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Multimodal inclusion complexes of ampicillin with β-cyclodextrins in aqueous solution

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Abstract

The inclusion complexation of ampicillin (ABPC) with β-cyclodextrin (βCD) and 2-hydroxypropyl-β-cyclodextrin (HPCD) in aqueous solution has been investigated by microcalorimetry, NMR spectrometry and molecular dynamics simulation (MDS). The heat of reaction of the complexation decreased as the pH value increased. ABPC and β-cyclodextrins formed one or two types of inclusion complexes by hydrophobic interactions and the complexation significantly depended on the species of ABPC based on the pH values of the solutions. Two different types of inclusion complexes with a 1:1 stoichiometry were realized for the cation species of ABPC in the strong acid solution. In the first type of inclusion with higher association constants of $(6.0–10) \times 10^3\ M^{-1}$, the penam ring of ABPC was inserted in the cyclodextrin cavity, whereas in the second type with lower association constants of $1.0 \times 10^3\ M^{-1}$, the phenyl ring seemed to penetrate into the cavity. The β-lactam ring of ABPC was included in both types to be protected from the acid-catalyzed hydrolysis. The zwitterion and anion species of ABPC formed only one type of inclusion complex with β-cyclodextrins, where the phenyl ring and the penam ring were included, respectively. These association constants and enthalpic changes were smaller than those for the first type inclusion complex of the cation species. These results indicate that the inclusion complexes of ABPC with β-cyclodextrins would be useful to the drug delivery system.

Keywords: Ampicillin; 2-Hydroxypropyl-β-cyclodextrin; β-Cyclodextrin; Microcalorimetry; Inclusion complex; Molecular dynamics simulation

1. Introduction

In studying inclusion complexes with cyclodextrins, structural information such as the stoichiometry, thermodynamics and geometry of the complex is necessary to clarify the complexation mechanism. Although the values of association parameters have been determined using a number of physicochemical methods such as spectroscopy and the solubility technique, these methods are mostly based on the typical inclusion complexation that forms only one inclusion type with a 1:1 stoichiometry and occasionally a stepwise reaction to form two types with 1:1 and 1:2 stoichiometries. Nevertheless, very little is known about the multimodal inclusion complexes with a 1:1 stoichiometry. When the molecules such as ampicillin (ABPC) and benzylpenicillin (PCG) are not unique species of an acid/base conjugate solution system, the obtained parameters would not be real and not reflect the contributions of different inclusion types. Isothermal titration microcalorimetry is an ideal analytical method for this purpose, since the values of the association parameters and the enthalpy changes are directly calculated from the data by computer for a single experiment at suitable temperature [1].

ABPC and PCG are β-lactam antibiotics used in the treatment of a variety of infectious diseases. However, they are hydrolyzed in aqueous solution and to form polymers, via nucleophilic attack by the side-chain amino group in one molecule upon the β-lactam carbonyl of a second molecule [2,3]. The degradation of ABPC in the gastric juice decreases bioavailability in human subjects and polymers in ampicillin preparations possess strong antigenic properties [4]. β-Cyclodextrins (βCDs) were found to inhibit the degradation and the polymerization via the formation of a complex with ABPC and PCG [5–8]. In this study, we investigated the mechanisms that inhibit the decomposition

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and polymerization of ABPC in aqueous solution by inclusion complexation.

2. Materials and methods

2.1. Materials

Ampicillin, ampicillin sodium (ABPC) and β-cyclodextrin (FW 1134.98) were purchased from Sigma (St. Louis, Mo.). 2-Hydroxypropyl-β-cyclodextrin (HPCD; FW = 1540, MS = 1.0) was obtained from Aldrich (Milwaukee, WI). The average molecular weight of HPCD and the degree of substitution of the hydroxypropyl group were 1538 and 6.95, respectively, as determined by FAB-mass Spectrometry using a JMS HX-110 spectrometer equipped with KMADA-7000 (JEOL< Tokyo, Japan). All other materials used were of analytical reagent grade.

2.2. Microcalorimetry and calculation of complex association constants and thermodynamic parameters

The heat of reaction was measured with an isothermal titration microcalorimeter, Thermal Activity monitor 2277 (ThermoMetric AB JörfUalla, Sweden) at 298.15 K. ABPC and cyclodextrins were dissolved in a 1/10 M HCl−1/10 M disodium hydrogen citrate buffer solution (pH 1.2–4.5) and in a 1/10 M NaOH−1/10 M disodium hydrogen citrate buffer solution (pH 5.0–8.0). The heat of dilution of cyclodextrins was measured separately and then subtracted from the heat of reaction.

