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PHYSICOCHEMICAL PROPERTIES OF LARGE-RING CYCLODEXTRINS (CD_{10}~CD_{17}) [1]

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Cyclomaltodecaose (CD_{10}), cyclomaltoundecaose (CD_{11}), cyclomaltododecaose (CD_{12}), cyclomaltotridecaose (CD_{13}), cyclomaltotetradecaose (CD_{14}), cyclomaltopentadecaose (CD_{15}), cyclomaltohexadecaose (CD_{16}) and cyclomaltoheptadecaose (CD_{17}) are cyclic oligosaccharides composed of 10, 11, 12, 13, 14, 15, 16 and 17 D-glucose units, respectively. This report describes the physicochemical properties of CD_{10}, CD_{11}, CD_{12}, CD_{13}, CD_{14}, CD_{15}, CD_{16} and CD_{17}, in terms of aqueous solubility, surface tension, specific rotation and acid-catalyzed hydrolysis.

Цикломальтодекаоза (CD_{10}), цикломальтоундекаоза (CD_{11}), цикломальтододекаоза (CD_{12}), цикломальтотридекаоза (CD_{13}), цикломальтотетрадекаоза (CD_{14}), цикломальтопентадекаоза (CD_{15}), цикломальтогексадекаоза (CD_{16}) и цикломальтогептадекаоза (CD_{17}) - циклические олигосахариды, состоящие из 10, 11, 12, 13, 14, 15, 16 и 17 D-глюкозных единиц соответственно. В сообщении описываются физико-химические свойства CD_{10}, CD_{11}, CD_{12}, CD_{13}, CD_{14}, CD_{15}, CD_{16} и CD_{17} циклодекстринов с точки зрения растворимости в воде, поверхностного напряжения, специфического вращения и кислото-катализируемого гидролиза.

Ցիկլոմալտոդեկաոզան (CD_{10}), ցիկլոմալտոունդեկաոզան (CD_{11}), ցիկլոմալտոդոդեկաոզան (CD_{12}), ցիկլոմալտոտրիդեկաոզան (CD_{13}), ցիկլոմալտոտետրադեկաոզան (CD_{14}), ցիկլոմալտոպենտադեկաոզան (CD_{15}), ցիկլոմալտոհեքսադեկաոզան (CD_{16}) և ցիկլոմալտոհեպտադեկաոզան (CD_{17}) ցիկլիկ օլիգոսախարիդներ են, կազմված համապատասխանաբար 10, 11, 12, 13, 14, 15, 16 և 17 D-գլյուկոզի միավորներից: Այս հաղորդության մեջ նկարագրվում են CD_{10}, CD_{11}, CD_{12}, CD_{13}, CD_{14}, CD_{15}, CD_{16} և CD_{17} ցիկլոդեքստրինների ֆիզիկաքիմիական առանձնահատկությունները ջրում լուծելիության, մակերեսային լարվածության, յուրահատուկ պտտման և թթվով կատալիզվող հիդրոլիզի տեսանկյունից:

Introduction

Cyclodextrin (CD) is a cyclic oligosaccharide produced by cyclodextrin glucanotransferase (CGTase), and α-CD (CD_6), β-CD (CD_7), γ-CD (CD_8) and their derivatives have been well studied. With regard to large-ring CDs (LR-CDs) composed of more than 9 D-glucose units, we have reported a method for preparation and purification from commercially available CD powder [2-5]. In particular, we characterized the physicochemical properties and inclusion complex formation abilities of CD_9 composed of 9 D-glucose units with several guest molecules [2,6,7]. Furthermore, inclusion complex formation constants of CD_{10}, CD_{11}, CD_{12} and CD_{13}, composed of 10, 11, 12 and 13 D-glucose units, respectively, were determined by capillary zone electrophoresis [8]. However, it is difficult to investigate the physicochemical properties and complexation abilities of

LR-CDs with more than 10 D-glucose units in detail, because of the low yields of LR-CDs prepared using commercially available CD powder as the starting material. Recently, it was reported that LR-CDs were preferentially produced in the initial stage of CGTase cyclization reaction and were subsequently converted into smaller CDs [9]. In this study, LR-CD (CD_{10}~CD_{17}) were prepared and purified from CD powder produced by the initial action of CGTase mentioned above. Their physicochemical properties, i. e. aqueous solubility, surface activity, specific rotation, and acid-catalyzed hydrolysis rate, were elucidated in detail in comparison with those of conventional α-, β-, γ-CD and other LR-CDs (CD_9).

