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MECHANISM FOR THE INHIBITION OF THE ACID DEGRADATION OF AMPICILLIN BY 2-HYDROXYPROPYL-β-CYCLODEXTRIN

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Abstract

The formation of inclusion complexes between amoxicillin (AMPC) and 2-hydroxypropyl-β-cyclodextrin (HPCD) was investigated by isothermal microcalorimetry and molecular dynamics simulation to evaluate the inhibitory effects on the degradation of AMPC in aqueous solutions at various pH. The process depended significantly on the ionic species of AMPC in the solution. In a strong acid solution, cationic AMPC and HPCD formed two different types of inclusion complexes with a 1:1 stoichiometry: the first-type had a high association constant K_1 of $4.0–8.0 \cdot 10^3$ M^{-1} and included the penam ring of AMPC in the HPCD cavity (Mode I), while the second-type with a K_2 of $1.0 \cdot 10^3$ M^{-1} contained the phenyl group of AMPC (Mode II). Furthermore, a complex with a 1:2 (AMPC:HPCD) stoichiometry was realized in a two-step reaction and was characterized by a smaller $K_{1:2}$ of $4.0 \cdot 10^2$ M^{-1} and larger negative enthalpy and entropy changes than the complexes with a 1:1 stoichiometry. Since the β-lactam ring of AMPC could be protected by inclusion with HPCD in the 1:2 complex and Mode I of 1:1 complexes, the degradation of AMPC in the presence of HPCD was approximately four times slower than in its absence at pH 1.2 and 37°C. In weak acid and neutral solutions, zwitterionic AMPC and HPCD formed only one type of inclusion complex with a 1:1 stoichiometry, where the phenyl group was included (Mode II). AMPC was very stable in these solutions ($t_{1/2}$=226 h at pH=6.0) and there is little significant difference in the degradation rate between complexed AMPC and uncomplexed AMPC. Thus, the results indicated that the inclusion complex of AMPC with HPCD, effectively increasing the stability of AMPC in a strong acidic solution like that the stomach, would be useful for eradicating *Helicobacter pylori* infection and as a drug delivery system.

Keywords: amoxicillin, 2-hydroxypropyl-β-cyclodextrine, inclusion complex, microcalorimetry, molecular dynamics simulation

Introduction

Helicobacter pylori is a Gram-negative bacterium that induces chronic gastritis and peptic ulcer. As one of the most effective medicine for treating *H. pylori* infection,

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amoxicillin (AMPC) is now used orally in combination with lansoprazole (a proton pump inhibitor) and clarithromycine (an antibiotics) but is unsuccessful to eradicate *H. pylori* entirely because of the instability of antibiotics in the strongly acidic environment of the stomach and the increase in antibiotic-resistant *H. pylori* [1–3]. AMPC, an aminopenicillin, exhibits a broad spectrum of antibacterial activity and is relatively stable in aqueous solutions compared to other types of penicillin [4, 5]. The stability of aminopenicillin has been attributed to the incorporation of an electron withdrawing substituent (NH_2) on its side chain [6]. Being amphoteric, AMPC in solution exists mainly as three different species; a cation, a zwitterion, and an anion. According to the pH profile for the degradation of AMPC, it was stable in aqueous solutions of pH 4 to 7 but unstable at lower and higher pH values [5, 7, 8]. This is because the β-lactam ring was more susceptible to hydrolytic degradation when the pH deviated significantly from the isoelectric point (pH 4.8) [5, 8]. Notably, AMPC was rapidly degraded under strong acidic conditions as in the stomach. It is as yet unclear whether AMPC acts against *H. pylori* directly after oral administration or via the systemic circulation. In any case, the stability of AMPC in the stomach is likely to be a key feature for the delivery system.

