Ability of non-cyclic oligosaccharides to form molecular complexes and its use for chiral separation by capillary zone electrophoresis

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Abstract

The binding constants (K) for complexation of the phenyl acetates with linear α-1,4-linked dextrins have been determined from the kinetics of the hydrolyses of the esters. The K value tends to increase with increasing the number of the glucopyranose units, suggesting hydrophobic interaction as a binding force. The weak ability of the linear dextrins to form the molecular complexes makes it possible to separate the enantiomers of binaphthyl derivatives such as 1,1'-binaphthyl-2,2'-dicarboxylic acid, 1,1'-binaphthyl-2,2'-diyl hydrogenphosphate and 2,2'-dihydroxy-1,1'-binaphthyl-3,3'-dicarboxylic acid in their anionic forms. Hydrogen bonding as well as hydrophobic interaction is suggested as an essential force for enantioselective complexation between saccharide and anionic binaphthyl.

1. Introduction

Although cyclic oligosaccharides, cyclodextrins (CDs), are well known to include various organic compounds in their cyclic cavities [1], the ability of non-cyclic oligosaccharides to form molecular complexes has scarcely been recognized. However, judging from the fact that α-1,4-linked linear polymer of glucose, amylose, forms complexes with various alcohols whose helical structures have been determined by X-ray analysis (see, for example, [2] and [3]), it is expected that linear α-1,4-linked oligosaccharides such as maltohexaose (G_6) and/or maltoheptaose (G_7), which may have a turn of a dextrin helix [2], complex with organic compounds. We found that (4Z,15Z)-bilirubin-IX (BR) is bound to maltose

(G_2), maltotriose (G_3) and G_7 to form chiral BR complexes through hydrogen bonding between the CO_2^- groups of BR and the OH groups of linear α-1,4-linked dextrin (G_n) [4]. Recently, the hydrophobic fluorescence probes such as 8-anilino-1-naphthalenesulfonate (ANS) and 6-toluidino-2-naphthalenesulfonate (TNS) form the molecular complexes with G_n ($n = 4$–7) while the binding constants (K) are extremely small (1.8–27 M^{-1}) [5]. Meanwhile, CDs with many chiral centers can recognize the chiralities of their guest molecules [6]. Linear oligomers of glucose are also expected to act as guest molecules which discriminate between the enantiomers of the guest molecules. Although the studies on the molecular recognition by non-cyclic oligosaccharides seem to be very important in connection with the role of oligosaccharides at biological cell surfaces (see, for example, [7]),

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little attention has been paid to chiral recognition of non-cyclic oligosaccharides. The present study deals with the ability of G_n ($n = 2-7$) to form molecular complexes and its use to separate the enantiomers of the binaphthyl derivatives such as 1,1'-binaphthyl-2,2'-dicarboxylic acid (BNC), 1,1'-binaphthyl-2,2'-diyl hydrogenphosphate (BNP), 2,2'-dihydroxy-1,1'-binaphthyl-3,3'-dicarboxylic acid (HBNC) and 1,1'-bi-2-naphthol (BN) by capillary zone electrophoresis (CZE) using G_n as the chiral separators.
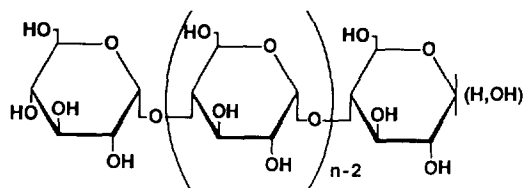
It is very important to know the ability of G_n to form molecular complexes and to clarify the mechanisms of molecular complex formation of G_n. In general, however, it is very difficult to study the molecular complex formation of G_n because of their very small K values. Since the determination of small K values by spectroscopic titrations is very difficult, we employed the kinetic method to evaluate the K values for complexation of p-nitrophenyl acetate (PNPA) and p-carboxyphenyl acetate (PCPA) with G_n. Both PNPA and PCPA should be regarded as the probe molecules. The hydrolyses of the esters were carried out in aqueous alkaline solutions containing G_n. The results obtained in this work suggest a hydrophobic interaction between the ester and G_n. The weak ability of G_n to form molecular complexes was applied to realize the optical resolution of the racemic binaphthyls using CZE. This may be the first case where the chiral recognition of the axial chiralities by non-cyclic oligosaccharides is proved.

