Gas chromatographic separation of amino acid enantiomers and their recognition mechanism on a 2,6-di-O-butyl-3-O-trifluoroacetylated-γ-cyclodextrin capillary column

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Abstract

Some amino acids and 2- and 3-hydroxybutyric acid derivatives were resolved on a chiral capillary column coated with 2,6-di-O-butyl-3-O-trifluoroacetylated-γ-cyclodextrin, and their thermodynamic data were obtained from this stationary phase. Obvious differences in $\Delta(\Delta H^\circ)$ and $\Delta(\Delta S^\circ)$ were observed among these amino acids. The enthalpy–entropy compensation and structural effects were analysed with consideration of chiral recognition. The results suggested that the induced conformation fit between enantiomers and cyclodextrin derivatives could be important in chiral recognition. The enantiomers with large negative $\Delta(\Delta H^\circ)$ and $\Delta(\Delta S^\circ)$ values could form inclusion complexes and the separation of enantiomers with small negative $\Delta(\Delta H^\circ)$ and $\Delta(\Delta S^\circ)$ values could be due to other types of interactions.

1. Introduction

During the past 5 years, differently modified cyclodextrins have been synthesized and found successful applications in gas chromatography [1–5]. A large number of enantiomers have been resolved on such modified cyclodextrins. Although modification of cyclodextrin has showed that substitution of the hydroxyl groups at positions 2, 3 and 6 has a great influence on enantioselectivity towards enantiomers, the mechanisms of interaction between enantiomers and cyclodextrin derivatives are still unclear. In some instances the experimental results are quite confusing, e.g., the 6-O-acyl derivative [3] and 2,6-

di-O-pentyl-3-O-methyl-β-CD [6] display almost no enantioselectivity.

In order to gain a better understanding of the separation mechanisms, more attention has recently been paid to the thermodynamic data of enantiomer separations on cyclodextrin derivatives [7–10]. Venema *et al.* [7] investigated enantiomeric separations of some alkanes and alkanoic acid esters substituted at the C-2 position on alkylated β-CD and concluded that the stereochemistry and hydrophobic interactions were the major factors affecting the enantioselectivity for substituted cyclodextrins. Berthod *et al.* [8] recently suggested that there may be two mechanisms contributing to the separation obtained on a trifluoroacetylated phase, the formation of inclusion complexes and some form of external association. Smith and Simpson [9]

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separated some alcohols and proposed that all these compounds interact by similar mechanisms. De Vries et al. [10] compared thermodynamic data for styrene oxide on four types of cyclodextrin derivatives and showed that both the size and the polarity of the CD have a great influence on the enantioselectivity. Several types of interactions have been suggested for chiral separations on cyclodextrin derivatives, including hydrophobic interactions, dipole–dipole interactions, geometry factors and the formation of inclusion complexes.

This work covers the enantiomeric separation of some amino acids, and 2- and 3-hydroxybutyric acid on 2,6-di-O-butyl-3-O-trifluoroacetylated-γ-cyclodextrin. Thermodynamic data measured on this stationary phase are given, and the possible separation mechanisms are discussed.

2. Experimental

2.1. Synthesis of 2,6-di-O-butyl-3-O-trifluoroacetylated-γ-CD (DB-TFA-γ-CD)

First, 1 g of γ-CD was converted into 2,6-di-O-butyl-γ-CD (DB-γ-CD) according to Li et al. [11]. The reaction mixture was extracted with $CHCl_3$. The organic layer was washed with water until neutral, then dried with Na_2SO_4, the solvent was carefully evaporated and drying was attempted at 60°C for 2 h under vacuum. The DB-γ-CD obtained was dissolved in tetrahydrofuran and a threefold molar excess of trifluoroacetic acid anhydride was added and refluxed for 4 h. After the extractive and drying work-up procedure, the raw product was purified by silica gel chromatography and finally a viscous oil of DB-TFA-γ-CD was obtained that was identified as described previously [12].

2.2. Preparation of chiral glass capillary column

The pretreatment and coating of glass capillary columns were as described previously [12]. Typi-

cally, a 25 m × 0.25 mm I.D. capillary column gives a column efficiency of 3740 plates/m.

2.3. Instrumentation

All chromatographic measurements were performed on a Model 1001 gas chromatograph (Shanghai Analytical Instrumentation Factory) equipped with a flame ionization detector. An HP-3390A integrator was used to record retention times and to calculate capacity factors (k') and separation factors (α). High-purity nitrogen was used as the carrier gas at a velocity of ca. 25 cm/s with a splitting ratio of 1:60–1:100.

2.4. Analytes and derivatization procedures

Most of the amino acids used were BDH products. 3-Aminobutyric acid, 2-hydroxybutyric acid and glutamic acid were obtained from Shanghai Chemical.

