Improvement of Dissolution Properties of a New *Helicobacter pylori* Eradicating Agent (TG44) by Inclusion Complexation with β-Cyclodextrin

Kinsei ANZAI,[a] Jun-ichi MIZOGUCHI,[a] Toshiharu YANAGI,[a] Fumitoshi HIRAYAMA,[b] Hidetoshi ARIMA,[c] and Kaneto UEKAMA*,[b]

[a] *Nagase ChemteX Corporation, Kobe Product Development Center; 2–2–3 Murotani, Nishi-ku, Kobe, Hyogo 651–2241, Japan:* [b] *Facutly of Pharmaceutical Sciences, Sojo University; 4–22–1 Ikeda, Kumamoto 860–0082, Japan: and* [c] *Graduate School of Pharmaceutical Sciences, Kumamoto University; 5–1 Oe-honmachi, Kumamoto 862–0973, Japan.*
Received May 2, 2007; accepted July 12, 2007

The interaction of a newly developed *Helicobacter pylori* eradicating agent (TG44, 4-methylbenzyl-4′-[*trans*-4-(guanidinomethyl)cyclohexylcarbonyloxy]biphenyl-4-carboxylate monohydrochloride) with β-cyclodextrin (β-CyD) in aqueous solution and in solid state was studied to gain insight into the high *in-vivo H. pylori* eradicating activity of TG44/β-CyD complex. The interaction was studied by the solubility method, spectroscopic methods, powder X-ray diffractometry and differential scanning colorimetry (DSC). TG44 gave A_L-type phase solubility diagram with β-CyD in water, showing a linear increase in solubility of the drug up to 8 mM β-CyD concentration. The solubility of TG44 (0.04 mM in water at 25 °C) increased about 70-folds at 8 mM β-CyD. Ultraviolet, circular dichroism, fluorescence and ^{1}H-nuclear magnetic resonance spectroscopic studies indicated that TG44 forms the inclusion complex with β-CyD in a 1 : 1 stoichiometry and the biphenyl moiety of TG44 is preferably included in the β-CyD cavity in water. The Giordano plot made by monitoring changes in the fusion enthalpy of TG44 (about 184 °C) suggested that TG44 forms the 1 : 1 complex with β-CyD in the solid state. The TG44/β-CyD solid complex in a 1 : 1 stoichiometry was prepared by the grinding and spray-drying methods and confirmed by powder X-ray diffractometry and DSC that the complex is in an amorphous state. The initial dissolution rate of TG44/β-CyD complex was significantly faster than those of the drug alone and the physical mixture of both components, maintaining higher supersaturated concentrations of the drug for a long time. The results suggested that the higher eradicating activity of TG44/β-CyD complex to *Helicobacter pylori*, compared with that of the drug alone, is attributable at least partly to the faster dissolving property of the complex and its ability to maintain the supersaturated state of the drug in the gastric fluid.

Key words TG44; β-cyclodextrin; inclusion complex; solubility; dissolution; *Helicobacter pylori* eradicating agent

Helicobacter pylori (*H. pylori*) is implicated in the etiology of duodenal and gastric ulcer diseases and gastric cancer in severe cases.[1)] Many studies have been demonstrated that *H. pylori* eradication treatment is indicated in all patients with active or recurrent peptic ulceration. Oral triple-therapy schedules using a proton inhibitor or an H_2-antagonist in combination of two antibiotics such as clarithromycin, metronidazole, amoxicillin and tetracycline have been proved to be highly effective in the treatment of *H. pylori*. However, such the triple therapy sometimes results in failure of the *H. pylori* therapy because of poor compliance with the treatment regimen, with the development of antibiotic resistance and with drug–drug interaction, *etc*.[2,3)] Therefore, it is desired to develop new strategies of *H. pylori* therapy, including the development of new drugs and multiple-combinations, *etc*.