Assuming that a guest ligand molecule (L) and a cyclodextrin (CD) form several types of inclusion complexes with 1:1 molar ratio in aqueous solution as follows:

$$\mathrm{L} + \mathrm{CD} \rightleftarrows \sum_{i=1}^{n} (\mathrm{L} - \mathrm{CD})_i \tag{1}$$

then, the heat effect (ΔQ) of the complexation is proportional to the quantity of complexes and can be expressed as a function of the free concentration of CD ($\mathrm{CD_f}$),

$$\Delta Q = \sum_{i=1}^{n} \frac{\Delta H_i \cdot K_i \cdot \mathrm{CD_f}}{1 + K_i \cdot \mathrm{CD_f}} \cdot L_\mathrm{t} \cdot V_\mathrm{r} \tag{2}$$

where L_t represents the total concentration of guest molecule (ABPC), V_r is the total volume in a reaction cell, and K_i and ΔH_i are the association constant and the enthalpy change for the ith type of inclusion complex $(L-\mathrm{CD})_i$, respectively. In the case of $i = 1$, CD forms only one type of inclusion complex with L at a molar ratio of 1:1. In the case where i is >2, it is assumed that several types of inclusion complexes with a 1:1 molar ratio are formed independently. A titration curve was obtained by plotting the values of ΔQ versus the total concentration of CD added. The best fit values of K_i and ΔH_i can be computed from the actual calorimetric data with an iterative nonlinear least-square method using a computer [1].

2.3. NMR spectrometry

^{1}H NMR spectra were taken on JEOL GX-400 interfaced with a DEC RSX-11 M computer operating at 25 °C. ABPC of 5 mg and βCD at various βCD/ABPC molar ratios were dissolved in D_2O or in 0.1 M DCl solutions (10 ml) and mixed for 30 min. The chemical shifts for free (δ_free) and complexed (δ_complex) ABPC were measured in the absence and presence of CD, respectively, and assigned based on the external standard sodium 2,2,3,3-d_4-3-trimethylsilylpropionate. Induced changes in the chemical shifts for ABPC ($\Delta\delta$) by complexation were calculated using the following equation:

$$\Delta\delta = \delta_\mathrm{complex} - \delta_\mathrm{free} \tag{3}$$

2.4. Molecular dynamic simulation

Molecular dynamic simulation (MDS) of inclusion complexes between ABPC and β-CD in aqueous solution was performed using the AMBER program (ver. 6) running on an SGI OCTANE-SE computer [9]. The geometries and EPS charges of ABPC and βCD were optimized using Gaussian programs [10]. In the most realistic model of the complex in aqueous solution, ABPC and βCD molecules were placed in the center of a 27 Å cubic box containing ca. 500 TIP3P water molecules. Then an inclusion complex was subjected to energy minimization to obtain more realistic, low-energy minimization starting structures of MDS using the Monte Carlo technique. The MDS was equilibrated by 200 ps ($\Delta t = 0.001$ ps and 200,000 time steps) with SHAKE [11] constraints for hydrogen atoms under conditions of constant pressure (1 atm) and temperature (293 K). Intermediate structures were saved in a file after every 50 steps to obtain representative, sequential structures generated during the simulation.

3. Results and discussion

3.1. Heat of reaction of complexation between ABPC and β-cyclodextrins

Since ABPC has two pKa$_2$ values, pKa$_1$ = 2.53 (–COOH) and pKa$_2$ = 7.24 (–NH_2), based on the structure, different species of cation, zwitterion and anion exist in aqueous solution depending on the pH value. To evaluate the inclusion type contributing most to the driving forces and stability of the complexation in aqueous solution, the reaction heats of ABPC and β-cyclodextrins were measured at various pH values. Figs. 1 and 2 show the calorimetric titrations of βCD and HPCD to ABPC with a constant concentration of 0.5 mM in aqueous solutions at 25 °C,

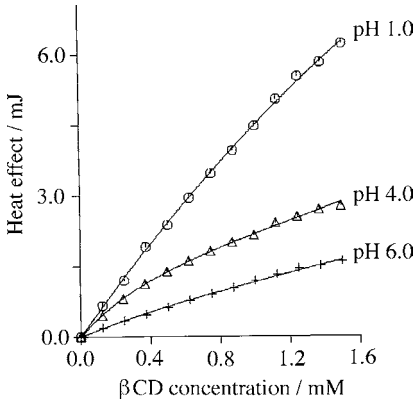

Fig. 1. Calorimetric titration curves for ABPC-βCD complexation at physiological pH. In all the experiments, the concentration of ABPC was 0.5 mM. Points show the means of experimental data from triplicate measurements and solid lines represent computer-generated best fit curves.