Cyclodextrins (CDs) are known to form non-covalently bonded inclusion complexes with a variety of drugs as the guest molecule (G) in both solution and a solid-state, thus improving their solubility, stability or bioavailability. 2-Hydroxypropyl-β-cyclodextrin (HPCD) used in this study is readily soluble in water and a pharmaceutically useful CD derivative [9, 10]. It was also found that the degradation and polymerization of benzylpenicillin and ampicillin were prevented by the formation of a complex with HPCD [11–13]. An important prerequisite for the preparation and use of inclusion complexes is understanding the mechanism by which the complex is formed. Structural information, such as the stoichiometry and geometry of the complex, and thermodynamic information, such as the changes in free energy (ΔG), enthalpy (ΔH), entropy (ΔS) and heat capacity (ΔC_p) of the inclusion system, are necessary to clarify the driving forces governing the interaction between G and CD. Association constants (K) have been determined using a number of chemical and physical methods, such as spectrometry, potentiometry, kinetics, and solubility techniques. These methods are mostly based on a typical reaction that produced only one inclusion type with a 1:1 stoichiometry and occasionally a stepwise reaction to form complexes with 1:1 and 1:2 stoichiometries. Nevertheless, very little is known about the multiple types of inclusion complexes with a 1:1 stoichiometry. In the case of amphoteric compounds like AMPC and ampicillin, the structure of the inclusion complex is very much dependent on the pH of the solution and pK_a of the species, since these parameters will decide the ratio between the species to be included by CD. Unfortunately, some of the K values and inclusion structures reported in the literature have been determined under uncontrolled conditions. Furthermore, inaccuracy and inconsistency in the thermodynamic parameters often lead to erroneous conclusions when ΔH is determined using a linearization method based on van't Hoff's equation [14, 15]. Isothermal microcalorimetry is an ideal analytical method for this

purpose, since the values of K and ΔH are directly estimated from a single experiment at a desired temperature.

In order to develop a formulation with an increased life span in the stomach to enhance the activity of AMPC against *H. pylori*, the effects of HPCD on the degradation of AMPC in aqueous solutions at pH 1.2 and 6.0 were clarified. The inhibitory mechanism was also discussed.

Materials and methods

Materials

Amoxicillin anhydride (AMPC) and β-cyclodextrin (βCD) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). 2-Hydroxypropyl-β-cyclodextrin (HPCD; *FW*=1540, *MS*=1.0) was purchased form Aldrich Chemical Co. (Milwaukee, WI, USA), and the average molecular mass 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 a KMADA-7000 (Jeol, Tokyo, Japan). All other materials used were obtained from Wako Pure Chemical Ind. Ltd. (Osaka, Japan) and were of analytical reagent grade. AMPC and HPCD were dissolved in buffer solutions: Clark–Lubs solution (1/5M HCl, 1/5M KCl, H_2O) at pH 1.2 and 2.0, and Sörensen solution (1/15M KH_3PO_4, 1/15M Na_2HPO_4) at pH 4.5, 6.0 and 8.0.

Stability of AMPC in buffered aqueous solutions

AMPC (0.5 mM) was dissolved in freshly prepared buffer solutions at pH 1.2 and pH 6.0, and HPCD corresponding to molar ratios of AMPC to HPCD from 1:1 to 1:10 was added to the solutions. The solutions were equilibrated in a water-bath maintained at 37±0.1°C. The AMPC remaining in the solution was analyzed at intervals by HPLC (Shimadzu LC10A; Shimadzu Co. Ltd, Kyoto, Japan) utilizing a UV detector at 254 nm. The analytical column used was TSK-GEL ODS-80TS (250×4.6 mm i.d.) (Tosoh, Tokyo, Japan). The mobile phase was composed of aqueous 0.05 M phosphate buffer (containing 0.1% *v/v* triethylamine and adjusted to pH 3.0 with orthophosphoric acid–methanol (90:10 *v/v*)). The flow rate was 1.0 mL min^{-1} and sample injection volume was 20 μL.

Microcalorimetry

All measurements were made in Thermal Activity monitor 2270 (ThermoMetric AB Jörf Ualla, Sweden) at 25.15±0.0001°C. Full details of the experimental method are given in an earlier paper [16]. The titrations were performed with a constant AMPC concentration of 0.5 mM. The concentration of HPCD was changed from 0.125 to 1.8 mM (after mixing). The heat of dilution of HPCD was measured for each sample and taken into account when the reaction heat was calculated. The reaction heat (ΔQ) represents a quantitative measure of the amount of product formed (A_p), and is

$$\Delta Q=\Delta H_{app}A_p \tag{1}$$

where ΔH_{app} is the apparent enthalpy change associated with the reaction.

