Effect of β-Cyclodextrin on the Degradation Rate of Amoxicillin in Acidic Solution

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The formation of an inclusion complex of amoxicillin (AMX) with β-cyclodextrin (β-CD) in aqueous solution was confirmed by a solubility method and proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy. The apparent stability constant for the inclusion complex was 10.72 M^{-1} in water at 25°C. The effect of α-CD, β-CD, and γ-CD on the degradation of AMX in a pH 1.2 solution at 37°C was investigated. β-CD and γ-CD reduced the rate of degradation. α-CD had no effect. These results were consistent with those of ^{1}H-NMR spectroscopy. The effect of β-CD on the degradation rate was studied in more detail. The apparent first order rate constant for the degradation of AMX in the pH 1.2 solution at 37°C was 0.1121 h^{-1} (t$_{1/2}$=6.18 h), which decreased with the addition of β-CD. The rate constants and t$_{1/2}$ values for the concentrations of β-CD added, corresponding to molar ratios of AMX to β-CD of 1 : 0.5, 1 : 1, 1 : 2, 1 : 5, and 1 : 10, were 0.1051 h^{-1} and 6.59 h, 0.0992 h^{-1} and 6.98 h, 0.0893 h^{-1} and 7.76 h, 0.0697 h^{-1} and 9.95 h, and 0.0509 h^{-1} and 13.61 h, respectively. The activation energy for the degradation of AMX in the pH 1.2 solution was increased from 6.9×10^4 J/mol (AMX alone) to 8.0×10^4 J/mol (AMX : β-CD=1 : 10).

Key words——amoxicillin; β-cyclodextrin; inclusion complex; stability; acidic solution

INTRODUCTION

Amoxicillin (AMX), an oral antibiotic with a wide spectrum of antibacterial activity, has recently been used to treat *Helicobacter pylori* (*H. pylori*) infections. Following this change of usage, a drug delivery system for the eradication of *H. pylori* was reported.[1,2] AMX is unstable in an acidic solution, and so a proton pump inhibitor must be administered for the eradication. Therefore, the stabilization of AMX may increase its effect in the stomach. Cyclodextrins (CDs) are generally used in pharmaceutical preparations to increase stability and dissolution by forming inclusion complexes with drugs.[3–6] Several presentations regarding the stabilization of AMX using CDs have been performed[7–9] In this study, the complexation of AMX with β-CD was confirmed by a solubility method and proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy. The effect of β-CD on the degradation of AMX in a pH 1.2 solution was also investigated.

EXPERIMENTAL

Materials AMX was purchased from Sigma Chemical Company (St. Louis, Mo, USA). α-Cyclodextrin (α-CD), β-CD, and γ-CD were purchased from Wako Pure Chemical Industries, Ltd. Other chemicals were of reagent or HPLC-grade.

Solubility Studies Fifty milligrams of AMX was added to 5 ml of β-CD solution at a concentration of 0.0, 5, 10, 15, or 20 mM. After being stored for 3 days to achieve equilibrium at 25°C, the solutions were filtered through a membrane with mean pore size of 0.45 μm, and the concentration of AMX in the filtrate was determined by high performance liquid chromatography (HPLC). An apparent stability constant (*K′*) of the complex of AMX with β-CD was calculated from the following equation.[10]

$$K' = \frac{Slope}{S_0(1 - Slope)}$$

NMR Spectroscopy This was conducted in D$_2$O using a JNM LA500 (JEOL, Ltd.). ^{1}H-Chemical shifts were given relative to external tetramethylsilane. The concentrations of AMX and CDs in sample solutions were 4 mM.

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Measurement of Stability of AMX in a pH 1.2 Solution   AMX was dissolved in a pH 1.2 solution, the 1st fluid for dissolution test of Japanese Pharmacopoeia XV (JP XV), to make a 5 mg/ml solution. A 4.9 ml aliquot of the 1st fluid for dissolution test of JP XV with or without CDs was pre-incubated for 15 min at 37, 50 and 60°C. The experiments were initiated by adding the AMX solution to produce a final concentration of 100 μg/ml that was 0.24 mM. A 100 μl aliquot of the sample solution was removed at appropriate intervals. The solution was added to 200 μl of a pH 6.8 buffer, the 2nd fluid for disintegration test of JP XV, and mixed well. A 20 μl volume of the solution was injected onto an HPLC column. In the comparison among CDs, the concentrations of α-CD, β-CD and γ-CD were 1.2 mM. The molar ratio of AMX to the CDs was 1 : 5. In the experiment on the effect of the amount of β-CD added, the concentrations of β-CD used were 0.12, 0.24, 0.48, 1.2 and 2.4 mM (1 : 0.5, 1 : 1, 1 : 2, 1 : 5, and 1 : 10, respectively).