2. Experimental

D-Glucose (G_1), G_2, G_3 (Nacalai), G_4, G_5, G_6 and G_7 (Hayashibara) were purchased and used without further purification. An antioxidant in α- and β-CDs (Nacalai) commercially obtained was

extracted with tetrahydrofuran (THF) using a Soxhlet extractor. PNPA (Nacalai) was recrystallyzed from aqueous ethanol. PCPA was prepared and purified according to the procedures described in the literature [8]. Optically active and racemic BNP and BN (Aldrich) were commercially obtained. The preparation of (±)-, (S)- and (R)-HBNCs were described before [9]. The hydrolyses of PNPA and PCPA were carried out using a UNISOKU stopped-flow apparatus with a multichannel spectrophotometer. The changes in absorbances of the phenolate ions were followed at 440 nm for PNPA and 295 nm for PCPA and the kinetic data were analyzed by a damping Gauss–Newton method (a non-linear least squares method) using a microcomputer.

The CZE experiments were performed with a Jasco capillary electrophoresis system CE-800 with a 300 mm (effective length) × 50 μm I.D. fused-silica capillary cartridge (non-coated). The capillary was filled with the buffer solution with G_n and the sample in the same buffer solution was introduced into the capillary by applying the potential for 10 s. The electropherogram was taken at the same potential using a Jasco 875-CE UV–Vis detector.
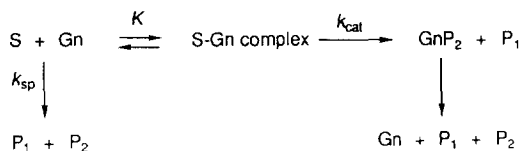
The molecular mechanics–molecular dynamics (MM-MD) calculations were carried out using an AMBER program system (version 4, presented by P. Kollman, University of California at San Francisco, USA) on a COMTEC 4D RPC XS24Z R4000 workstation at 285.1–302.6 K for 10–11,8 ps. The calculations include the effects of water as a solvent. Before MM-MD calculations, the information of the charge of the glucopyranose was prepared by a MOPAC (version 6 developed by J.J.P. Stewart, US Airforce Academy, USA) calculation.

3. Results and discussion

3.1. Interaction between G_n and esters

Spectroscopic titration using UV–Vis, fluorescence, circular dichroism or NMR spectroscopy is the most common way to determine the K value. However, the spectroscopic titration can-

not be applied for most systems having very small K values. In the present study, we used a kinetic method to evaluate the K value for complexation of benzene derivatives with G_n. It is well known that CD accelerates the hydrolyses of phenyl acetates [1,10]. The hydrolysis of the ester catalyzed by saccharide is as follows:

$$S + Gn \underset{}{\overset{K}{\rightleftharpoons}} \text{S-Gn complex} \xrightarrow{k_{cat}} GnP_2 + P_1$$

$$S \xrightarrow{k_{sp}} P_1 + P_2$$

$$\text{S-Gn complex} \rightarrow Gn + P_1 + P_2$$

where S and GnP_2 represent the substrate (ester) and acylated G_n, respectively, and k_{sp} and k_{cat} are the pseudo first-order rate constant for the hydrolysis of the ester in the absence of G_n and the first-order rate constant for the reaction of S–G_n complex, respectively. In the case of CD, the dissociated secondary OH group of CD attacks to the carbonyl carbon of the ester to promote acyl transfer reaction. However, since the pK_a of the secondary OH group of G_n is much higher than that of CD, the nucleophilic attack of the dissociated OH group does not occur in the G_n-catalyzed hydrolysis under the present conditions. VanEtten et al. [11] reported the G_1-catalyzed hydrolyses of the phenyl acetates and assumed a hemiacetal alkoxide anion is an active species which attacks to the carbonyl group of the ester. The K and k_{cat} values can be evaluated from the Eadie-type plot [12]:

$$k_{obs} - k_{sp} = -(k_{obs} - k_{sp})/(K[G_n]) + k_{cat} - k_{sp}$$

(1)