Materials and methods

Materials. α- and β-CD were gifts from Nihon Shokuhin Kako Co., Ltd. (Tokyo, Japan). γ-CD was supplied by Wacker Chemie GmbH (Munich, Germany). They were used after recrystallization from water. Other chemicals were obtained from commercial sources and were used without further purification. Milli-Q Water (Milford, MA, USA) was used in all experiments.

Method for production and purification of LR-CD (CD_{10}~CD_{17}). The production of LR-CD powder by the initial action of CGTase on synthetic amylose was carried out as described previously [9]. The CD mixture obtained was dissolved in water. Then, tetrachloroethane and bromobenzene were added and shaken at 4°C for 20h to remove α-, β- and γ-CD. After centrifugation, the supernatant was subjected to HPLC to separate CD_{10}, CD_{11}, CD_{12}, CD_{13}, CD_{14}, CD_{15}, CD_{16} and CD_{17}. HPLC consisted of two main steps using an ODS column and an amino column. The details of the purification conditions and identification of purified products were almost the same as described previously [2]. The yields of each LR-CD (CD_{10}~CD_{17}) were as follows: CD_{10}, 1.33%; CD_{11}, 1.20%; CD_{12}, 1.34%; CD_{13}, 1.01%; CD_{14}, 0.58%; CD_{15}, 0.39%; CD_{16}, 0.36%; CD_{17}, 0.35%.

Physicochemical properties of CDs [2,10,11]. *Solubility.* For the solubility measurement of CD_{10} and CD_{14}, saturated solutions of CD_{10} and CD_{14} were prepared according to the standard procedure at 25°C. The supernatants were subjected to HPLC on an ODS column (YMC-Pack ODS-AQ, 4.6Φ×250mm) with methanol-water as the mobile phase (3:97 for CD_{10}, 4:96 for CD_{14}) at a flow rate of 1.0mL/min at 30°C. The solubilities of CD_{11}, CD_{12}, CD_{13}, CD_{15}, CD_{16} and CD_{17} were determined using other methods. Water was carefully added to a glass vessel containing 100mg of LR-CDs. The quantity of water varied progressively from 0.01 to 0.1mL. The samples were vigorously shaken for 1min at 10min intervals at 25°C. The cycle was continued until CDs had dissolved completely. The total volume of water added was measured, and the saturated solubility was calculated.

Surface tension. Surface tension measurements were taken on a Wilhelmy surface tensiometer (Kyowa Kaimenkagaku Co., Ltd., Tokyo, Japan) with an accuracy of ± 0.2mN/m. The glass vessels used were treated with 20% sulfuric acid before each measurement.

Specific rotation. Specific rotation measurements were taken on a SEPA-200 digital polarimeter (Horiba, Kyoto, Japan) with an accuracy of ± 0.002. The digital polarimeter was calibrated with sucrose solution before measurement.

Acid-catalyzed hydrolysis of CDs. Aliquots of 100mg of each CD were dissolved in 5mL of 1mol/L HCl, and the reaction solution was heated in a water bath at 50°C. Samples of the reaction solution were taken at appropriate intervals and neutralized by addition of 1mol/L NaOH containing an internal standard for HPLC. The samples were determined quantitatively on HPLC. The internal standards used were as follows: γ-CD for measurement of α-CD, β-CD, CD_{10}, CD_{11}, CD_{12}, CD_{13}, CD_{14}, CD_{15}, CD_{16}; α-CD for measurement of CD_{17}; α-CD for measurement of γ-CD; and β-CD for measurement of CD_9. HPLC was conducted under the following conditions: column, YMC-Pack ODS-AQ (4.6Φ×250mm); eluent, methanol-water (3:97 or 4:96); flow rate, 1.0mL/min; column temperature, 30°C.