Determination of AMX by HPLC   The concentration of AMX in a sample solution was determined by HPLC with a Model LC-9A pump, equipped with a Model SCL-6B system controller, a Model SPD-6A UV spectrophotometric detector, a Model CTO-6B column oven, a Model C-R4A Chromatopac; and a Model SIL-6B autoinjector, all from Shimadzu Corporation (Kyoto, Japan). The mobile phase was acetonitrile-water-perchloric acid (60%)-sodium perchlorate monohydrate (128 : 872 : 1 : 5, v/v/v/w). The chromatographic column was an YMC Pack AM312 ODS (150 mm×6 mm I.D., particle diameter 5 μm) obtained from YMC Co., Ltd. (Kyoto, Japan).

The flow rate, the wavelength for determination, and the temperature of the column were 1 ml/min, 254 nm and 40°C, respectively.

RESULTS AND DISCUSSION

Evidence of Inclusion Complexation   Table 1 shows the results of ^{1}H-NMR spectroscopy. The position of each proton in the chemical structure of AMX is shown in Fig. 1. The effect of α-CD on the chemical shifts of AMX was very small. In the case of β-CD, H5 showed a high-field shift. When γ-CD was added, almost all protons of AMX shifted to the high-field region. These results suggested that AMX could form inclusion complexes with β-CD and γ-CD.

Figure 2 shows the effect of α-CD, β-CD and γ-CD on the rate of degradation of AMX in the pH 1.2 solution at 37°C. In this experiment, the concentrations of AMX and CDs were 0.24 and 1.2 mM, respectively. Each plot in Fig. 2 is considered to be linear, which shows that the degradation of AMX under the conditions is a pseudo first-order reaction. The apparent first-order rate constant for each plot is summarized in Table 2. The rate constant of AMX was well
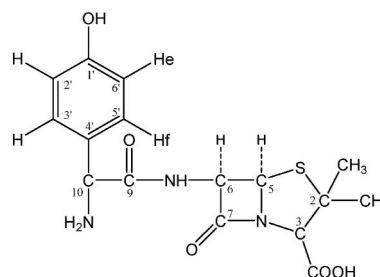
Fig. 1.   Chemical Structure of AMX

Table 1.   ^{1}H-NMR Chemical Shifts of AMX in the Presence or Absence of CDs

Proton	Chemical shift (ppm)						
	AMX	AMX +α-CD	Δ [a]	AMX +β-CD	Δ	AMX +γ-CD	Δ
2α-CH3	1.203s	1.216	0.013	1.203	0.000	1.208	0.005
2β-CH3	1.228s	1.227	−0.001	1.228	0.000	1.226	−0.002
H3	3.943s	3.948	0.005	3.943	0.000	3.942	−0.001
H10	4.903s	4.905	0.002	4.906	0.003	4.874	−0.029
H5	5.276q	5.278	0.002	5.259	−0.017	5.258	−0.018
H6	5.301q	5.302	0.001	5.317	0.016	5.300	−0.001
eH	6.770q	6.772	−0.002	6.772	0.002	6.766	−0.004
fH	7.161q	7.163	0.002	7.163	0.002	7.157	−0.004

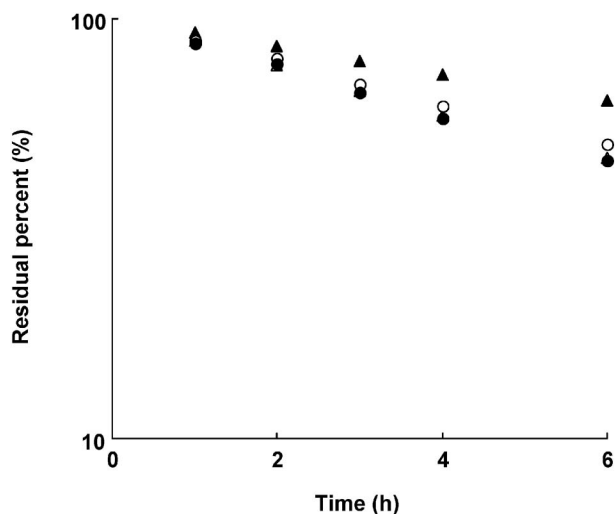a) Change of chemical shifts, calculated from (a chemical shift of AMX with CDs−that of AMX without CDs).