The hydrolyses of PNPA were carried out in the pH 11.7 phosphate buffer (0.1 M) containing various amounts of G_n at 25°C. The plot of k_{obs} vs. [G_n] is not linear for each saccharide (Fig. 1), indicating that the complexation between PNPA and G_n occurs. Good linear relationship (correlation factor $r > 0.999$) between ($k_{obs} - k_{sp}$) and ($k_{obs} - k_{sp}$)/[G_n] provided K and k_{cat}. The results are summarized in Table 1. In contrast with the CD-catalyzed hydrolyses, the k_{cat} values are almost the same in the hydrolyses in the presence of various G_n compounds. In the case of the PNPA–α-CD complex, the active site (the
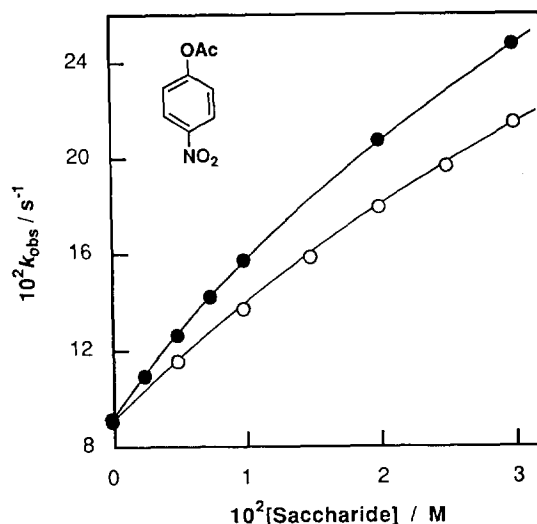

Fig. 1. Effects of the concentrations of G_3 (○) and G_5 (●) on k_{obs} for hydrolyses of PNPA in 0.1 M phosphate buffer at pH 11.7 and at 25°C.

dissociated secondary OH group of α-CD) is located far from the carbonyl group of PNPA leading to smaller k_{cat}. Meanwhile, the PNPA molecule can alter its position in larger cavity of β-CD. The PNPA molecule may move freely in the PNPA–G_n complex leading to relatively large and constant k_{cat} in the G_n-catalyzed hydrolyses. Except for the G_2 complex, the K value increases with increasing the number of the glucopyranose units of G_n. Similarly, very small K values have been obtained in complexation of

Table 1
Linear dextrin-catalyzed hydrolyses of PNPA in 0.1 M phosphate buffer at pH 11.7 and at 25°C

Saccharide	k_{obs} (s^{-1})	k_{cat} (s^{-1})	K (M^{-1})
None	0.09	–	–
G_1	0.12	0.56	7.9
G_2	0.14	0.48	13.4
G_3	0.14	0.67	9.0
G_5	0.15	0.60	14.2
G_7	0.18	0.62	20.0
α-CD	0.15	0.24	74.0
β-CD	0.49	0.76	147

The hydrolysis of PNPA (0.1 mM) was carried out in the phosphate buffer at pH 11.7 containing 0.01 M saccharide.

G_n with ANS or TNS [5]: the K values for the TNS–G_5 and TNS–G_7 complexes being reported to be 1.9 and 27 M^{-1}, respectively.

The dissociated PCPA was chosen as another substrate which can interact with G_n through hydrogen bonding between the CO_2^- group of PCPA and the OH group of G_n. The results are shown in Table 2. In the case of G_1, a good linear relationship was observed between k_{obs} and $[G_1]$, indicating that no complex of G_1 and PCPA is formed. The k_{cat} value for each G_n is much larger than those for α- and β-CDs. Very small k_{cat} for β-CD suggests the difference between the structures of the β-CD complexes of PNPA and PCPA. Similar to the case of PNPA, the K value increases with increasing the number of the glucopyranose units of G_n and no significant difference is found between the K values for PNPA and PCPA. This may suggest that hydrogen bonding does not participate in complexation of PCPA and G_n. Our results are in good agreement with those obtained by Aoyama et al. [5] and indicate the hydrophobic nature of linear α-1,4-linked dextrins by which the molecular complexes are formed. Recent studies on solubilization, Diels–Alder reactions and denaturation of protein also demonstrate that linear α-1,4-linked dextrin shows an amphiphilic nature while only hydrophilic character is shown for β-1,4-linked glucoside cellulose and α-1,4-linked dextran [13].