Results and discussion

Solubility. Table 1 lists the physicochemical properties of α-, β-, γ-CD and LR-CDs. The aqueous solubilities of CDs increased in the order of: β-CD < CD_{14} < CD_{10} < CD_9 < α-CD < γ-CD < CD_{15} ≈ CD_{16} ≈ CD_{17} < CD_{11} ≈ CD_{12} ≈ CD_{13}. The solubility of CD was suggested to be correlated with differences in structural flexibility [12]. The low solubility of β-CD may be a consequence of the rigid structure caused by intramolecular hydrogen bonds and its high crystal lattice energy [13]. In β-CD, all glucose residues are in *syn* orientation, forming systematic interglucose $O(3)_n \cdots O(2)_{n+1}$ hydrogen bonds. On the other hand, the molecular structure of CD_{14} is characterized by typical "band flip" in which diametrically opposed glucose residues are in *anti* rather than in the common *syn* orientation, this conformation being stabilized by interglucose $O(3)_n \cdots O(6)_{n+1}$ hydrogen bonds [5,14]. X-ray crystal structure analysis of CD_{10} also revealed that it had the band flip structure [15]. In addition, CD_{10} and CD_{14} are as readily crystallized from aqueous solution as β-CD [5,14,15]. The crystallization may be caused by intermolecular hydrogen bonds. Therefore, the relatively low solubilities of CD_{10} and CD_{14} may be a consequence of intramolecular and intermolecular hydrogen bonds.

The aqueous solubilities of CD_{15}, CD_{16} and CD_{17} were as high as those of CD_{11}, CD_{12} and CD_{13} in contrast to those of α-, β- and γ-CD. Intra- and intermolecular hydrogen bonds are difficult to form in CD_{11}, CD_{12}, CD_{13}, CD_{15}, CD_{16} and CD_{17}. In general, the nucleation rates, which must precede crystal growth, of sugars are very low, so supersaturated solutions of sugars are often syrup-like liquids [16,17]. The solubilities of CD_{11}, CD_{12}, CD_{13}, CD_{15}, CD_{16} and CD_{17} could not be measured precisely, because the solubility behaviors of CD_{11}, CD_{12}, CD_{13}, CD_{15}, CD_{16} and CD_{17} were similar to those of sugars.

Table 1. Physicochemical Properties of CDs

	Glucose unit	Aqueous[a] solubility (g/100mL)	Surface[a] tension (mN/m)	Specific rotation $[\alpha]_D^{25}$	Half-life of[b] ring opening (h)
α-CD	6	14.5[c]	72	+147.8[d]	33[d]
β-CD	7	1 85[c]	73	+161.1[d]	29[d]
γ-CD	8	23.2[c]	73	+175.9[d]	15[d]
CD_9	9	8 19[c]	73	+187.5[d]	4.2[d]
CD_{10}[d]	10	2.82	72	+204 9	3 2
CD_{11}[d]	11	>150	72	+200 8	3 4
CD_{12}[d]	12	>150	72	+197.3	3.7
CD_{13}[d]	13	>150	72	+198 1	3.7
CD_{14}	14	2 30	73	+199.7	3.6
CD_{15}	15	>120	73	+203.9	2 9
CD_{16}	16	>120	73	+204.2	2.5
CD_{17}	17	>120	72	+201.0	2.5

a) Observed at 25 C
b) In 1mol/L at 50 C
c) I.Miyazawa, H. Ueda, H. Nagase, T. Endo, S. Kobayashi, T. Nagai. Eur. J. Pharm., 3, 153-162 (1995).
d) Presednted in part at the 17th Cyclodextrin Symposium of Japan, Osaka, Japan, October 1999, procceding, p. 69-70.