4-Methylbenzyl-4′-[*trans*-4-(guanidinomethyl)cyclohexylcarbonyloxy]biphenyl-4-carboxylate monohydrochloride (TG44, Fig. 4 for the chemical structure) is a newly synthesized *H. pylori* eradicating agent with high selectivity to *H. pylori,* compared with other gram-negative and gram-positive bacteria.[4)] The structure of TG44 is closely related to an antiulcer agent, benexate, which is formulated in the form of β-CyD complex and on market as a trade name of Ulgut or Lonmiel, although the pharmacological indication is different between them. Because benezate is commercially available as a β-CyD complex, we used β-CyD as a solubilizing agent for TG44, among various CyDs and their derivatives. The *H. pylori* eradicating activity of TG44 was markedly enhanced when it was orally administered in the form of β-CyD complex, compared with TG44 alone, which will be reported elsewhere. The enhanced antimicrobial activity of TG44/β-CyD complex may be ascribed to improved dissolving properties of the β-CyD complex, but the detailed mechanism is not yet fully elucidated. This study dealt with the inclusion complexation of TG44 with β-CyD in water and in solid state, to gain insight into the high *in-vivo H. pylori* eradicating activity of TG44/β-CyD complex.

Experimental

Materials TG44 and β-CyD were supplied by Nagase ChemteX Corporation (Osaka, Japan) and Nihon Shokuhin Kako Co., Ltd. (Tokyo, Japan), respectively. All other chemicals and solvents were of analytical reagent grade, and deionized distilled water was used throughout the study.

Solubility Measurements Solubility studies were carried out according to the method of Higuchi and Connors.[5)] The screw-capped vials containing TG44 in excess amounts (0.3 g/50 ml) in aqueous CyD solutions at various concentrations (2×10^{-3}—8×10^{-3} M) were stirred for 24 h at 25 °C. The suspension was filtered through a syringe with a membrane-filter, and the filtrate was adequately diluted and analyzed for TG44 using high-performance liquid chromatography (HPLC) under the following conditions: a Shimadzu Class LC-10A HPLC system, (Kyoto, Japan), a column of CERI L-column ODS 5 μm (Tokyo, Japan), a mobile phase of phosphate buffer (pH=3) of H_2O/acetonitrile (1 : 1 v/v), a flow rate of 1.0 ml/min, and a detection of 278 nm. The 1 : 1 apparent stability constant (Kc, M^{-1}) of TG44/β-CyD complex was calculated from the initial straight line portion of the phase solubility diagram according to the equation of Kc=slope/[So(1–slope)], where So is the intrinsic solubility of TG44.[5)]

Spectroscopic Studies Ultraviolet (UV) and fluorescence spectra of TG44 in the absence and presence of β-CyD (7.6×10^{-4}—3.8×10^{-3} M) in water were recorded with Shimadzu UV-2500PC (Kyoto, Japan) and Shi-

* To whom correspondence should be addressed. e-mail: uekama@ph.sojo-u.ac.jp

madzu RF-5300PC fluorescence spectrometers (Kyoto, Japan), respectively, at 25 °C and the drug concentrations were 2.2×10^{-5} M and 4.5×10^{-5} M for UV and fluorescence measurements, respectively. The 1 : 1 stability constant of TG44/β-CyD complex was determined by analyzing changes in UV and fluorescence intensities of TG44 at 278 nm and 370 nm (excitation wavelength 278 nm), respectively, by the Scott equation.[6] The Job's plot[7] was made by monitoring changes in UV intensity at 278 nm at a total concentration of the host and guest molecules of 5.0×10^{-5} M. Circular dichroism (CD) spectra were measured using a Jasco J-720 polarimeter (Tokyo, Japan) at 25 °C, and the concentrations of TG44 and β-CyD were 5.2×10^{-5} M and 5.2×10^{-5} M, respectively, in water. ^{1}H-NMR spectra of TG44 in deuterium oxide (D_2O) were measured with a Bruker AVANCE-400 instrument (400 MHz) at 30 °C. The proton signals of TG44 were assigned according to the ^{1}H-NMR spectrum and ^{1}H–^{1}H correlation spectroscopy (^{1}H–^{1}H COSY), and those of β-CyD were assigned according to the reference.[8]