Fig. 2. Effect of CDs on the Degradation of AMX in the Solution of pH 1.2 at 37°C
●; AMX alone, △; α-CD, ▲; β-CD, ○; γ-CD.

Fig. 3. Effect of Concentrations of β-CD on the Solubility of AMX in Water at 25°C

Table 2. Effect of CDs on the Degradation of AMX in pH 1.2 Solution at 37°C

	k (h^{-1})	$t_{1/2}$ (h)
AMX	0.1316	5.27
AMX : α-CD=1 : 5[a]	0.1288	5.38
AMX : β-CD=1 : 5	0.0757	9.17
AMX : γ-CD=1 : 5	0.1164	5.95

Each value is calculated from a set of data. The initial concentration of AMX is 100 μg/ml (0.24 mM). a) The initial molar ratio in the solution.

consistent with values previously reported.[11,12] It was found from these results that α-CD had no stabilizing effect on AMX, and that β-CD had a stronger effect than γ-CD.

CDs have been reported to suppress or enhance chemical reactions in aqueous solutions by forming inclusion complexes.[5,13,14] The degradation rate for AMX in the acidic solution was decreased by the addition of β-CD and γ-CD, indicating that inclusion complexes of AMX with β-CD and γ-CD formed in the solution. These results were supported by those of ^{1}H-NMR spectroscopy. Therefore, the complex of AMX and β-CD was studied using a solubility method. Figure 3 shows an equilibrium phase solubility diagram for the AMX/β-CD system in water. The solubility of AMX increased from 5.88 to 7.09 mM with the addition of β-CD from 0 to 20 mM showing a linear relationship. The apparent stability constant, K', of the complex in water at 25°C was estimated as 10.72 M^{-1} from the straight line.
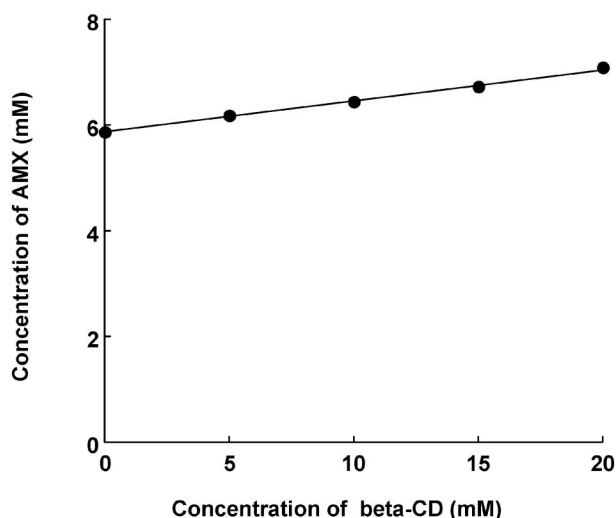
Effect of β-CD Concentration and Temperature on the Degradation of AMX AMX is unstable in acidic solution, therefore a proton pump inhibitor is inevitably used for the eradication therapy. Improving the stability of AMX is considered important, so further investigation with β-CD was performed.

Figure 4 shows the effect of the concentration of β-CD on the degradation of AMX in a solution of pH 1.2 at 37, 50 and 60°C. Each plot was linear, which shows that the degradation is a pseudo first-order reaction. The apparent first-order rate constant was calculated from this relationship. The apparent first-order rate constants are summarized in Table 3. The rate constants of AMX alone and AMX : β-CD=1 : 5 in Table 3 were smaller than those in Table 2. This difference was considered to be caused by the different final sampling time in the experimental conditions, which were 6 h for the experiment of Table 2 and 8 h for that of Table 3. The ratios of the rate constant, AMX : β-CD=1 : 5/AMX alone, in Tables 2 and 3 were about 0.6, which indicated that the relative relationship was maintained between the data in Tables 2 and 3. The stabilizing effect of γ-CD was confirmed from this relationship, although the effect was not detected by the comparison of the rate constant between AMX : γ-CD=1 : 5 in Table 2 and AMX alone in Table 3.