Surface tension. CD_{10}, CD_{11}, CD_{12}, CD_{13}, CD_{14}, CD_{15}, CD_{16} and CD_{17} showed no surface activity. We assumed that the surface activity of LR-CDs, composed of more than 18 D-glucose units, does not change with increasing D-glucose content.

Specific rotation. The specific rotation increased in the order : α-CD < β-CD < γ-CD < CD_9 < CD_{12} ≈ CD_{13} ≈ CD_{14} ≈ CD_{11} ≈ CD_{17} < CD_{15} ≈ CD_{16} ≈ CD_{10}. In homologous compounds with the different molecular weight, the evaluation of molecular rotation is suited for their rotatory power [18]. Molecular rotation [ϕ] is given by:

$$[\phi]_\lambda^t = \frac{M}{100}[\alpha]_\lambda^t$$

where M is molecular weight, [α] is specific rotation, t is temperature and λ is wavelength. Figure 1 shows the calculated molecular rotations of CDs (CD_6~CD_{17}). van't Hoff proposed "optical superposition", in which the rotation of optically active substances with several asymmetric carbon atoms is expressed with the algebraic sum of optical rotation contributed by individual asymmetric carbon atoms in the molecule. If there are no structural differences affecting optical rotatory power, molecular rotation must increase linearly with the increase in number of glucose units. In Figure 1, the variation in molecular rotation of CDs with number of glucose units is expressed as two straight lines. One is plotted against α-, β-, γ-CD and CD_9, and the other is plotted against CD_{10}~CD_{17}. Therefore, it was suggested that there are specific structural changes such as band flip in the CD_{10}~CD_{17} molecule, that do not occur in α-, β-, γ-CD and CD_9.