Preparation of Solid Complexes TG44/β-CyD solid complexes were prepared by the cogrinding and spray-drying methods.[9] The ground products of TG44 and β-CyD in different molar ratios were prepared using a vibrational mill of a Chuo Kakohki Model MB-1 (Aichi, Japan) at a total mass amount of about 450 g, a grinding time of 10 h and temperature of 25 °C. The spray-dried products of TG44 and β-CyD were obtained by dissolving the components (10.0 g TG44 and 21.2 g β-CyD) in 1 mM hydrochloric acid solution (1000 ml), followed by spray-drying under the following conditions: an Okawara Model L-8 spray-drier (Kanagawa, Japan), a liquid flow rate of 100 ml/min, an air pressure of 1.0 kgf/cm^3, and inlet and outlet temperatures of 110 °C and 65 °C, respectively. Powder X-ray diffraction patterns were measured using a Rigaku Miniflex under the following conditions: Ni-filtered Cu–$K\alpha$ radiation (1.542 Å), a voltage of 30 kV, a current of 15 mA. The differential scanning calorimetry (DSC) analyses were carried out using a Rigaku Thermo Plus DSC 8230 (Tokyo, Japan) with a data analysis system (Thermo Plus 2, Japan), operated with a sample weight of 5 mg in an aluminium pan under nitrogen purge (50 ml/min) and a scanning rate of 5 °C/min.

Dissolution Studies The dissolution rate was measured according to the paddle method using tablets or the dispersed amount method using powders.[10] In the case of the paddle method, the test powder (1.5 g as the complex (TG44 0.48 g and β-CyD 1.02 g), <100 mesh) was compressed into cylindrical tablets (diameter 7 mm), using a Perkin-Elmer hard compressing machine (Kanagawa, Japan). The tablets were put into 100 ml water and the dissolution medium was stirred at 50, 100 or 200 rpm of the paddle at 25 °C. At appropriate intervals, an aliquot (2.0 ml) was withdrawn, filtered through a membrane filter (cellulose acetate, 0.2 μm), diluted appropriately with water/acetonitrile (1 : 1), and analyzed for TG44 spectrophometically at 277 nm. In the case of the dispersed amount method, a fixed amount (1.5 g as the complex, <100 mesh) of test powder was put into 100 ml water and stirred at 900 rpm and 25 °C. At appropriate intervals, an aliquot (2—4 ml) was filtered through the membrane filter and the filtrate was analyzed for TG44 by HPLC under the above conditions.

Results and Discussion

Solubility Studies Figure 1 shows the phase solubility diagram of TG44 with β-CyD in water at 25 °C, showing a A_L-type phase solubility diagram[5] where the solubility of the drug increased linearly as a function of β-CyD concentration (up to 8 mM). The solubility of TG44 increased according to the equation (Concn. of TG44, mol/l)$=0.309\times$(Concn. of β-CyD, mol/l)$+4.05\times10^{-5}$, giving the apparent 1 : 1 stability constant (Kc)$=12400\pm1000$ M^{-1} (see Experimental). As is apparent from Fig. 1, the low solubility of TG44 (0.036 ± 0.004 mM in water at 25 °C) increased about 70-folds in the presence of 8 mM β-CyD. On the other hand, the solubility of TG44 decreased above 10 mM β-CyD concentrations, probably due to the precipitation of the solid complex (data not shown). However, detailed solubility studies at higher β-CyD concentrations (10 mM) were not conducted, because it may take much longer time to convert completely the excess amount of TG44 to the solid complex, and the longer shaking more than 2 d induces the hydrolysis of ester groups of TG44.