For a one-step reaction, assuming that a guest molecule (G) and a cyclodextrin (CD) form several types (structures) of inclusion complexes with a 1:1 stoichiometry in aqueous solution:

$$G + CD \leftrightarrows \sum_{i=1}^{n}(G–CD)_i \tag{2}$$

The reaction heat can then be expressed as a function of the $(G–CD)_i$ concentration ($[G–CD_i]$) or free concentration of CD ([CD]).

$$\Delta Q = V\{[(G–CD)_1]\Delta H_1+[(G–CD_2]\Delta H_2+...+[(G–CD)_n]\Delta H_n\} \tag{3}$$

$$\Delta Q = [G_t]\,V\sum_{i=1}^{n}\frac{\Delta H_i K_i[CD]}{1+K_i[CD]} \tag{4}$$

where K_i and ΔH_i are the association constant and enthalpy change for the *i*th type of inclusion complex $(G–CD)_i$, respectively, and V is the volume in a reaction cell. The total concentrations of G and CD ($[G_t]$ and $[CD_t]$, respectively) are represented by the following equations.

$$[G_t]=[G]+\left[\sum_{i=1}^{n}(G–CD)_i\right] \tag{5}$$

$$[CD_t]=[CD]+\left[\sum_{i=1}^{n}(G–CD)_i\right] \tag{6}$$

In the case of i=1, G and CD form only one type of inclusion complex at a molar ratio of 1:1. In the case where i is more than 2, it is assumed that several types of inclusion complexes with a 1:1 stoichiometry are formed independently [16].

In two-step reactions expressed by Eq. (7), G and CD form a 1:1 complex (G–CD) and a 1:2 complex ($G–CD_2$) in the reaction system:

$$\begin{aligned} G+CD &\leftrightarrows G–CD && K_{1:1},\ \Delta H_{1:1} \\ G–CD+CD &\leftrightarrows G–CD_2 && K_{1:2},\ \Delta H_{1:2} \end{aligned} \tag{7}$$

where $\Delta H_{1:1}$ and $\Delta H_{1:2}$ are enthalpy changes for the interactions of the first and the second step, respectively. And $K_{1:1}$ and $K_{1:2}$ represent the association constants for the formation of (G–CD) and ($G–CD_2$), respectively.

$$K_{1:1}=\frac{[G–CD]}{[G][CD]} \tag{8}$$

$$K_{1:1}K_{1:2}=\frac{[G–CD_2]}{[G][CD]^2} \tag{9}$$

The reaction heat measured by microcalorimetry can be expressed as follows:

$$\Delta Q = V\{[G–CD]\Delta H_{1:1}+[G–CD_2](\Delta H_{1:1}+\Delta H_{1:2})\} \quad (10)$$

$$\Delta Q = [G_t]V\frac{\Delta H_{1:1}K_{1:1}[CD]+(\Delta H_{1:1}+\Delta H_{1:2})K_{1:1}K_{1:2}[CD]^2}{1+K_{1:1}[CD]+K_{1:1}K_{1:2}[CD]^2} \quad (11)$$

The experimentally measured ΔQ values were plotted against CD_t when G_t was fixed. The values of ΔH and K for each complexation can be simultaneously obtained by solving Eqs (4) and (11) using a nonlinear least-squares curve-fitting program [16, 17].

Molecular dynamics simulation

A molecular dynamics simulation (MDS) of the formation of inclusion complexes between AMPC and β-cyclodextrin (βCD) in aqueous solution was performed using the AMBER program (ver. 6) running on an SGI OCTANE-SE computer. The geometries of AMPC and βCD were optimized using Gaussian programs [18]. In the most realistic model of the complex in aqueous solution, these molecules were placed in the center of a 27 Å cubic box containing ca. 500 TIP3P water molecules. Then the 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 (Δt=0.001 ps and 200.000 time steps) with Shake [19] constraints for hydrogen atoms under conditions of constant pressure (1 atm) and temperature (25°C). Intermediate structures were saved in a file every 50 steps to obtain representative, and sequential structures generated during the simulation.

