enβCD/AZ-B (2.8 mg), or dienβCD/AZ-B (2.8 mg) complex, respectively. The solutions were kept at room temperature and analyzed by UV/vis spectra each day. The solution absorbance kept stable and did not change after 25 days. The initial values (A_0) of absorbance peak at 215 nm were recorded as 100% and a plot of absorbance against time are given in Fig. (**6**).

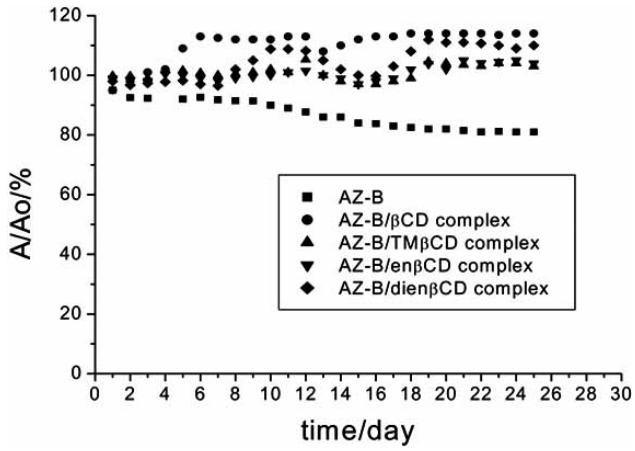

Fig. (6). Decomposition against time of AZ-B and its complexes in a pH 7 aqueous solution at room temperature (Measurement was taken by UV-Vis absorption at 215 nm).

The absorbance of the complex was nearly unchanged during the time course, indicating that only a little amount of the encapsulated AZ-B was decomposed. However, the absorbance of AZ-B decreased obviously, which indicates the decomposition of AZ-B. This result consequently supported the conclusion drawn from the spectral and NMR experiments. That is, most of the AZ-B would be encapsulated in the CD cavity in aqueous solvent, which is important to the application of CDs, as carriers for these biopesticides and herbal medicines or healthcare products.

4. CONCLUSION

In summary, the inclusion complexation behavior of βCD and its derivatives with AZ-B were investigated. The results showed that CDs could enhance not only the water-solubilities, but also the stabilities of AZ-B. Considering the shortage of the application of AZ-B, these complexes should be regarded as an important choice in the design of novel formulation of AZ-B for the biopesticide and herbal medicine or healthcare products.

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ABBREVIATIONS

AZ-A = Azadirachtin

AZ-B = 3-tigloyl-azadirachtol

βCD = β-cyclodextrin

TMβCD = Heptakis (2,3,6-tri-O-methyl)-βCD

enβCD = Mono (6-ethylene-diamino-6-deoxy)-βCD

dienβCD = Mono (6-diethylene-triamino-6-deoxy)-βCD

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