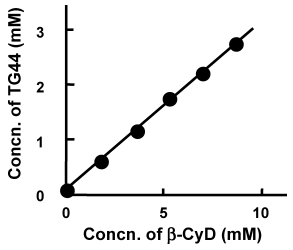

Fig. 1. Phase Solubility Diagram of TG44/β-CyD System in Water at 25 °C

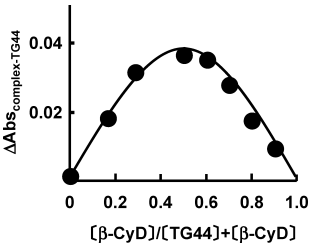

Fig. 2. Continuous Variation Plot of TG44/β-CyD System (Total Concentration of the Host and Guest: 5.0×10^{-5} M) in Water at 25 °C

Interaction in Aqueous Solution TG44 gave a UV spectrum with a maximum wavelength (λ_{max}) at 278 nm and an extinction coefficient (ε=26500) in water, and this absorption intensity was significantly increased by the addition of β-CyD. Further, TG44 gave a fluorescence spectrum with a maximum wavelength at 370 nm in water, when excited at 278 nm, and this fluorescence intensity was also increased by the addition of β-CyD. The continuous variation plot (Job's plot[7]) made by monitoring the UV intensity at λ_{max} gave a maximum at a 0.5 guest/(host+guest) molar ratio as shown in Fig. 2, indicating that TG44 forms the inclusion complex with β-CyD in a molar ratio of 1 : 1 in water. Therefore, the changes in UV and fluorescence intensities of TG44 as a function of β-CyD concentration were quantitatively analyzed according to the Scott equation (Eq. 1)[6] to obtain Kc value of the complex:

$$(a\cdot b)/d=1/(Kc\cdot I_c)+b/I_c \quad (1)$$

where a is the total concentration of TG44, b is the total concentration of β-CyD, I_c is the difference in intensity of UV or fluorescence for free and complexed TG44, and d is the change in intensity of UV or fluorescence of TG44 by the addition of β-CyD. The plots of the Scott equation gave the following first-order equations: (y)$=1.166\times10^{-4}$ (b)$+1.687\times10^{-8}$ (correlation coefficient (r)$=0.994$) for the UV change and (y)$=9.954\times10^{-8}$ (b)$+1.069\times10^{-11}$ (r=0.983) for the fluorescence change, where (y) stands for the left-hand term of Eq. 1. The Kc values of the complex obtained from the linear plots of Eq. 1 were 6900 ± 700 M^{-1} and 9300 ± 1000 M^{-1} for the UV and fluorescence methods, respectively, and the linear plots suggested the 1 : 1 complexation of TG44 with β-CyD under the present experimental conditions. In the CD spectra, a negative and positive Cotton effects were induced at 225 nm and 275 nm, respectively, by the addition of β-CyD, indicating TG44 molecule is included within the asymmetric locus of β-CyD cavity.

^{1}H-NMR spectroscopic studies were carried out to gain insight into the inclusion structure of TG44/β-CyD complex in