respectively. The heat effect of ABPC with βCD was smaller than that with HPCD because of the solubility of βCD. In the complexation between ABPC and β-cyclodextrins, the heat effect was highest at pH 1.2, where ABPC existed almost entirely as cation species, and decreased suddenly at higher pH values (≥4.2), where the carboxyl group on the penam ring of ABPC was fully ionized (zwitterion or anion species).

The calorimetric findings were directly fitted to Eq. (2), where i was varied from 1 to 2 assuming a 1:1 complex formation with one to two types of inclusion. In the titration curves at pH 1.2 and 2.0, the data did not fit to the one-type inclusion model ($i = 1$) but fit to the two-type inclusion model ($i = 2$) with mean of squared errors (MSE) being less than 0.01. While, the titration curves at pH 4.2 (4.0), 6.0 and 8.0 fitted to one and two types of inclusion models with MSE of less than 0.01. The estimated values of the association and thermodynamic parameters for the two-type inclusion model are listed in Table 1. Although the association constants (K_1 and K_2) of the complex formation at pH 4.2 (4.0), 6.0 and 8.0 were computed in the two types of inclusion model, the values of K_2 for the complexation were much smaller than those of K_1 to stabilize the inclusion complexes. Thus, it seemed that only cation species of ABPC formed two types of the inclusion complex at lower pH than pKa_1. In the first type of inclusion complex with a higher association constant K_1 at pH 1.0 and 1.2, the small negative values of ΔH_1 and the large positive ΔS_1 indicated hydrophobic interaction between ABPC and the hydrophobic cavity of β-cyclodextrins. The values of K_2 for the second type of inclusion complex at pH 1.0 and 1.2 were almost equal to K_1 values for the first type at pH 4.2, 6.0 and 8.0.

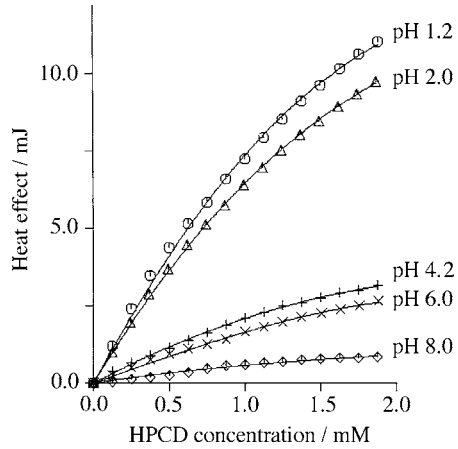

Fig. 2. Calorimetric titration curves for ABPC–HPCD complexation at physiological pH. In all the experiments, the concentration of ABPC was 0.5 mM. Points show the means of experimental data from triplicate measurements and solid lines represent computer-generated best fit curves.

3.2. NMR evidence of inclusions

The results of the calorimetric experiments revealed the coexistence of two types for inclusion complexes between ABPC and β-cyclodextrins in aqueous solution. To identify the interaction, ^{1}H NMR spectra of ABPC for the inclusion complexes were measured in D_2O and in 0.1 M DCl solutions. Fig. 3 shows the induced changes ($\Delta\delta$) in the chemical shifts for ABPC-βCD. The ^{1}H-chemical shifts of ABPC in D_2O solution moved slightly upfield by forming complexes and $\Delta\delta$ reached to plateau levels around a βCD/ABPC molar ratio of 1.0 (Fig. 3a). While, the chemical shift in 0.1 M DCl solutions also moved upfield on the addition of βCD, and $\Delta\delta$ values of 3H, 5H and 2-CH_3 on the penam ring especially increased as the molar ratio of βCD/ABPC increased (Fig. 3b). High upfield shifts have generally been noted for complexes in which the penam ring is entrapped by deep penetration in the hydrophobic cyclodextrin cavity. However, the chemical shifts of the phenyl ring underwent upfield shifts to keep constant above the molar ratio of 1.0. The shielding effect on the phenyl ring was due to interaction with the ring current as a consequence of complete inclusion within the cyclodextrin cavity. This could be considered the result of both hydrophobic interactions and a ring current effect caused by inclusion of the phenyl ring moiety. Considering the ABPC structure, it appears unlikely for two molecules of ABPC to penetrate into the cavity of β-cyclodextrins. Thus, it seemed that two types of complexes were formed by the inclusion of either a penam ring or a phenyl ring of ABPC within the cavity.