Table 2
Linear dextrin-catalyzed hydrolyses of PCPA in 0.1 M phosphate buffer at pH 11.7 and at 25°C

Saccharide	k_{obs} (s^{-1})	k_{cat} (s^{-1})	K (M^{-1})
None	0.019	–	–
G_1	0.022	–	–
G_2	0.025	0.079	13.3
G_3	0.026	0.078	15.5
G_5	0.029	0.096	16.0
G_7	0.032	0.094	21.0
α-CD	0.025	0.044	29.2
β-CD	0.020	0.022	160

The hydrolysis of PNPA (0.1 mM) was carried out in the phosphate buffer at pH 11.7 containing 0.01 M saccharide.

3.2. Chiral separation of binaphthyls by CZE using G_n as separator

Rapid development has been achieved in chiral separation by HPLC [14] and CZE [15] using CDs as chiral separators. The enantiomers of amino acid derivatives [16–18] and drugs and related compounds [17,19–23] have been separated by CZE using α-, β- and γ-CDs and their methylated derivatives. 5-Aminonaphthalene-2-sulfonic acid derivatives of monosaccharide enantiomers are also separated by CD CZE [24]. We also reported excellent separation of the enantiomers of BN, HBNC and BNC by CZE using heptakis(2,3,6-tri-O-methyl)-β-CD (TMe-β-CD) as a chiral additive in the running buffer electrolyte [9]. The present study on CZE reveals that linear dextrins (G_n) also discriminate between the enantiomers of the binaphthyl derivatives.

The electropherograms of BNC are shown in Fig. 2 and the numerical data are summarized in Table 3. The separation factor α is defined as

$$\alpha = (t_2 - t_0)/(t_1 - t_0) \qquad (2)$$

where t_1 and t_2 represent the retention times of the first and second peaks, respectively, and t_0 is the retention time of the coexisting compound which does not interact with G_n. We used methanol (2%, v/v) to determine t_0. G_n (0.4 M) in 0.04 M carbonate buffer (pH 9–9.5) were used as the separators. Except for the case of G_5, baseline or partial separation of the enantiomers of BNC was achieved by CZE. In CZE using G_2, G_3 and G_4, the retention times of the (S)-enantiomer are shorter than those of the (R)-enantiomer. The enantiomers of BNC are partially resolved by G_2 ($\alpha = 1.02$) and baseline separation is realized by G_3 CZE ($\alpha = 1.10$). The retention times of the BNC enantiomers are close again in G_4 CZE ($\alpha = 1.09$) and no peaks separation occurs in the case of G_5 ($\alpha = 1.00$). The enantiomers of BNC are separated again by G_6 ($\alpha = 1.03$) and G_7 ($\alpha = 1.08$). In contrast with the cases of G_2, G_3 and G_4, the retention times of the (S)-enantiomer become longer than those of the (R)-enantiomer in CZE using G_6 and G_7. In these experiments, the currents were
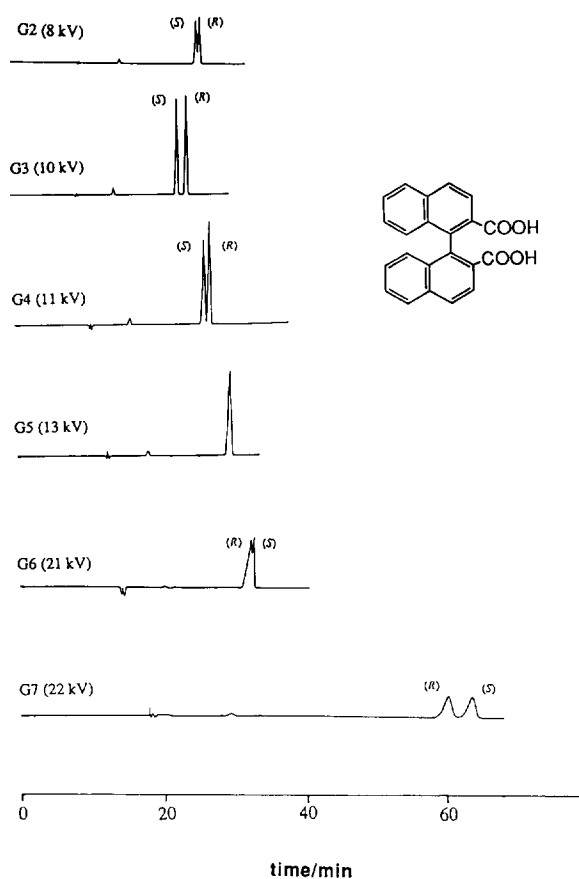
Fig. 2. Electropherograms of (±)-BNC (0.1 mM) obtained by CZE using 0.04 M carbonate buffer (pH 9–9.5) containing G_n (0.4 M); current 17–18 μA, detection wavelength 225 nm.