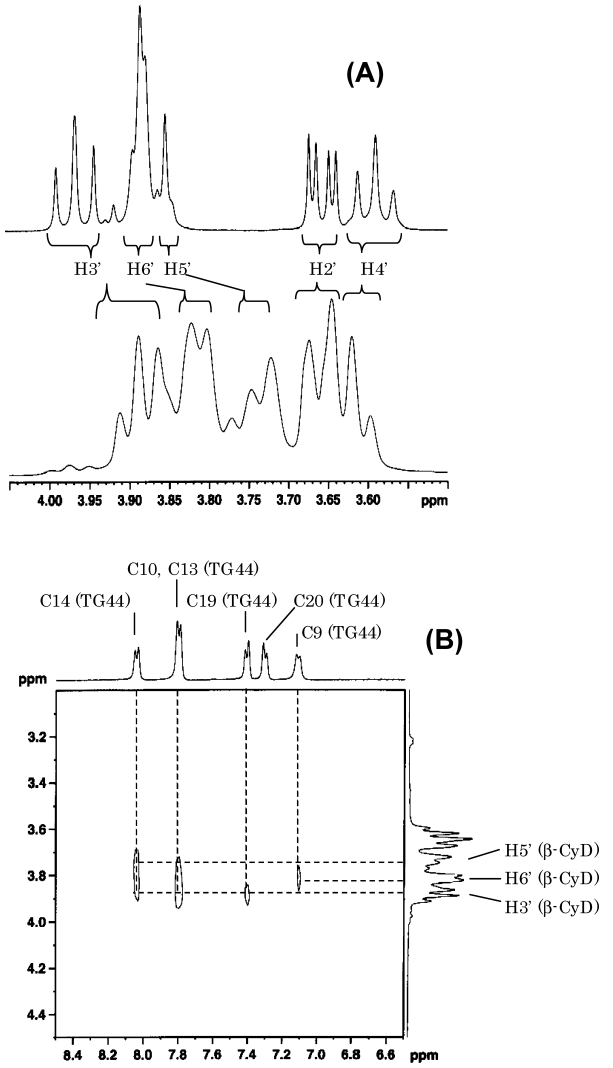

Fig. 3. (A) ^{1}H-NMR Spectra of β-CyD (3.0×10^{-3} M) in the Absence and Presence of TG44 (3.0×10^{-3} M) in D_2O and (B) Partial Contour Plot of ROESY Spectrum of TG44 (3.0×10^{-3} M)/β-CyD (3.0×10^{-3} M) System in D_2O

water. Figure 3A shows the ^{1}H-NMR spectra of β-CyD in the absence and presence of TG44 in D_2O. It was apparent that the ^{1}H-signals of the inner protons, H3′ and H5′ protons, of β-CyD shifted significantly upfield, and further the H6′ signal of the primary alcohol side shifted upfield. On the other hand, the outer H2′ and H4′ protons changed negligibly or only slightly. These results suggested that TG44 molecule is embedded in the β-CyD cavity, and the upfiled shift of the inner H3′ and H5′ protons of β-CyD is induced by the ring-current effect of the aromatic moieties of TG44 molecule.[11] The primary H6′ proton may be magnetically affected by a part of the guest molecule extruded from the cavity, because TG44 is structurally a relatively long-shaped molecule. Figure 3B shows a partial contour plot of ROESY spectrum of TG44/β-CyD system in D_2O, where the protons of the biphenyl moiety of TG44 gave cross peaks with the inner H3′ and H5′ protons of β-CyD, *i.e.*, both inner H3′ and H5′ protons of β-CyD gave the cross peaks with the C10, C13 and C14 protons of TG44. These results indicate that β-CyD includes preferably the central biphenyl moiety of TG44 molecule in water as shown in Fig. 4. Further, two inclusion

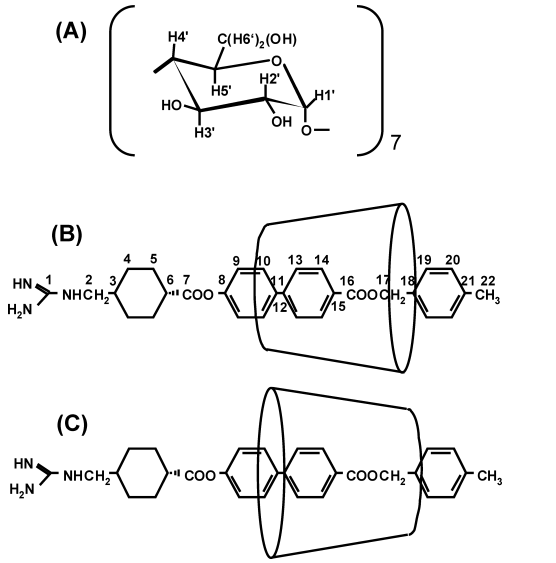

Fig. 4. (A) Structure of β-CyD and Its Proton Numbering, (B) Proposed Inclusion Mode of TG44/β-CyD Complex, the Methylbenzene Moiety Entering from the Narrow Primary Hydroxyl Side of the β-CyD Cavity and the Carbon Numbering of TG44 and (C) Proposed Inclusion Mode of TG44/β-CyD Complex, the Methylbenzene Moiety Entering from the Wider Secondary Hydroxyl Side of the β-CyD Cavity

modes of the guest may be possible, the methylbenzene moiety entering from both wider secondary hydroxyl and narrow primary hydroxyl sides of the cavity, where the population of the latter mode (Fig. 4B) may be higher than that of the former mode (Fig. 4C), because 1. the H3′ proton located at the wider rim of β-CyD gave the cross peak with the C19 proton of the guest molecule and 2. the H6′ proton of the primary hydroxyl group located at the narrow side gave the cross peak with the C9 proton of the guest molecule.