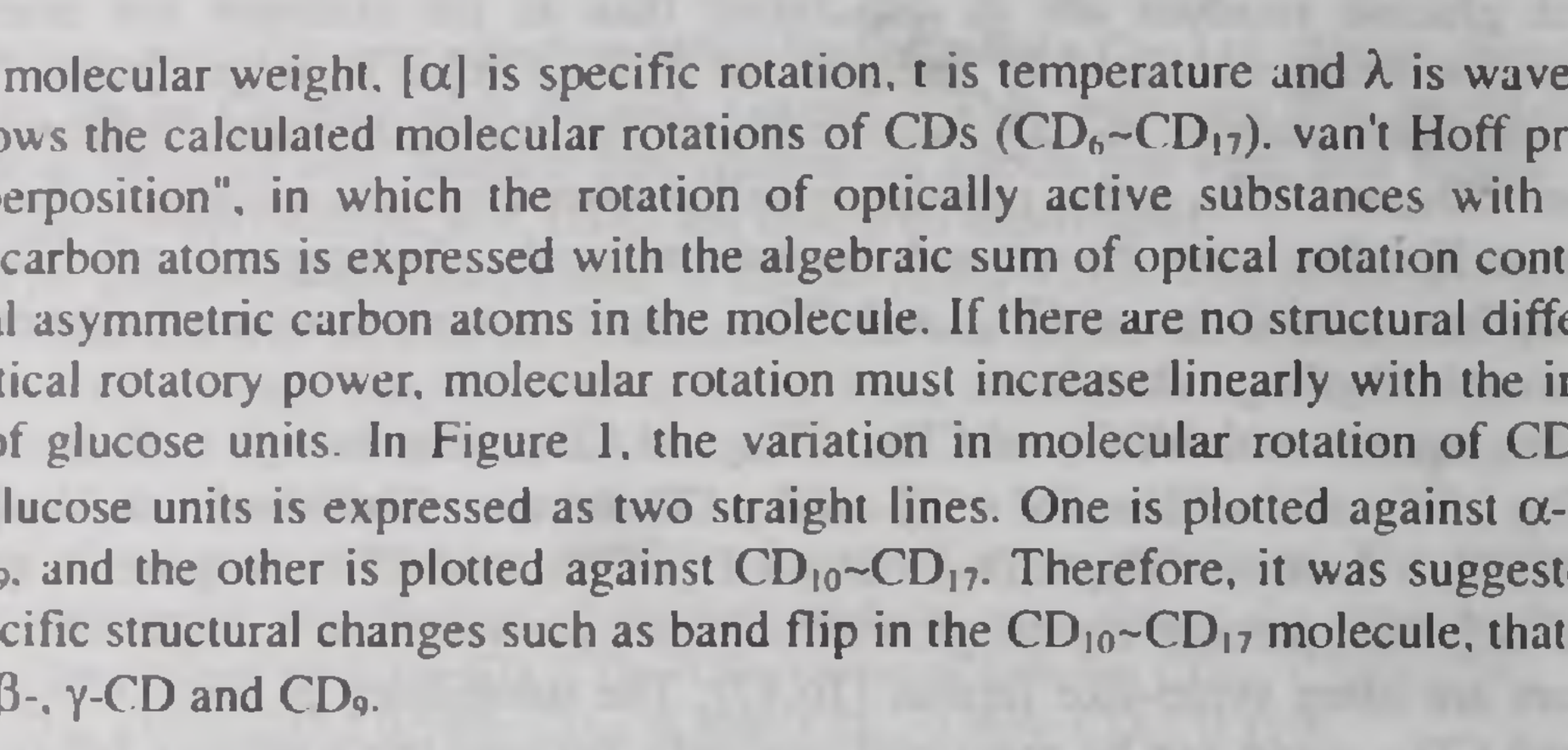

Figure 1. Variation in Molecular Rotation of CDs with Number of Glucose Units

^{13}C-NMR spectroscopy on CD with 6 ~ 17 D-glucose units shows only one sharp signal for each of the six glucose carbon atoms indicating the glucose residues of the individual molecules [3]. In the chemical shifts of C1 and C4 concerned with binding to two glucose residues, the signals for CD with 10 ~ 17 D-glucose units were shifted further upfield than those of α-, β- and γ-CD [3,19]. These results suggested that there are specific structures in CD_{10}~CD_{17}. Furthermore, X-ray analyses showed that there were band flip structures in CD_{10}, CD_{14} and CD_{26} [5,12,15]. Thus, we considered that there are band flip structures in LR-CDs composed of more than 10 D-glucose units, other than CD_{10}, CD_{14} and CD_{26}.

Acid-catalyzed hydrolysis.

Figure 2 shows the acid-catalyzed hydrolysis of CDs. The acid-catalyzed hydrolysis rates of CD_{14}, CD_{15}, CD_{16} and CD_{17} were as fast as those of the other LR-CDs (CD_9~CD_{13}) in contrast to those of α-, β- and γ-CD. There were no marked differences among LR-CDs (CD_{10}~CD_{17}). The above results showed that increases in simple disorder and decomposition sites (α-1,4 linked parts) of their intact rings with increasing number of the D-glucose units did not markedly influence the breakdown of LR-CDs. The hydrolysis rates of CD_{11}, CD_{12}, CD_{13} and CD_{14} were lower than that of CD_{10}. Saenger et al. proposed that the excessive steric strain of the CD ring was relieved by the band flip structures [12], so the steric strains of CD_{11}, CD_{12}, CD_{13} and CD_{14} were probably weaker than that of CD_{10}.