Results and discussion

Influence of HPCD on the stability of AMPC in aqueous solutions

In this study, the effect of HPCD on the degradation of AMPC at physiological temperature and pH (1.2 as gastric juice and 6.0 as small intestinal juice) was investigated. Introduction of HPCD did not affect the observed kinetic order. Figure 1 shows the pseudo first-order plots of the AMPC remaining in the buffer in the absence and presence of HPCD solutions at 37°C. AMPC was degraded rapidly at pH 1.2, and the observed half-life of 5.3 h was in agreement with previous studies [7, 8]. The influence of HPCD on the stability of AMPC was evaluated by comparing half-lives determined in the presence of HPCD with those in its absence. At pH 1.2, the degree of stabilization was dependent on the concentration of HPCD, and a maximum increase in stability of AMPC, of 4.0-fold, was observed at an AMPC to HPCD molar ratio of 1:5. There is little difference in the stability at a molar ratio more than 1:5 (Fig. 1a). On the other hand, AMPC was very stable in aqueous solution of pH 6.0, where the half-life was 225 h. In the presence of HPCD at a ratio of 1:1 or 1:2 (AMPC:HPCD) and in its absence, there was little significant difference among the

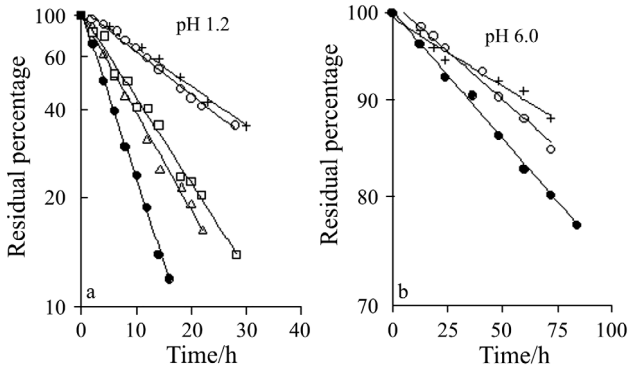

Fig. 1 Degradation of AMPC in the presence of HPCD at 37°C; ● – AMPC only; △ – AMPC:HPCD, 1:1 (molar ratio); □ – AMPC:HPCD, 1:2; ○ – AMPC:HPCD, 1:5; + – AMPC:HPCD, 1:10

degradation rates. Up to a molar ratio of 1:10, the half-life of AMPC was observed to be about 400 h. Thus, the degradation of AMPC was more effectively inhibited by HPCD in the strong acidic solution than in the weak acid and neutral solutions.

Complex of AMPC with HPCD in aqueous solutions at various pH

The calorimetric titrations of AMPC with HPCD in aqueous solutions with various pH values at 25°C are shown in Fig. 2. The reaction heat (ΔQ) increased exothermically as the concentration of HPCD increased but decreased as the pH of solution increased. At first, the calorimetric findings were analyzed using a one-step reaction system expressed by Eq. (4), where i was varied from 1 to 2 assuming a 1:1 complex with one to two inclusion types, respectively. The estimated values of the association constants and thermodynamic parameters for the complexation model with two inclusion types (i=2)

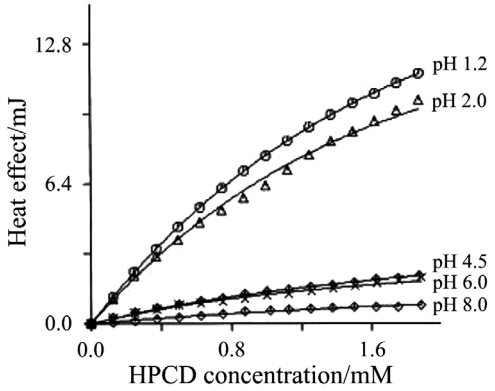

Fig. 2 Calorimetric titration curves for AMPC-HPCD complexation at various pH. In all the experiments, the concentration of AMPC was 0.5 mM. Points show the experimental data and solid lines represent computer-generated best fit curves

Table 1 Association constants and thermodynamic parameters for 1:1 multimodal inclusion complexes between AMPC and HPCD

pH	$K_1 \cdot 10^3/M^{-1}$	$-\Delta G_1$/ kJ mol^{-1}	$-\Delta H_1$/ kJ mol^{-1}	ΔS_1/ J mol^{-1} K^{-1}	$K_2 \cdot 10^2/M^{-1}$	$-\Delta G_2$/ kJ mol^{-1}	$-\Delta H_2$/ kJ mol^{-1}	ΔS_2/ J mol^{-1} K^{-1}
1.2	8.0±1.6	11.2	3.4±0.8	28	11.4±0.4	8.8	9.3±0.3	–1.6
2.0	4.2±0.5	10.4	3.3±0.2	23	8.5±0.2	8.4	7.2±0.3	4.0
4.6	1.2±0.05	8.9	1.6±0.2	24	0.098	–	–	–
6.0	1.0±0.05	8.7	1.4±2.2	24	0.095	–	–	–
8.0	0.99±0.1	8.6	0.6±0.1	27	0.084	–	–	–