Interaction in the Solid State The inclusion complexation of TG44 with β-CyD in the solid state was investigated by powder X-ray diffractometry and DSC measurements. Figure 5A shows thermograms of TG44/β-CyD complexes prepared by the cogrinding method in different molar ratios of the host and guest molecules. TG44 alone showed two endothermic peaks at 153 °C and 184 °C, where the former peak is attributable to the phase change from the solid to liquid crystal phases because TG44 forms liquid crystals between about 150—180 °C as is apparent from its chemical structure, *i.e.,* biphenyl skeleton substituted with hydrophobic moieties at both *para*-position. The latter peak is due to the melting of the liquid crystal of TG44. With increasing the ratio of β-CyD in the complex, these endothermic peaks shifted to lower temperature with decrease in its intensity, and disappeared almost completely at the guest/host molar ratio of 1 : 1. Giordano *et al.* reported that the stoichiometry of solid CyD complexes can be determined by DSC measurements of the complexes containing excess amounts of guest, where the free mole fraction of guest (FGMF) is expressed by Eq. 2[12]

$$\mathrm{FGMF}=\mathrm{TGMF}(1+R)-R \quad (2)$$

where TGMF and R stand for the total mole fraction of guest and the stoichiometry (guest/host) of the complex, respectively. Therefore, DSC curves of TG44/β-CyD complexes with different molar ratios were analyzed by Eq. 2. Figure 5B

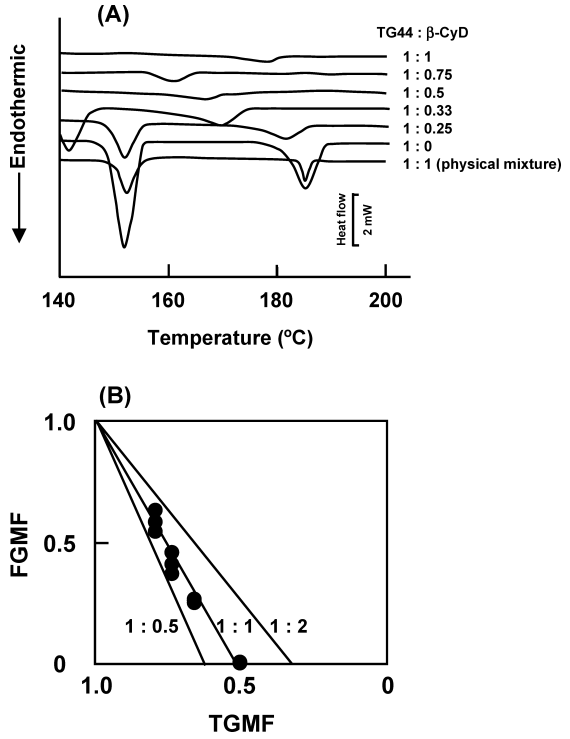

Fig. 5. (A) DSC Thermograms of Ground TG44/β-CyD Products with Different Molar Ratios and (B) Giordano Plot

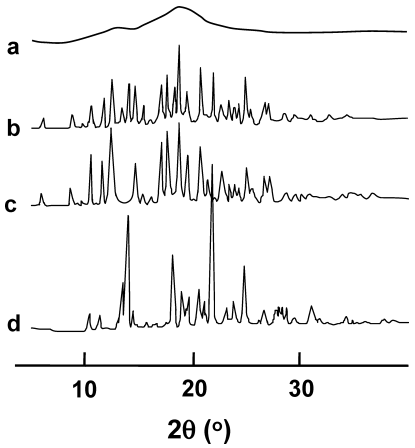

Fig. 6. Powder X-Ray Diffractograms of TG44/β-CyD System

a, TG44/β-CyD complex prepared by the cogrinding method in a molar ratio of 1 : 1; b, physical mixture of TG44 and β-CyD in a molar ratio of 1 : 1; c, β-CyD alone; d, TG44 alone.