3.3. Molecular dynamics simulation (MDS) of ABPC-βCD inclusion complexes

The above results showed that either the phenyl ring or the penam ring of ABPC was probably included by one mole of β-cyclodextrins. Both ring systems would be suitable as inclusion groups according to a preliminary inspection of the structure of ABPC (Fig. 3) and the void volume of the β-cyclodextrin cavity. In the study of the inclusion behavior of ABPC using the MDS, βCD was adopted as a host molecule to easily determine the stable structure of

Table 1
Association constants and thermodynamic parameters of ABPC−β-cyclodextrins complexation in at 25 °C

	K_1 (10^3 M^{-1})	$-\Delta H_1$ (kJ mol^{-1})	$-\Delta G_1$ (kJ mol^{-1})	ΔS_1 (J mol^{-1} K^{-1})	K_2 (10^2 M^{-1})	$-\Delta H_2$ (kJ mol^{-1})	$-\Delta G_2$ (kJ mol^{-1})	ΔS_2 (J mol^{-1} K^{-1})
ABPC−HPCD								
pH 1.2	10.15 ± 1.0	5.45 ± 0.9	22.9	58.5	12.4 ± 1.9	9.00 ± 1.3	17.6	29.0
pH 2.0	5.80 ± 0.4	4.99 ± 0.4	21.5	55.5	11.5 ± 1.3	9.04 ± 1.6	17.5	28.3
pH 4.2	1.28 ± 0.2	2.86 ± 0.3	17.8	50.1	0.1 ± 0.04	2.48 ± 0.2	6.2	12.6
pH 6.0	1.80 ± 0.5	1.51 ± 0.2	18.6	57.4	0.7 ± 1.0	0.69 ± 0.1	10.4	32.7
pH 8.0	0.64 ± 0.1	1.02 ± 0.1	16.1	50.5	0.1 ± 0.03	0.72 ± 0.0	5.8	17.0
ABPC−βCD								
pH 1.2	4.83 ± 0.5	3.13 ± 0.9	21.1	60.3	10.8 ± 2.2	4.02 ± 0.7	17.3	44.6
pH 4.0	1.15 ± 0.2	1.67 ± 0.4	17.5	53.2	0.2 ± 0.06	1.82 ± 0.5	7.6	19.4
pH 6.0	1.24 ± 0.4	1.13 ± 0.1	1.42	59.0	0.05	0.63 ± 0.1	4.1	11.8

Each value represents an average ±S.D. of triplicate experiments.

ABPC-βCD complex. βCD should be regarded as a truncated cone rather than a cylinder, where all the primary hydroxy groups are on the narrower base and the secondary hydroxy groups are on the wider base of the toroid. Considering the molecular size of ABPC, it appears unlikely for one molecule of ABPC to be simultaneously introduced into two molecules of βCD in solution. Thus, two types of initial geometries (Mode I and Mode II) for MDS of the inclusion complexes in aqueous solution were settled on for cation, zwitterion and anion species of ABPC. In Mode I, the penam ring of ABPC was initially placed in the βCD cavity and the phenyl ring was situated at the rim on the secondary hydroxy side. The phenyl ring was initially located in the center of the cavity in Mode II, whereas the penam ring was also situated at the rim of the secondary hydroxy side. The behaviors of the inclusion complexes in aqueous solution were simulated for 200 ps using the AMBER program and reached equilibrium by 50 ps. Fig. 4a–c show the snapshots and total energies of inclusion complexes of ABPC–βCD at 200 ps for cation, zwitterion and anion species of ABPC, respectively. However, the values of the total energies did not help to determine which Mode was stable, because the different numbers of TIP3P water molecules were containing in every cubic box.

