Carbohydrate Research, 192 (1989) 351–356
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

Note

Interaction of nitroglycerin with 6-*O*-α-maltosylcyclomaltoheptaose

KAZUO TOMONO, HIROKO GOTOH, MAKOTO OKAMURA, TAROH SAITOH,

Department of Pharmaceutics, College of Science and Technology, Nihon University, 8-14, Kanda Surugadai 1-chome, Chiyoda-ku, Tokyo 101 (Japan)

HARUHISA UEDA*, AND TSUNEJI NAGAI

Faculty of Pharmaceutical Sciences, Hoshi University, 4-41, Ebara 2-chome, Shinagawa-ku, Tokyo 142 (Japan)

(Received January 3rd, 1989; accepted for publication, May 2nd, 1989)

The application of cyclomaltoheptaose (β-cyclodextrin, βCD) in the pharmaceutical field is limited by its relatively low aqueous solubility. Many kinds of branched CDs have been studied[1] because they are significantly more soluble in water than the natural CDs, and some have found pharmaceutical applications particularly in injection preparations[2,3]. We now report on the complexation of the vasodilator (coronary) glycerol trinitrate (nitroglycerin, TNG) with 6-*O*-α-maltosyl-β-cyclodextrin (G_2-βCD) in comparison with the complexation of βCD[4,5] and other βCD derivatives[6].

The formation of a complex between TNG and G_2-βCD in aqueous solution was studied using the solubility method. Fig. 1 shows an equilibrium-phase solubility diagram obtained for the TNG/G_2-βCD system at 5°. The plot shows a typical A_L-type solubility curve [*cf.* the B_S-type curve for the TNG/βCD system[4,5] and the A_P-type curve of the TNG/water-soluble βCD polymer (CDPS) system[6]]. The apparent stability constant (K') of the TNG/G_2-βCD system, assuming that a 1:1 complex was formed, was 67.95 M^{-1} at 5° (*cf.* 29.4 M^{-1} for the TNG/CDPS complex, and 181 M^{-1} for the TNG/βCD complex).

^{1}H-N.m.r. techniques were used to elucidate the interactions of TNG/G_2-βCD in aqueous solution (see Table I). On complexation, four signals of TNG were shifted to higher field and two were shifted to lower field. The chemical shift data were similar for the TNG/G_2-βCD and TNG/βCD systems, and suggest that the TNG molecule is included partially in the cavity of G_2-βCD and βCD.

*Author for correspondence.

0008-6215/89/$ 03.50

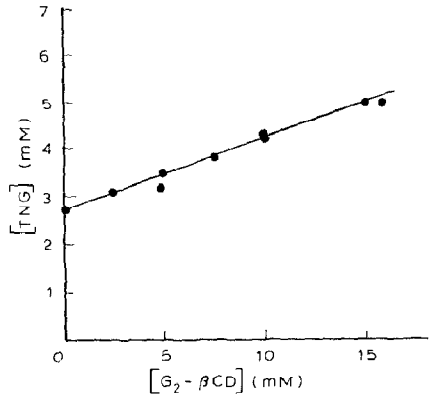
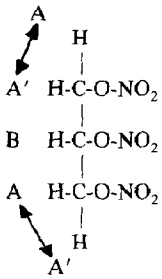


Fig. 1. Phase solubility diagram of TNG/G_2-βCD in water at 5°.

TABLE I

^{1}H-N.M.R. DATA FROM TNG IN PRESENCE OR ABSENCE OF βCD OR G_2-βCD[a]



Proton[b]	*Chemical shift (p.p.m.)*				
	TNG (δ_0)	*Plus CD* (δ_1)	($\delta_1 - \delta_0$)	*Plus* G_2-β*CD* (δ_2)	($\delta_2 - \delta_0$)
A	4.5963[c]	4.5872	−0.0091	4.5847	−0.0116
	4.6293[c]	4.6201	−0.0092	4.6180	−0.0113
A′	4.7407[c]	4.7444	0.0037	4.7450	0.0043
	4.7737[c]	4.7774	0.0037	4.7780	0.0043
B	5.5566[d]	5.5517	−0.0049	5.5499	−0.0067

[a]βCD, 0.01M; G_2-βCD, 0.01M; TNG, 0.005M; in D_2O. [b]The protons A and A′ were not assigned individually, because of their mobility and symmetrical structure. [c]Centre point of a doublet. [d]Central main peak of multiplet.

The complexation of TNG with G_2-βCD and βCD by the grinding method is shown in Fig. 2. The velocity for G_2-βCD was slightly lower, possibly because of steric hindrance by the maltose moiety[7]. On the basis of the results of differential scanning calorimetry (Fig. 3), there was no significant difference between the

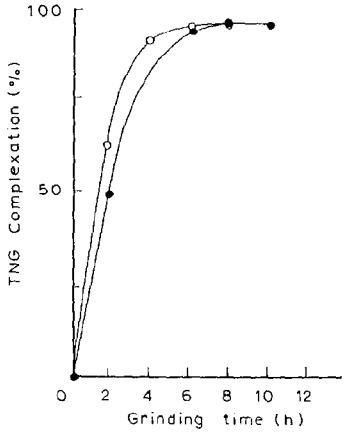


Fig. 2. Effect of time of grinding on the complexation of TNG with βCD (○) and G_2-βCD (●).