shows the theoretical lines of Eq. 2, assuming $R=1:0.5$, $1:1$ and $1:2$, together with FGMF values obtained experimentally from the fusion enthalpy at 184 °C of TG44. It was apparent from Fig. 5B that the experimental data fitted well to the theoretical line of $R=1:1$, indicating a 1 : 1 stoichiometry of the solid TG44/β-CyD complex. Figure 6 shows powder X-ray diffractogram of the TG44/β-CyD complex prepared by the cogrinding method in a molar ratio of 1 : 1, in comparison with those of each component and their physical mixture. TG44 gave diffraction peaks at $2\theta=13.6°$, 21.2°, 22.0°, 25.0° and 28.8°. The physical mixture of TG44 and β-CyD gave the superimposed diffractogram of that of each component. On the other hand, these diffraction peaks were

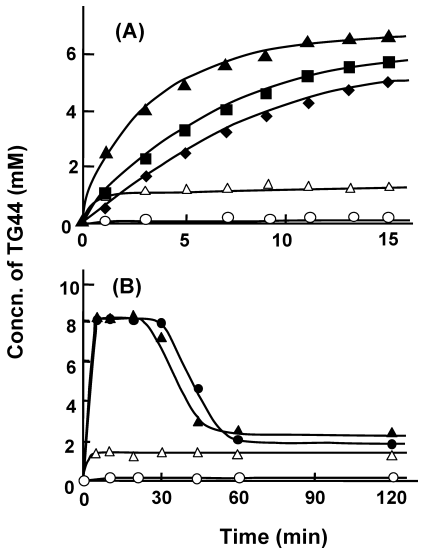

Fig. 7. (A) Dissolution Profiles of Ground TG44/β-CyD Complex (1.5 g, <100 mesh) and Their Physical Mixture with a 1 : 1 Molar Ratio in 100 ml Water, Measured by the Paddle Method at 50, 100 and 200 rpm at 25 °C

◆, complex at 50 rpm; ■, complex at 100 rpm; ▲, complex at 200 rpm; △, physical mixture of TG44 and β-CyD in a 1 : 1 molar ratio at 200 rpm; ○, TG44 alone at 200 rpm.

(B) Dissolution Profiles of Ground and Spray-Dried TG44/β-CyD Complexes (1.5 g, <100 mesh) and Their Physical Mixture with a 1 : 1 Molar Ratio, Measured by the Dispersed Amount Method at 900 rpm in Water (100 ml) at 25 °C

●, ground complex; ▲, spray-dried complex; △, physical mixture of TG44 and β-CyD in 1 : 1 Molar Ratio; ○, TG44 alone. The values are the averages of 2–3 experiments, which coincided with each other within 5%.

completely disappeared in the 1 : 1 solid β-CyD complex, giving a halo-pattern. The same results were obtained for the complex prepared by spray-drying method. These results indicated that TG44 forms the 1 : 1 solid complex with β-CyD in the solid state and the resulting complex is in an amorphous state. In solid state two-dimensional ^{13}C–^{1}H heteronuclear correlation NMR spectroscopic studies of the TG44/β-CyD complex, we observed clear correlation peaks between the carbon signals of the biphenyl moiety of TG44 and the inner H3 and H5 protons of β-CyD, suggesting that the host molecule includes preferably the biphenyl moiety of the guest molecule.[13)]

Dissolution Behavior The solid TG44/β-CyD complexes in a molar ratio of 1 : 1 were prepared and the dissolution behavior of the complexes in water was investigated. Figure 7A shows dissolution profiles of TG44/β-CyD complex from tablets, measured by the paddle method at paddle-rotating speeds of 50, 100 and 200 rpm. The complex was prepared by the cogrinding method. It is apparent that the dissolution rate of the complex was much faster than that of the drug alone, and the dissolution rate of the complex increased with the rotation speed of the paddle. Figure 7B shows the dissolution profiles of powders of TG44/β-CyD complexes prepared by the cogrinding and spray-drying methods, in comparison with that of the physical mixture of TG44 and β-CyD in the same molar ratio. The dissolution rate was measured by the dispersed amount method[10)] at a high rotating speed of 900 rpm of the paddle. It is apparent that both complexes rapidly dissolved and maintained the high supersaturated concentration (about 5 times higher than