When β-CD was present in the reaction system, the apparent first-order rate constant, k, was smaller at all three temperatures as shown in Table 3. The results indicate that the stabilizing effect increased
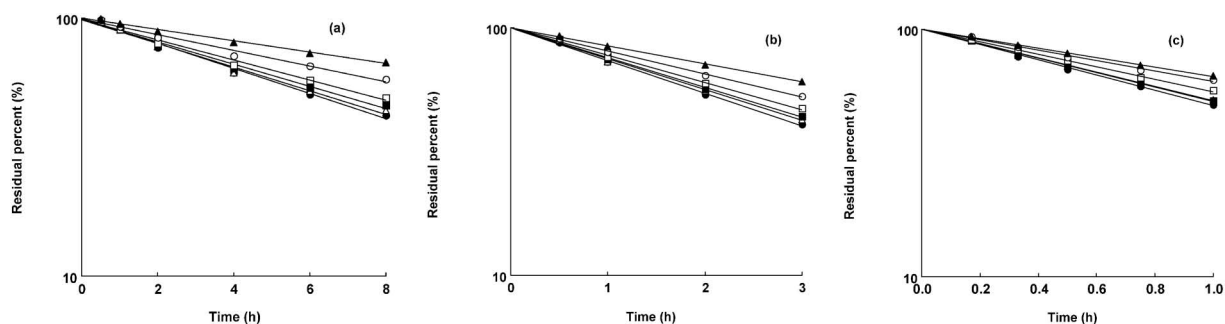
Fig. 4. Effect of Concentrations of β-CD on the Degradation of AMX in the Solution of pH 1.2 at 37 (a), 50 (b), and 60℃ (c)
●; AMX alone, △; AMX: β-CD＝1 : 0.5, ■; AMX : β-CD＝1 : 1, □; AMX : β-CD＝1 : 2, ○; AMX : β-CD＝1 : 5, ▲; AMX : β-CD＝1 : 10.

Table 3. Effect of Concentrations of β-CD on the Apparent First-order Rate Constants of the Degradation of AMX in pH 1.2 Solution at 37℃, 50℃, and 60℃

AMX : β-CD[a]	k (h^{-1})					
	37℃		50℃		60℃	
	Mean	SD	Mean	SD	Mean	SD
AMX alone	0.1121	0.0013	0.3021	0.0019	0.7126	0.0653
1 : 0.5	0.1051	0.0007	0.2871	0.0020	0.6658	0.0117
1 : 1	0.0992	0.0002	0.2764	0.0040	0.6763	0.0149
1 : 2	0.0893	0.0028	0.2530	0.0043	0.5809	0.0219
1 : 5	0.0697	0.0007	0.2148	0.0018	0.4897	0.0096
1 : 10	0.0509	0.0010	0.1686	0.0027	0.4410	0.0070

Each value represents the mean and S.D. of 3 determinations. The initial concentration of AMX in the solution is 100 μg/ml (0.24 mM). a) Initial molar ratio in the solution.

Table 4. Effect of the Concentration of β-CD on the Activation Energy for the Degradation of AMX in the Solution at pH 1.2

AMX : β-CD[a]	Ea (J/mol)
AMX alone	6.9×10^4
1 : 1	7.1×10^4
1 : 5	7.3×10^4
1 : 10	8.0×10^4

a) Initial molar ratio in the solution.

Fig. 5. Arrhenius Plots Based on the Rate Constants Measured in the Solution at pH 1.2
●; AMX alone, □; AMX : β-CD＝1 : 1, ○; AMX : β-CD＝1 : 5, ▲; AMX : β-CD＝1 : 10.

β-CD dependeds on the formation of an inclusion complex. These results should be useful for designing dosage forms of AMX.

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with the concentration of β-CD in the system.

Figure 5 shows Arrhenius plots of the degradation of AMX with or without β-CD. All plots showed good linearity. The activation energy of the degradation was calculated from the slope of each plot. The results are shown in Table 4.

AMX, which is unstable in acidic solution, was found to be stabilized in the presence of β-CD. It was indicated from our results that the stabilizing effect of
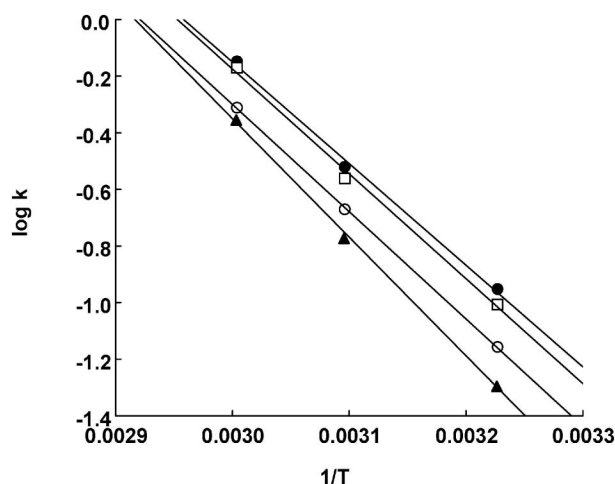
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