controlled to be 17–18 μA by changing applied voltages. Table 4 shows the results on CZE examined at constant voltage (10 kV). Essential-

ly the same results were obtained. In CZE of BN, BNC, BNP and HBNC using TMe-β-CD or β-CD as the separator, the retention time of the enantiomer having a larger K for complexation with CD is shorter than that of another enantiomer having a smaller K [9]. Although we have not verified the correlation between the retention times of the enantiomers and the K values for complexation of the enantiomers with G_n, the enantiomer having a shorter retention time seems to have a larger K. Therefore, it can be concluded that G_3 has the highest enantioselectivity amongst G_2, G_3 and G_4 which prefer the (S)-enantiomer of BNC as the guest and the (S)-selectivity is weakened and the (R)-selectivity is gradually enhanced with increasing the number of the glucopyranose units of the G_n. It is quite reasonable to assume that the macroscopic structure of G_n as a polymer is preferable to complex with (R)-BNC while the microscopic structure of G_n prefers the (S)-enantiomer of BNC.

In order to explain the effect of the chain length of G_n on the chiral recognition of BNC, the MM-MD calculations were carried out for G_n ($n = 2–7$) in water. The most stable structures derived from the calculations are shown in Fig. 3. As can be expected, a helical structure becomes remarkable as increasing the number of the glucopyranose units of G_n. The primary OH groups of G_n of $n \geqslant 3$ are directed to the outside of the helix and the hydrophobic environment is constructed inside of the helix. One turn of the helix is prepared in G_7, which is in good agreement with the result of the X-ray analysis [2].

Table 3
Chiral separation of (±)-BNC (0.1 mM) by CZE at 17–18 μA using linear dextrins (0.4 M) as separators

G_n	Voltage (kV)	t_0 (min)	t_1 (min)	t_2 (min)	α
G_2	8	9.6	26.4 (S)	26.8 (R)	1.02
G_3	10	9.2	23.6 (S)	25.0 (R)	1.10
G_4	11	10.6	27.0 (S)	27.9 (R)	1.09
G_5	13	12.0	29.8 (S)	29.8 (R)	1.00
G_6	21	13.8	32.2 (R)	32.7 (S)	1.03
G_7	22	17.8	60.3 (R)	63.7 (S)	1.08

The carbonate buffer (0.04 M) was used as a background electrolyte.

Table 4
Chiral separation of (±)-BNC (0.1 mM) by CZE at 10 kV using linear dextrins (0.4 M) as separators

G_n	Current (μA)	t_0 (min)	t_1 (min)	t_2 (min)	α
G_2	21	7.4	20.3 (S)	20.6 (R)	1.02
G_3	17	9.2	23.6 (S)	25.0 (R)	1.10
G_4	15	13.0	33.0 (S)	34.0 (R)	1.05
G_5	13	16.4	40.8 (S)	40.8 (R)	1.00
G_6	8	33.6	71.6 (R)	72.5 (S)	1.02

The carbonate buffer (0.04 M) was used as a background electrolyte.