Since the carboxyl group on the penam ring was un-ionized in the cation species, Mode I and Mode II of

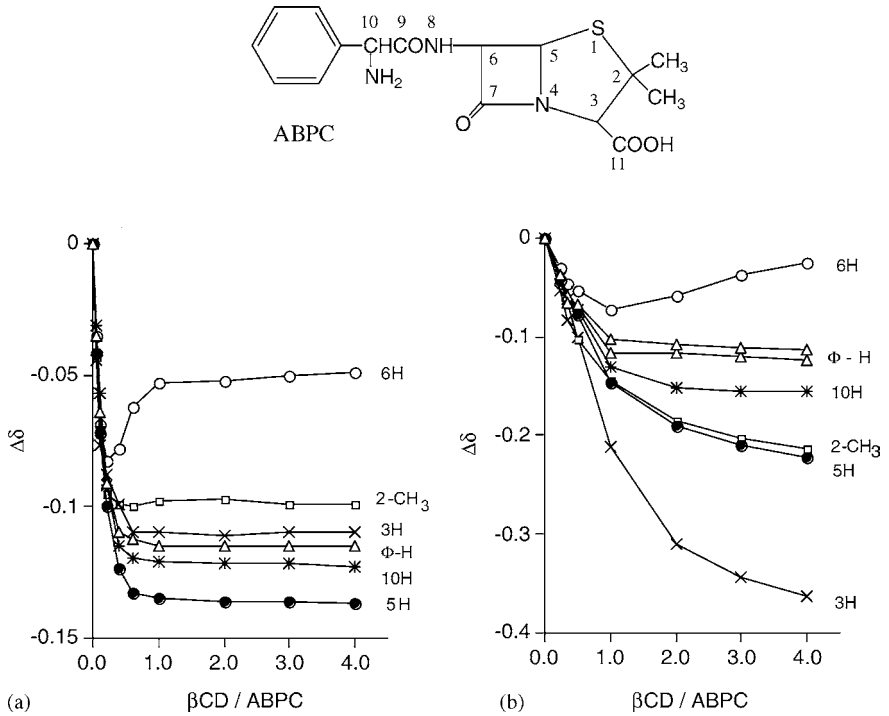

Fig. 3. Changes in ^{1}H NMR chemical shifts of ABPC caused by the complexation between ABPC and βCD in D_2O (a) and in 0.1 M DCl (b) as a function of the molar ratio of βCD/ABPC.