About 5 mg of each analyte were mixed with 0.5 mL of methanolic hydrochloric acid solution [acetyl chloride–methanol (1:10, v/v)] in a capped glass vial. The mixture was reacted at 80°C for 0.5 h, then the solvents was evaporated with a flow of nitrogen. The residue was dissolved in 0.2 ml of dry acetonitrile and 0.2 ml of trifluoroacetic acid anhydride was added. After the mixture had been kept at room temperature for 0.5 h, the excess reagents were removed. The final residue was dissolved in 0.4 ml of dichloromethane and was ready for chromatographic analysis.

3. Results and discussion

3.1. Enantiomeric separation of amino acids and 2- and 3-hydroxybutryic acid

Amino acids have been well separated on both Chirasil-Val [13] and 2,6-di-O-pentyl-3-O-butyryl-γ-cyclodextrin stationary phase [14]. 2,6-Di-O-pentyl-3-O-butyryl-γ-cyclodextrin seems to have displayed better enantioselectivity for almost all amino acids. Unfortunately, the recognition mechanism and effect of substitution of CD are not well understood. In our experiments,

most of the amino acids were resolved on DB-TFA-CD with high enantioselectivity. The D-configuration was first eluted for all resolved amino acids. However, a few amino acids, such as arginine, lysine, tryptophan and proline, were not resolved on this phase. Probably the entry of these amino acids into the cavity of CD was hindered by the relatively large groups linked at the C-3 position, thus reducing the enantioselectivity. We observed that 2- and 3-aminobutyric acid gave longer retention times than the corresponding 2- and 3-hydroxybutryic acid. This increased retention could be due to hydrogen bond formation between the amino acid and DB-TFA-γ-CD. A good separation was also observed for 2- and 3-hydroxybutyric acid, although the hydrogen bond could not be formed after derivatization. In this case, it is suggested that the dipole–dipole interactions might mainly contribute to the separation.

3.2. Thermodynamic parameters for enantiomeric resolution

In general, the absolute free energy $\Delta G° = -RT \ln K$, where R is the gas constant, T is the absolute temperature and K is the thermody-namic stability constant of the association. To a first approximation, K may be considered as the partition coefficient between the mobile and the stationary phases, so it can be expressed as ln $k' = -\Delta H°/RT + \Delta S°/R + \ln \beta$, where $K = k'/\beta$, k' is the capacity factor and β is the phase ratio. For each enantiomer pair, its $\Delta H°$ and $\Delta S° + R$ ln β values can be obtained by plotting ln k' versus $1/T$. In this experiment, k' was measured at intervals of 10°C in the temperature range $T_c \pm 20°C$ (see Table 1) and all straight lines were obtained with good regression ($r > 0.994$). The slope is $-\Delta H°$ and the intercept is $\Delta S° + R$ ln β. The isoenantioselective temperature T_{iso} was calculated by the equation $T_{iso} = \Delta(\Delta H°)/\Delta(\Delta S°)$ [15]. Table 1 gives both separation and thermodynamic data for amino acids and 2- and 3-hydroxybutyric acid measured on DB-TFA-γ-CD. Table 1 shows obvious differences in $\Delta(\Delta H°)$ and $\Delta(\Delta S°)$ among the resolved amino acids. These $\Delta(\Delta H°)$ and $\Delta(\Delta S°)$ values are of approximately the same magnitude as those reported by Berthod et al. [8] and De Vries et al. [10]. From these data, it seems that the enantiomers investigated may fall into two groups. Group I includes $\Delta(\Delta S°)$ values <1.15 cal/mol · K and group II has $\Delta(\Delta S°)$ values ≥1.15 cal/

Table 1
Thermodynamic parameters of some enantiomers calculated from GC measurements on DB-TFA-γ-CD

Racemate	T_c (°C)	k_1'	α	$-\Delta H_1°$ kcal/mol	$-\Delta H_2°$ kcal/mol	$-\Delta(\Delta H°)$ kcal/mol	$-\Delta(\Delta S°)$ cal/(mol · K)	T_{iso} (°C)
Group I								
Serine	100	12.23	1.057	17.15	17.18	0.03	0.038	500
Cysteine	130	5.41	1.029	16.20	16.25	0.05	0.080	350
Threonine	80	19.69	1.052	17.75	17.94	0.19	0.44	150
β-Phenylalanine	130	19.12	1.038	15.78	16.04	0.26	0.57	180
Glutamic acid	130	13.11	1.066	16.21	16.64	0.43	0.94	190
Group II								
Methionine	130	12.31	1.084	15.96	16.49	0.53	1.15	150
Valine	100	6.87	1.221	14.29	15.56	1.27	3.01	150
3-Aminobutyric acid	100	12.35	1.248	16.37	17.75	1.38	3.26	150
Leucine	110	3.00	1.174	15.01	16.48	1.47	3.51	180
Aspartic acid	130	6.85	1.240	16.35	17.94	1.59	3.52	180
Alanine	90	7.286	1.400	14.97	16.56	1.59	3.71	150
2-Hydroxybutyric acid	80	4.230	1.373	14.10	15.81	1.71	4.21	130
2-Aminobutyric acid	100	5.327	1.587	14.51	17.45	2.94	6.96	150
3-Hydroxybutyric acid	100	1.505	1.449	12.12	15.43	3.14	7.68	130