TNG/G_2-βCD and TNG/βCD complexes formed by the grinding and solvent evaporation methods.

The stabilities of the TNG/G_2-βCD and TNG/βCD complexes at 37° are shown in Fig. 4 and those at 37°/3 mmHg in Fig. 5. The volatility of TNG was reduced greatly on complexation and there was no significant difference between the complexes.

The interaction of TNG with G_2-βCD in 0.02M sodium hydroxide was studied in order to evaluate the effect on the degradation of TNG using parameter K, based on semi-log plots of $\ln(C/C_0)$–time. As shown in Fig. 6, the rate of degrada-

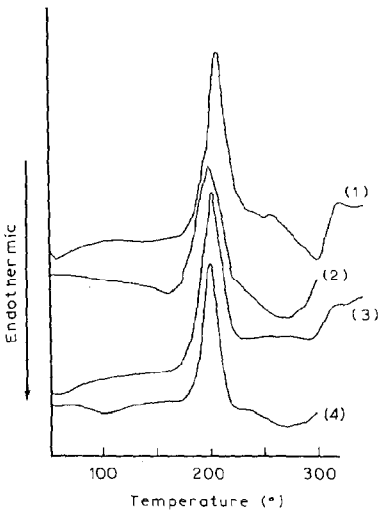


Fig. 3. Differential scanning calorimetry at 5°/min of TNG/G_2-βCD prepared by evaporation (1) and grinding (2) methods, and TNG/βCD prepared by evaporation (3) and grinding (4) methods.

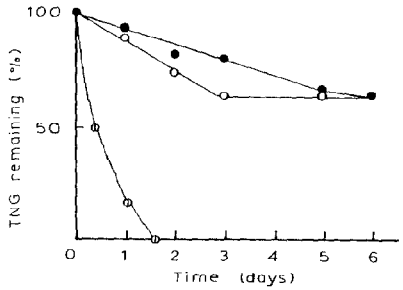


Fig. 4. Loss of TNG at 37° from TNG/βCD (○), TNG/G_2-βCD (●), and TNG/pullulan (Ⓓ) (the pullulan was used as an inactive additive).

tion increased in the series βCD<G_2-βCD<DM-βCD<CDPS. Experiments at various temperatures and the activation energies of TNG degradation were calculated from the Arrhenius plot. Fig. 7 shows the relationship between activation energy and stability constant. The acceleration of phenyl ester cleavage by CDs has been explained on the basis that the secondary hydroxyl groups are the active sites[8]. In TNG/CD systems, however, the hydroxyl groups seem not to play an important role in the degradation of TNG because reduction of the number of hydroxyl groups increased the rate of degradation. The mechanism of degradation of TNG by CDs is being investigated further. The loss of isosorbide dinitrate in medications is reduced[9] by βCD. The data presented herein demonstrate that the addition of any kind of CD in alkaline solution in order to prevent the loss of TNG will promote the degradation of TNG.

EXPERIMENTAL

Materials. — G_2-βCD, kindly donated by the Nikken Chemical Co. Ltd., was a white powder that was 100% pure (h.p.l.c.), had $[\alpha]_D^{20}$ +167° (water), and a solubility in water (20°) of ~1.7 g/mL. βCD and DM-βCD [hexakis(2,6-di-*O*-methyl)cyclomaltoheptaose] were supplied by Nihon Shokuhin Kako Co. Ltd.

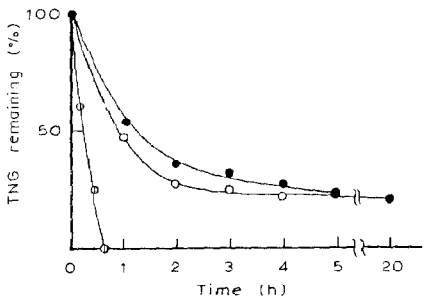


Fig. 5. Loss of TNG at 37°/3 mmHg from TNG/βCD (○), TNG/G_2-βCD (●), and TNG/pullulan (Ⓓ).

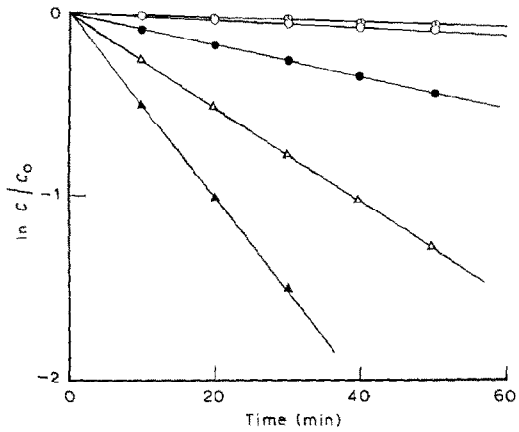


Fig. 6. Degradation of TNG at 27° in 0.02M NaOH: alone (①) and in the presence of βCD (○), G_2-βCD (●), CDPS (▲), and DM-βCD (△).