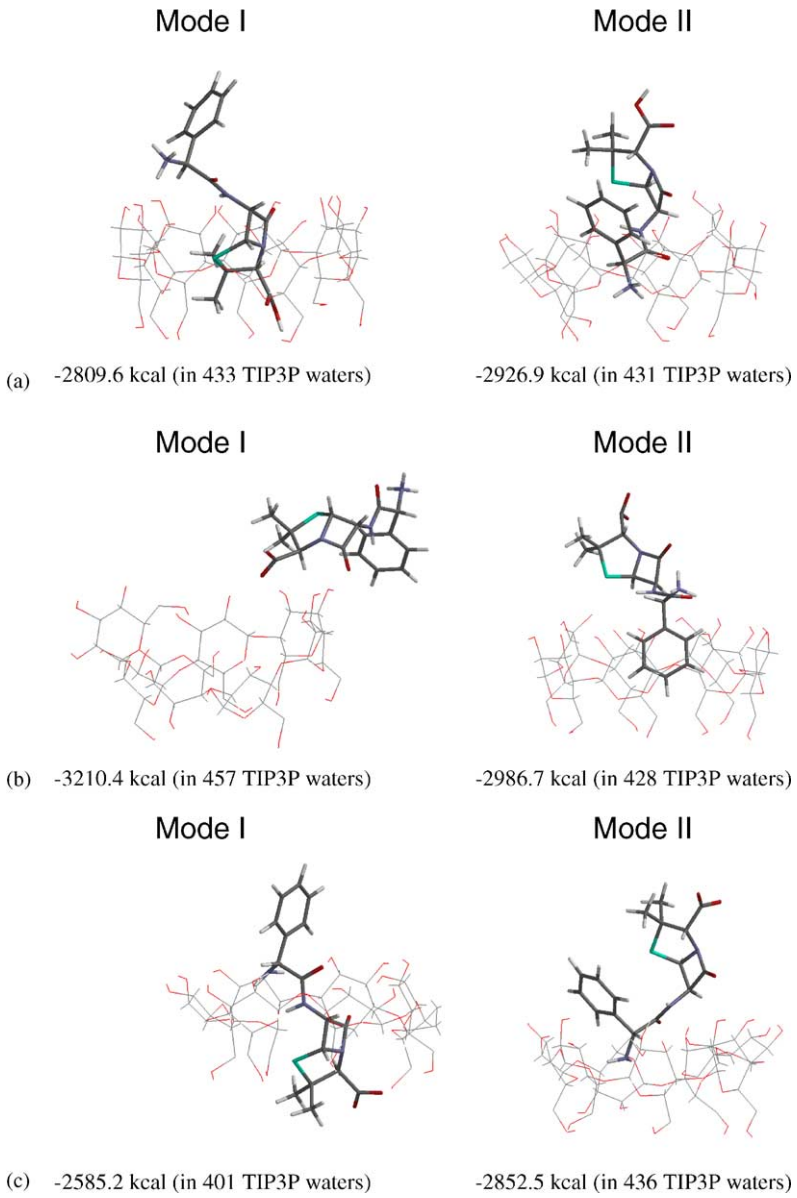

Fig. 4. Snap shots of two types of inclusion complex for (a) cation, (b) zwitterion and (c) anion species of ABPC at 200 ps. See text for an explanation of Mode I and Mode II. Each value under the snap shot represents the average of total energies for the last 50 ps.

inclusion complexes were kept stable. The β-lactam ring of ABPC was inserted in the βCD cavity in both models. In the zwitterion species, ABPC in Mode I gradually slipped out of the grip of the cyclodextrin cavity after about 20 ps. Mode II maintained the initial geometries of the complexes for 200 ps. The structure of the Mode II complex appeared to be stable with the CH/π interaction between the phenyl group of ABPC and hydrophobic C–H bonds in the cavity of the βCD. The anion species of ABPC migrated from the center of the cavity to the rim on the primary hydroxy side and from the cavity to the rim on the secondary hydroxy side of βCD to form Mode I and Mode II, respectively. Thus, the NH_2 group of ABPC was favorably located at the rim to form hydrogen bonds with the secondary hydroxyl groups of βCD.

3.4. Mechanisms of inclusion complexation to inhibit the decomposition and polymerization of ABPC

For the inclusion complexes between ABPC and β-cyclodextrins with a molar ratio 1:1 in aqueous solution, one or two types existed and the complexation significantly depended on the species of ABPC at the pH of the solution. Marked inflections in the heat effect of inclusion complexation were shown around the pKa_1 and pKa_2 (Figs. 1 and 2). In a strong acid solution at a lower pH than pKa_1, the penam ring with the un-ionized carboxyl group and phenyl ring could be inserted into the β-cyclodextrin cavity to form Mode I and Mode II complexes (Fig. 4a), respectively and to stabilize ABPC. The values of K_1 were $(10.15 \pm 1.0) \times 10^3\,M^{-1}$ at pH 1.2 and reduced in half to

$(5.80 \pm 0.4) \times 10^3\,M^{-1}$ at pH 2.0 (Table 1), since about 50% of carboxyl group was ionized at pH 2.0, making the penam ring more hydrophilic and as a result of which hydrophobic interactions decreased. From the results of ^1H NMR spectroscopy, the chemical shifts of 3H, 5H and 2-CH_3 on the penam ring moved upfield more largely than those of the protons on the phenyl ring (Fig. 3b). Thus, the first type of inclusion complex with a higher association constant (K_1) would be suited to Mode I.

In a weak acid and neutral solution, only one type of inclusion complex (Mode II in Fig. 5b) was formed with an association constant of 1.0×10^3 M and small negative ΔH. The values of K_1 were almost equal to those of K_2 of the second type of inclusion complex in the strong acid solution. Small changes of the chemical shifts of 3H, 5H and 2-CH_3 on the penam ring (Fig. 3a) reflect the interaction between the penam ring and the secondary hydroxy groups on the rim of βCD. Since the degradation in zwitterion species of ABPC is very slow [12,13], the study of stability is less significant than under strong acid conditions. At higher pH values (>8.0), the inclusion complex (Mode I in Fig. 4c) would be formed with the weak association constant (Table 1). However, the polymerization of ABPC in alkaline solution could be inhibited by the formation of hydrogen bonds between the NH_2 group of ABPC and the secondary hydroxyl groups of β-cyclodextrins as shown in Mode I and Mode II in Fig. 4c.

4. Conclusions

The inclusion complexation of ABPC and β-cyclodextrins (HPCD and βCD) was dependent on the pH value of the solution. The complexation was entropy driven to reflect hydrophobic interactions between the ABPC guest molecule and the cavity of β-cyclodextrins. In a strong acid solution, ABPC and β-cyclodextrins formed two types of inclusion complexes with a 1:1 stoichiometry, where the penam ring and phenyl ring were penetrated. Thus, the β-lactam ring of ABPC was inserted in the cyclodextrin cavity in both types to be protected from degradation in gastric juice, indicating that the inclusion complexes of ABPC with β-cyclodextrins were useful to the drug delivery system.

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