mol · K. However, neither relationships nor differences were observed with regard to the iso-enantioselective temperature in groups I and II. Serine and cysteine, with small $\Delta(\Delta H^\circ)$ and $\Delta(\Delta S^\circ)$ values, had much higher T_{iso} than other enantiomers. The decrease in the $\Delta(\Delta H^\circ)$ values reflects the affinity of the cyclodextrin derivative for the enantiomers. The large negative entropy $\Delta(\Delta S^\circ)$ may imply the loss of degree of freedom for enantiomers included in the cavity of the cyclodextrin derivative. The decrease in entropy from serine to 2-hydroxybutryic acid in Table 1 indicates that enantiomers are more ordered in the cavity of the cyclodextrin derivative than they are in the mobile phase in their inclusion complexes. Therefore, it is reasonable to suggest that the enantiomers with large negative $\Delta(\Delta S^\circ)$ values in group II could form inclusion complexes during separation.

3.3. Enthalpy–entropy compensation and induced conformation fit

For enantiomers belonging to groups I and II, the differences in the chiral recognition mechanism were further investigated using enthalpy–entropy compensation. According to refs. 8 and 16, similar physico-chemical behaviour or interaction can be shown for a family of compounds at a compensation temperature T_Φ if enthalpy–entropy compensation occurs. ΔH° and $\ln k'$ are related by $\ln k'_T = -\Delta H^\circ / R \ (T_\Phi - 1/\Phi) + \ln \beta$. Hence a straight line should be obtained on plotting $\ln k'$ *versus* $-\Delta H^\circ$ for the enantiomers which have enthalpy–entropy compensation. The enthalpy–entropy compensation plots for both the first- and second-eluting peaks of these enantiomers are shown in Fig. 1. It can be seen from these plots that relatively good linearity was observed in group I. In contrast, a large dispersion was found for group II. This shows that enantiomers in group I follow enthalpy–entropy compensation and possibly interact by a mechanism that is completely different from that for enantiomers in group II. In group I, the amino acids at the C-3 position link with hydroxy, carboxy, thiol and phenyl groups, whereas in group II, the C-3 position of amino acids is,
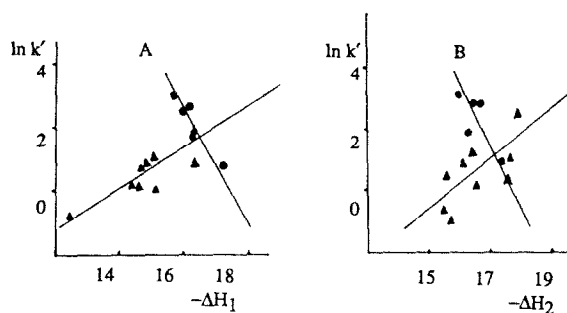

Fig. 1. Enthalpy–entropy compensation plots for (A) the first-eluted peaks and (B) the last-eluted peaks on DB-TFA-γ-CD at 130°C. ● = Group I; ▲ = group II.

in general, occupied by alkane groups. Therefore, it is possible that hydrophobic interaction between the alkane group and the non-polar cavity of γ-CD resulted in the enantiomers approaching the γ-CD cavity closer by induced conformational fit and consequently the inclusion complex could be formed more easily. Whether or not the inclusion complex or other interaction occurred during separation, the conformational fit may play an important role in separation. That is why some amino acids display good enantioselectivity and some others may not on the same phase. Further investigation of the retention of amino acids on other cyclodextrin derivatives is in progress.

4. Conclusions

2,6-Di-O-butyl-3-O-trifluoroacetylated-γ-cyclodextrin displays variable enantioselectivity for amino acids having different functions at the C-3 position. The obvious thermodynamic data differences suggest that there may be more than one recognition mechanism on derivatized cyclodextrin stationary phases for amino acids in GC. Of these, the induced conformational fit between enantiomers and the cyclodextrin derivative could be the chief factor affecting enantioselectivity.

Acknowledgements

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