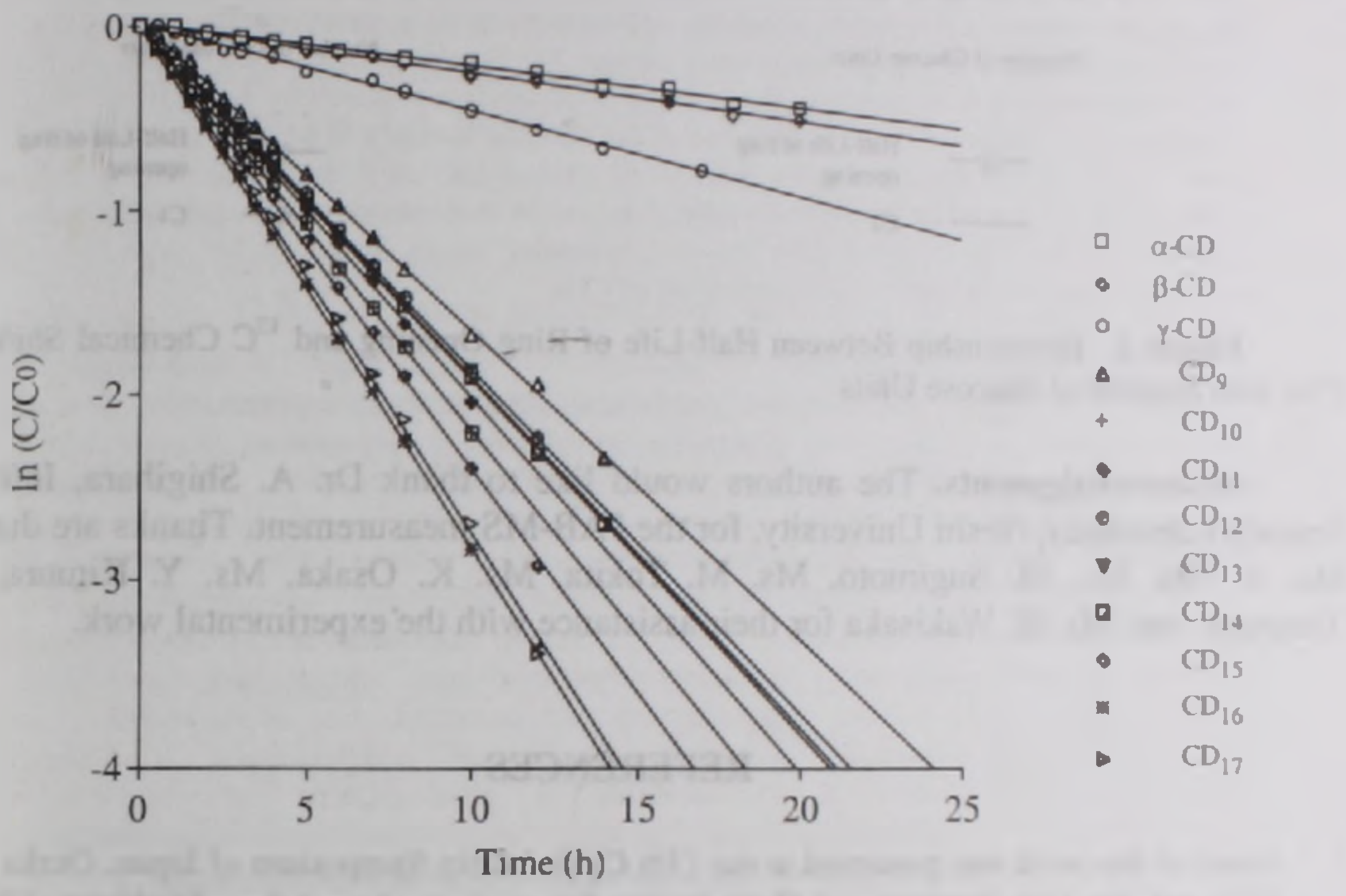

Figure 2. Time-Conversion Profiles of Acid Hydrolysis for CDs in 1mol/L HCl at 50°C

As shown in figure 3, the half-lives of ring opening paralleled ^{13}C chemical shifts of C1 and C4 with number of D-glucose units. We assumed that the signals of C1 and C4 were shifted upfield with the strength of steric strain, and the CD_{10} ring was most distorted among the series of CDs, composed of less than 14 D-glucose units examined in this study. The hydrolysis rates of CD_{15}, CD_{16} and CD_{17} were faster than that of CD_{10} because of the increase in decomposition sites with increasing number of D-glucose units. However, this hypothesis should be confirmed by further experiments using LR-CDs composed of more than 18 D-glucose units.