K_1 and K_2: Association constants of the first-type and the second-type complexes, respectively.

are listed in Table 1. At pH 1.2 and 2.0, the data did not fit the model forming only one type of inclusion complex (i=1) but fitted the model forming two inclusion types (i=2). The data obtained at pH 4.5, 6.0 and 8.0 fitted both models. However, the association constants of K_2 were much smaller than those of K_1 to stabilize the inclusion complexes as shown in Table 1. Thus, it seemed that only the cationic AMPC formed two types of inclusion complex. In the first type with a higher association constant K_1, the small negative value of ΔH_1 and the large positive ΔS_1 indicated hydrophobic interaction between AMPC and the hydrophobic cavity of HPCD. The value of K_1 decreased as the pH value increased, and the K_1 values at pH 4.5, 6.0 and 8.0 were equal to the K_2 values for the second type of inclusion complex at pH 1.2 and 2.0. The second type of inclusion complex with a smaller K_2 value at pH 1.2 and 2.0 was characterized by a relatively large negative ΔH_2 and small ΔS_2, and would be formed by a combination of hydrophobic interaction, van der Waals interaction between AMPC and the CD cavity and enthalpy gain due to the release of the 'high-energy' water from the cavity instead of including AMPC [17].

The calorimetric findings at pH 1.2 and 2.0 were further analyzed using a two-step reaction system (Eq. (11)) assuming the formation of 1:1 and 1:2 (AMPC:HPCD) complexes. The association constants ($K_{1:1}$ and $K_{1:2}$) and the thermodynamic parameters are listed in Table 2. The $K_{1:1}$ value for the 1:1 complex was estimated to be close to the averages of K_1 and K_2 in the two types of inclusion complexes with a 1:1 stoichiometry. In the 1:2 complex, the $K_{1:2}$ was smaller than K_2 and the large negative values of $\Delta S_{1:2}$ for the 1:2 complex were less favorable than those of ΔS for the 1:1 complex. The addition of the second HPCD molecule to the 1:1 complex significantly restricted the motional freedom of the AMPC molecule in the CD cavity and resulted in an unfavorable change in entropy. From a structural point of view, AMPC would be able to form a 1:2 complex with HPCD. To support this explanation, an AMPC-βCD complex was prepared using a freeze-drying method. On measuring the FAB-mass spectrum of the complex, a high intensity peak of the 1:1 complex and a far smaller intensity peak of the 1:2 complex were observed.

Molecular dynamics simulation (MDS) of AMPC-HPCD inclusion complexes

In the AMPC-HPCD complex with a 1:1 stoichiometry, either the phenyl group or the penam ring of AMPC was probably included by HPCD. Both ring systems would be suitable as inclusion groups according to a preliminary inspection of the structure of AMPC and the void volume of the CD cavity. As HPCD was a heterogeneous product and the structure had not been clearly determined, βCD was adopted as a host molecule to easily determine the stable structures of the complex in the study of the inclusion behavior of AMPC using the molecular dynamics. Two initial geometries (Mode I and Mode II) of the inclusion complexes in aqueous solution were settled on for MDS. In Mode I, the penam ring of AMPC was initially placed in the βCD cavity and the phenyl group was situated outside the rim on the secondary hydroxy group side with the wider base of the toroid. On the other hand, the phenyl group was initially located in the center of the cavity in Mode II, whereas the penam ring was

Table 2 Association constants and ΔH for 1:1 and 1:2 inclusion complexes between AMPC and HPCD

pH	$K_{1:1}\cdot 10^3/M^{-1}$	$-\Delta G_{1:1}$/ kJ mol^{-1}	$-\Delta H_{1:1}$/ kJ mol^{-1}	$\Delta S_{1:1}$/ J mol^{-1} K^{-1}	$K_{1:2}\cdot 10^2/M^{-1}$	$-\Delta G_{1:2}$/ kJ mol^{-1}	$-\Delta H_{1:2}$/ kJ mol^{-1}	$\Delta S_{1:2}$/ J mol^{-1} K^{-1}
1.2	3.5±0.7	10.2	5.3±1.2	16	4.0±0.3	7.5	16±1.3	–29
2.0	1.7±0.9	9.3	3.0±1.4	21	4.6±0.4	7.6	14±2.1	–21