the solubility of the corresponding physical mixture) of the drug at least for about 30 min even under the high rotation speed of 900 rpm. On the other hand, the physical mixture of TG44 and β-CyD gave the only slight increase in the dissolution at the initial stage (within 10 min), after which no increase in the dissolution was observed. The fast dissolution of TG44 followed by the supersaturation may be attributable to the amorphous complex formation of the drug with β-CyD. The decrease in the drug concentration after 30 min may be due to the dissociation of the complex into each component, resulting in precipitation of TG44 crystals. These results indicated that β-CyD not only improves the solubility and dissolution properties of TG44, but also maintains the supersaturated state of the drug in water. These dissolution properties of the complex clearly reflected in the *in-vivo* antimicrobiological activity, *i.e.,* when the 1 : 1 complex (938 mg/kg) was orally administered to *H. pylori* infected gerbils three times a day, *H. pylori* in all of 5 animals were completely eradicated, whereas the administrations of TG44 alone (300 mg/kg) and β-CyD alone (638 mg/kg) failed to eradicate the microbiology, which will be reported elsewhere.

Conclusion

The present results indicated that TG44 forms the inclusion complex with β-CyD in a molar ratio of 1 : 1 in aqueous solution and in the solid state. The TG44/β-CyD solid complex has the fast-dissolving property and maintains the high supersaturated concentration of the drug in water. The high *in-vivo* antimicrobial activity of TG44/β-CyD complex to *H. pylori*, compared with that of TG44 alone, may be attributable to its fast-dissolving property and its ability to maintain the supersaturated state in the gastric fluid, *i.e.,* the prolonged exposure afforded by the higher supersaturated state of TG44 could result in an increased antimicrobial activity against *H. pylori*, although other various factors such as dispersion property and residence time of the solid complex in the stomach and effects of β-CyD on *H. pylori* membranes and its permeation property may contribute to the enhanced antimicrobial activity. The present results will provide useful information for development of new *H. pylori* eradicating agents and their oral preparations.

References

1) Peura D. A., Graham D. Y., *Am. J. Gastroenterol.*, **89**, 1137—1139 (1994).
2) Dore M. P., Osato M. A., Realdi G., Mura I., Graham D. Y., Sepulveda A. R., *J. Antimicrob. Chemother.*, **43**, 47—54 (1999).
3) D.-Ossenkopp Y. J., Herscheid A. J., Pot R. G. J., Kuipers E. J., Kusters J. G., V.-Grauls C. M. J. E., *J. Antimicrob. Chemother.*, **43**, 511—515 (1999).
4) Kamoda O., Anzai K., Mizoguchi J., Shiojiri M., Yanagi T., Nishino T., Kamiya S., *Antimicrob. Agents Chemother.*, **50**, 3062—3069 (2006).
5) Higuchi T., Connors K. A., *Adv. Anal. Chem. Instr.*, **4**, 117—212 (1965).
6) Scott R. L., *Rec. Trav. Chim.*, **75**, 787—789 (1956).
7) Job P., *Ann. Chem.*, **9**, 113—203 (1928).
8) Saka W., Yamamoto Y., Inoue Y., Chujo R., Takahashi K., Hattori K., *Bull. Chem. Soc. Jpn.*, **63**, 3175—3182 (1990).
9) El-Gendy G. A., Terada K., Yamamoto K., Nakai Y., *Int. J. Pharm.*, **31**, 25—31 (1986).
10) Nogami H., Nagai T., Yotsuyanagi Y., *Chem. Pharm. Bull.*, **17**, 499—509 (1969).
11) Bender M. L., Komiyama M., "Cyclodextrin Chemistry," Springer-Verlag, Berlin, 1978.
12) Giordano F., Bruni G., Bettinetti G. P., *J. Thermal Anal.*, **38**, 2683—2691 (1992).
13) Anzai K., Kono H., Mizoguchi J., Yanagi T., Hirayama F., Arima H., Uekama K., *Carbohydr. Res.*, **341**, 499—506 (2006).