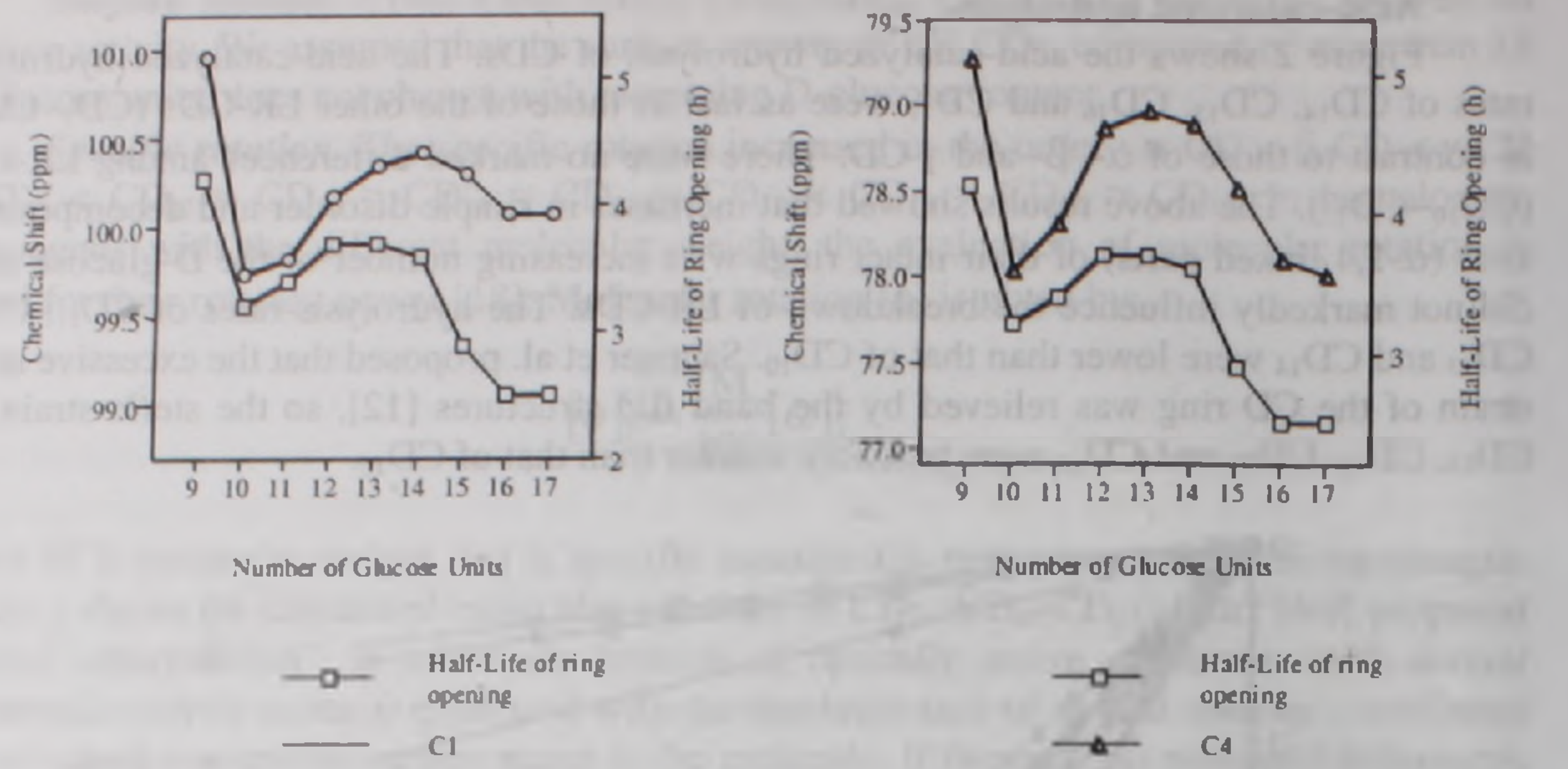

Figure 3. Relationship Between Half-Life of Ring Opening and ^{13}C Chemical Shifts of LR-CDs with Number of Glucose Units

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