$K_{1:1}$ and $K_{1:2}$: Association constants for 1:1 and 1:2 inclusion complexes of AMPC/HPCD, respetively. $\Delta H_{1:1}$ and $\Delta H_{1:2}$: Enthalpy changes in forming 1:1 and 1:2 inclusion complexes of AMPC/HPCD, respectively.

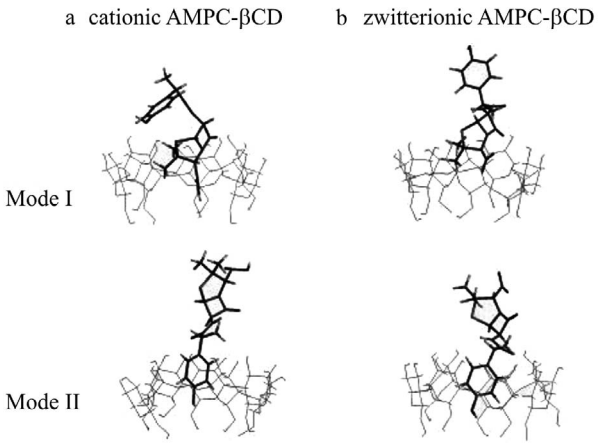

Fig. 3 Snap shots of two types of inclusion complexes between AMPC and βCD at 200 ps. See text for an explanation of Mode I and Mode II

situated outside the rim of the secondary hydroxy group. The inclusion behavior in aqueous solution was simulated for 200 ps using the AMBER program. Figures 3a and 3b show snapshots of inclusion complexes of AMPC-βCD for the cation and zwitterion of AMPC, respectively, at 200 ps.

In Mode I, the complex formed between cationic AMPC and βCD was kept stable for 200 ps, with the β-lactam ring favorably included near the rim of βCD. But the penam ring of the zwitterionic AMPC-βCD complex in Mode I was gradually driven out from the βCD cavity, so that the ionized carboxyl group on the penam ring formed hydrogen bonds with the secondary hydroxyl groups of βCD to keep the complex stable. In Mode II the complexes of cationic and zwitterionic AMPC with HPCD maintained their initial geometries for 200 ps. The complexes appeared to be stable with CH/π interaction between the hydrophobic C-H in the cavity of βCD and the phenyl group of AMPC. However, the β-lactam ring in Mode II could not be included in βCD.

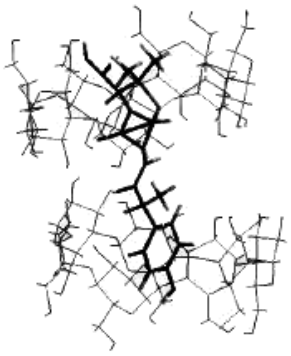

Fig. 4 Snap shot of a 1:2 inclusion complex between cationic AMPC and βCD at 200 ps

Figure 4 shows a snapshot of the cationic AMPC-βCD complex with a 1:2 stoichiometry at 200 ps. The phenyl group and lactam ring of AMPC were inserted into the cavities of two moles of β-CD with the initial geometry. As the motional freedom of AMPC was very restricted, the initial structure of the inclusion complex was maintained for 200 ps.

Mechanism of inclusion complexation inhibiting the decomposition of AMPC

AMPC exists as four different ionic species, a cation, a zwitterion, an anion, and a dianion in aqueous solution depending on the pH. The degradation of AMPC followed first-order kinetics and the following equation explained the kinetics of the reaction [5, 8].

$$k_{pH}=k_H[H^+]\ fA^+ + k_1' fA^+ + k_2\ fA^{\pm} + k_3'\ fA^- + k_{OH}\ [OH^-]fA^-$$
$$=(k_H\ [H^+]+k_1')\ fA^+ + k_2\ fA^{\pm} + (k_3'+k_{OH}\ [OH^-])fA^-$$
$$=k_1 fA^+ + k_2\ fA^{\pm} + k_3 fA^- \quad (12)$$

where, fA^+, $fA^{\pm}$ and fA^- represented the mole fractions of the cationic, zwitterionic and anionic species of AMPC, respectively; k_H and k_{OH} were second-order rate constants for hydrogen and hydroxide ion-catalyzed reactions, respectively; and k_1, k_2, and k_3 represented apparent first-order rate constants for the hydrolytic degradation of fA^+, $fA^{\pm}$ and fA^-, respectively.