The structures of G_6 and G_7 very much resemble those of α- and β-CDs, respectively, though the catalyses of G_6 and G_7 for the hydrolyses of PNPA and PCPA are quite different from those of α- and β-CDs. It is reasonable to assume that a hydrophobic environment is prepared inside of the helix of G_n to which hydrophobic or amphiphilic compounds such as PNPA, PCPA and BNC are bound to form molecular complexes.

The fact that G_n having larger n forms more stable molecular complexes of the phenyl acetates can be interpreted in terms of the helical structure of G_n. In the previous paper [9], we reported that β-CD as well as TMe-β-CD prefers the (R)-enantiomer of BNC as the guest which is included in the cavity of CD. Pseudocyclic oligosaccharides such as G_6 and G_7 may recognize the chirality of BNC through the similar mechanism which is applied for CD.

The electropherograms of other (±)-binaphthyls are shown in Fig. 4. CZE was performed at 15 kV and 28 μA by using 0.4 M G_2 in the 0.04 M carbonate buffer at pH 9. The enantiomers of the anionic binaphthyls such as BNC, BNP and HBNC can be separated while no peak separation is achieved in the case of BN without charge. This seems to suggest the participation of hydrogen bonding between the anionic sample and the OH group(s) of G_2 in chiral recognition. Further study is now in progress to clarify the mechanism for chiral recognition by G_n.
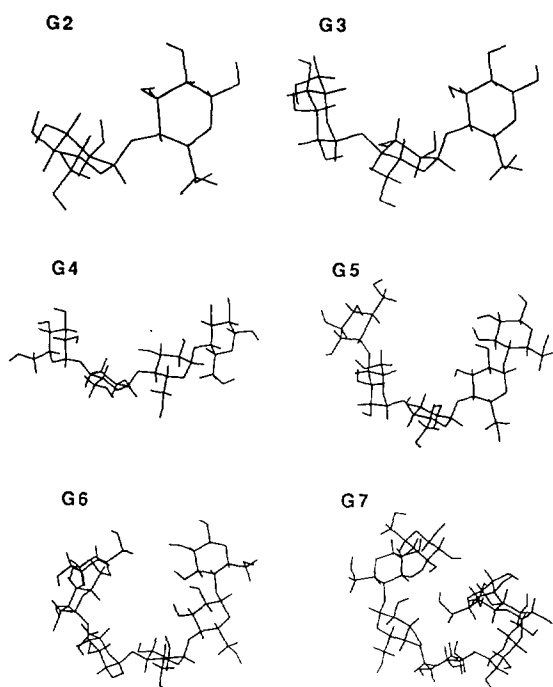

Fig. 3. Optimized structures of G_n in water computed by the MM-MD calculations.
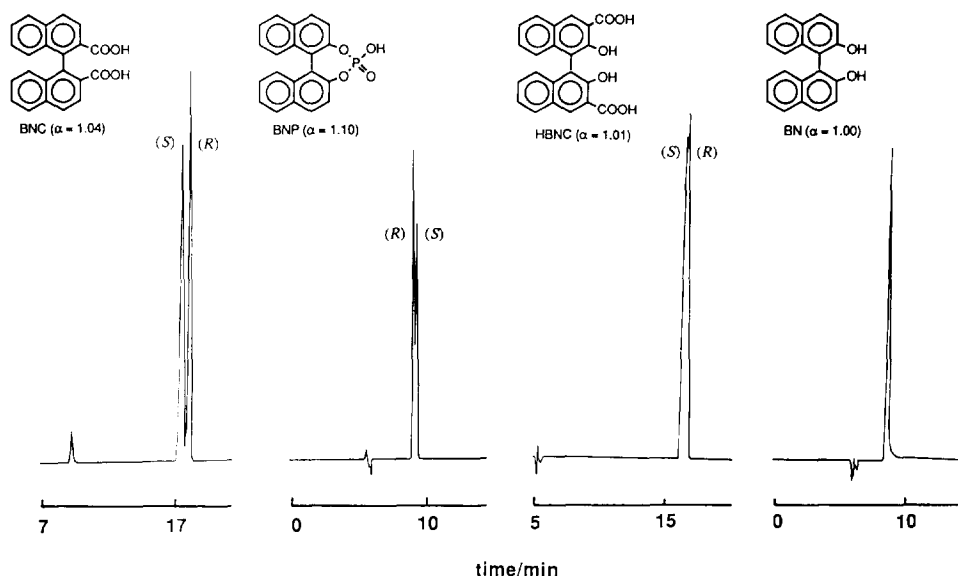
Acknowledgements