Water-soluble CD polymer was prepared as described[10]. Nitroglycerin was gift from Nippon Kayaku Co. Ltd.

Solubility studies. — The method of Higuchi and Connors[11] was used. The concentration of TNG in solution was determined by h.p.l.c.

Preparation of inclusion compounds. — (*a*) TNG and G_2-βCD or βCD (molar ratio 1:1) were added to water, and each mixture was concentrated using a rotary evaporator.

(*b*) TNG and G_2-βCD or βCD (molar ratio 1:1) were ground[12] at 85 r.p.m. for an appropriate time, using a Boll mill (Irie Shokai, Model V-1).

Physical methods. — Differential scanning calorimetry was performed at 5°/min with a Rigaku Denki TAS-100 instrument.

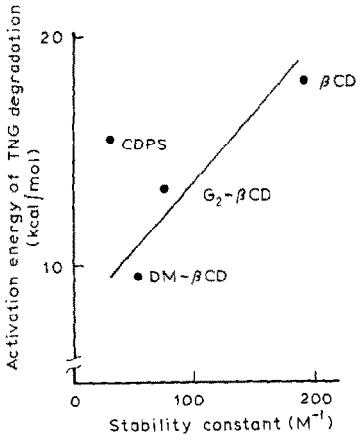


Fig. 7. Relationship between the activation energy for the degradation of TNG in 0.02M NaOH and the stability constants of the TNG/CD systems.

^{1}H-N.m.r. spectra (400 MHz) were recorded at 37° with a JEOL GX-400 spectrometer (external Me_4Si).

Kinetics of the degradation of TNG. — Aliquots (5 mL) of 1.06mM TNG and 2.11mM G_2-βCD in 0.02M NaOH were kept in sealed vessels at 17°, 27°, and 37° (±0.5°). At appropriate intervals, the concentrations of TNG in solution were determined by h.p.l.c. using a Jasco Model 800 system equipped with a variable-wavelength u.v. detector at 210 nm, and a column (4.5 mm × 15 cm) of Chemcosorb® ODS-H (7 μm) (Chemko Scientific Co. Ltd.) with aqueous 50% methanol as the mobile phase.

ACKNOWLEDGMENTS

We thank Mr. Y. Kogure (Nikken Chemicals Co. Ltd.) for supplying the G_2-βCD, and Dr. H. Ninomiya (Nippon Kayaku Co. Ltd.) for supplying the nitroglycerin.

REFERENCES

1 M. Yamamoto, A. Yoshida, F. Hirayama, and K. Uekama, *Int. J. Pharm.*, 49 (1989) 163–171.
2 K. Koizumi, Y. Okada, Y. Kubota, and T. Utamura, *Chem. Pharm. Bull.*, 35 (1987) 3413–3418.
3 Y. Okada, Y. Kubota, K. Koizumi, S. Hizukuri, T. Ohfuji, and K. Ogata, *Chem. Pharm. Bull.*, 36 (1988) 2176–2185.
4 K. Yamaoka, Y. Yamagishi, K. Takayama, N. Nambu, and T. Nagai, *Yakuzaigaku*, 41 (1981) 231–236.
5 K. Tomono, H. Ueda, T. Saitoh, and T. Nagai, *Yakuzaigaku*, 47 (1987) 133–140.
6 K. Tomono, H. Ueda, T. Saitoh, and T. Nagai, *Yakuzaigaku*, 48 (1988) 9–16.
7 Y. Nakanishi, Y. Ishizuka, and S. Kobayashi, *Proc. Cyclodextrin Symp. Jpn., 6th, Chiba, 1987*, p. 6.
8 R. L. Van Etten, J. F. Sebastian, G. A. Clowes, and M. L. Bender, *J. Am. Chem. Soc.*, 89 (1967) 3242–3253.
9 S. Nakajima, K. Kawano, K. Nakazawa, and K. Suzuki, *Yakuzaigaku*, 47 (1987) 248–253.
10 E. Fenyvesi, M. Szilai, B. Zadon, J. Szejtli, and F. Tudos, in J. Szejtli (Ed.), *Proc. Int. Symp. Cyclodextrins, 1st*, Reidel, Dordrecht, 1981, pp. 345–356.
11 T. Higuchi and K. A. Connors, *Adv. Anal. Chem. Instrum.*, 4 (1965) 117–212.
12 Y. Nakai, K. Yamamoto, K. Terada, and K. Akimoto, *Chem. Pharm. Bull.*, 32 (1984) 685–691.