The apparent pK_a values of fA^+, $fA^{\pm}$ and fA^- were estimated to be 2.63, 7.16, and 9.55, respectively [5]. Since the β-lactam ring was more susceptible to hydrolytic degradation when the pH was lower and higher than the isoelectric point (pH 4.8), the hydrolysis of cationic AMPC was much faster than that of zwitterionic AMPC ($k_1 \geq k_2$). An inhibitory effect of HPCD on the degradation was observed with the cationic rather than zwitterionic AMPC (Fig. 1). This could be explained by the different structures of the inclusion complexes formed.

The reaction heat effect (ΔQ) was highest at pH 1.2, where AMPC existed entirely as a cation (fA^+=0.97), decreased suddenly at pH values greater than 4.5, where the carboxyl group on the penam ring of AMPC was fully ionized to be a zwitterion ($fA^{\pm}$=0.97 at pH 6.0), and was lowest at pH 8.0, where AMPC exist almost as an anion (fA^-=0.85, $fA^{\pm}$=0.13, and fA^{2-}=0.02). In a strong acid solution, the penam ring with the unionized carboxyl group could be easily inserted into the hydrophobic cavity of CD to form a Mode I type complex of cationic AMPC and HPCD. The free energy (ΔG_1) was –11.2 kJ mol^{-1} at pH 1.2 and increased to –10.4 kJ mol^{-1} at pH 2.0 (Table 1). About 20% of carboxyl groups were ionized at pH 2.0, making the penam ring more hydrophilic, and as a result hydrophobic interactions decreased. In contrast, the ΔG_2 value was unchanged, being –8.8 and –8.4 kJ mol^{-1} at pH 1.2 and 2.0, respectively. Thus, the first-type of cationic AMPC-HPCD complex would be suited to Mode I, and could include the β-lactam ring in the HPCD cavity, thus protecting the β-lactam cleavage from hydrolysis. It is also possible that the second-type of Mode II geometry including the phenyl group of AMPC would subsequently form a

1:2 complex to inhibit the degradation of AMPC at higher concentrations of HPCD. In weak acid and neutral solutions, the carboxyl group on the penam ring of AMPC is completely ionized and only one type of inclusion complex (Mode II in Fig. 3b) was formed by hydrophobic interaction between the phenyl group of AMPC and the hydrophobic cavity of HPCD. The values of K_1 (about $1.0 \cdot 10^3$ M^{-1}) were almost equal to those of K_2 of the second-type of inclusion complex between cationic AMPC and HPCD (Mode II in Fig. 3a). The inhibitory effect of the complexation on the stability of AMPC was less significant in the weak acid and neutral solutions than in the strong acid solution, since the degradation in zwitterion species of AMPC was very slow.

Conclusions

The formation of a complex between AMPC and HPCD was dependent on the pH value of the solution. In a strong acid solution, two types of inclusion complexes with a 1:1 stoichiometry were mainly formed. The first-type with a high association constant was entropy driven and had the penam ring of AMPC included in the hydrophobic cavity of HPCD (Mode I). In the second-type of complex, the phenyl group of AMPC was included in HPCD (Mode II), and it would be possible to form a 1:2 complex between AMPC and HPCD in the two-step reaction, where both the penam ring and phenyl group of AMPC were included. The formation of Mode I and a 1:2 complex including the β-lactam ring would contribute to the stability of AMPC. The degradation rate of cationic AMPC in the presence of HPCD was approximately fourfold slower than in its absence. In weak acid and neutral solutions, zwitterion species of AMPC formed only one type of complex in which the phenyl group was inserted into the HPCD cavity (Mode II). There was little significant difference in the degradation rate between complexed and uncomplexed AMPC. Thus, HPCD was effective in stabilizing AMPC in a strong acid solution such as gastric juice, indicating that the complex of AMPC with HPCD would be useful for *H. pylori* infection and as a drug delivery system in an oral dosage form.

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