Fig. 4. Electropherograms of (±)-BNC, (±)-BNP, (±)-HBNC and (±)-BN (0.1 mM) obtained by CZE using 0.04 M carbonate buffer at pH 9.0 containing G₂ (0.4 M); applied voltage 15 kV, current 28 μA, detection wavelength 225 nm for BNC, 215 nm for BNP, 235 nm for HBNC, 227 nm for BN.

References

[1] M.L. Bender and M. Komiyama, Cyclodextrin Chemistry, Springer, Berlin, 1978.
[2] R.E. Rundle and F.C. Edwards, J. Am. Chem. Soc., 65 (1943) 2200.
[3] Y. Yamashita, J. Polym. Sci., Part A, 3 (1965) 3251.
[4] K. Kano, K. Yoshiyasu and S. Hashimoto, J. Chem. Soc., Chem. Commun., (1988) 801.
[5] Y. Aoyama, J. Otsuki, Y. Nagai, K. Kobayashi and H. Toi, Tetrahedron Lett., 33 (1992) 3775.
[6] K. Kano, in H. Dugas (Editor), Bioorganic Chemistry Frontiers, Vol. 3, Springer, Berlin, 1993, Ch. 1, p. 1.
[7] I. Eggens, B. Fenderson, T. Toyokuni, B. Dean, M. Stround and S. Hakomori, J. Biol. Chem., 264 (1989) 9476.
[8] F.D. Chattaway, J. Chem. Soc., (1931) 2495.
[9] K. Kano, Y. Tamiya, C. Otsuki, T. Shimomura, T. Ohno, O. Hayashida and Y. Murakami, Supramolecular Chem., 2 (1993) 137.
[10] O.S. Tee, C. Mazza and X.-X. Du, J. Org. Chem., 55 (1990) 3603; and references cited therein.
[11] R.L. VanEtten, J.F. Sebastian, G.A. Clowes and M.L. Bender, J. Am. Chem. Soc., 89 (1967) 3242.

[12] G.S. Eadie, J. Biol. Chem., 146 (1942) 85.
[13] D. Balasubramanian, B. Raman and C.S. Sundari, J. Am. Chem. Soc., 115 (1993) 74.
[14] L. Coventry, in W.J. Lough (Editor), Chiral Liquid Chromatography, Blackie & Son, Glasgow, 1989, Ch. 8, p. 148.
[15] R. Kuhn and S. Hoffstetter-Kuhn, Chromatographia, 34 (1992) 505.
[16] A. Guttman, A. Paulus, A.S. Cohen, N. Grinberg and B.L. Karger, J. Chromatogr., 448 (1988) 41.
[17] S. Fanali and P. Boček, Electrophoresis, 11 (1990) 760.
[18] M. Tanaka, S. Asano, M. Yoshinago, Y. Kawaguchi, T. Tetsumi and T. Shono, J. Anal. Chem., 339 (1991) 63.
[19] S. Fanali, J. Chromatogr., 474 (1989) 441.
[20] R. Kuhn, F. Stoecklin and F. Erni, Chromatographia, 33 (1992) 32.
[21] K.D. Altria, D.M. Goodall and M.M. Rogan, Chromatographia, 34 (1992) 19.
[22] I.D. Cruzado and G. Vigh, J. Chromatogr., 608 (1992) 421.
[23] M.W.F. Nielen, Anal. Chem., 65 (1993) 885.
[24] M. Stefansson and M. Novotny, J. Am. Chem. Soc., 115 (1993) 11573.