Optimization Strategies and Modeling of Separations of Dansyl-Amino Acids by Cyclodextrin-Modified Capillary Electrophoresis

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Abstract

Presented in this study is an approach to optimize conditions for capillary electrophoresis separations of multianalyte enantiomeric pairs (D- and L-dansyl (Dns)-amino acids) that involves the rational use of combinations of cyclodextrins (CDs) as enantioselective running buffer additives. Migration data is experimentally obtained for a range of concentrations for native CDs used individually and employed to determine inclusion constants for the Dns-amino acids of interest. An expression for the mobility of the amino acids when multiple (two in this work) CDs are present in the running buffer is used to simulate separations for more complex CD systems. A chromatographic response function involving predicted resolution is generated to gauge the quality of these separations. Simplex methods are then employed for the first time to optimize conditions for the separation of amino acid enantiomers. The validity of this approach is demonstrated for separations of five Dns-amino acid enantiomers using γ- and β-CDs at various concentrations. Extending the dual-CD approach to other CDs and increasing the number of CDs beyond two should be possible. To this end, preliminary experiments are performed by using several available single-isomer, derivatized CDs (individually) to determine if they have potential for further studies. Although results with these particular derivatized CDs are not encouraging, we did find that molecular mechanics modeling is useful in interpreting those cases in which low inclusion constants possibly contributed to the ineffectiveness of the CDs.

Introduction

The enantiomeric separation of amino acids is significant for peptide chemistry, amino acid biochemistry, and the dating of fossils. The different biological activities of stereoisomers also make their separation important. A number of chiral selectors have been used for the separation of enantiomers in capillary electrophoresis (CE) (1–2). Wistuba et al. (3) used bovine serum albumin (BSA), α_1-acid glycoprotein, ovomucoid, and casein as chiral selectors, but found it necessary to use extensive rinse procedures every ten runs to remove the wall-adsorbed proteins. A dextran polymer network containing a chiral additive has been used to separate amino acids and drugs (4). Another approach used by Kuhn et al. (5–6) was the use of the chiral crown ether (18-crown-6-tetracarboxylic acid) for enantiomeric separations. The macrocyclic antibiotic vancomycin has been employed as a chiral buffer additive in CE because of the advantages that it provides such as high solubility and the ability to provide excellent separation selectivity and efficiency (7–8). Coated columns are necessary to minimize the wall adsorption of vancomycin that leads to poor separation efficiency and problems with migration time reproducibility. In our laboratory, we have obtained a migration time reproducibility of less than 1% for the separation of amino acids with cyclodextrins (CDs) (9). Micellar electrokinetic chromatography (MEKC) with CDs as selectors has been employed for the separation of many analytes, but the analysis times can be somewhat lengthy (10–15). The use of derivatized CDs with MEKC has been demonstrated to be an excellent alternative to β-CD for the separation of naphthalene-2,3-dicarboxaldehyde derivatized amino acids, because an optimum concentration can be obtained without solubility problems (11).

An advantage of the use of nonaqueous media is that it can allow for higher concentrations of chiral selectors that are poorly soluble in water (16–17). Nevertheless, the most common approach for separating amino acids is the use of CDs in aqueous media (18). Vigh and coworkers (19) synthesized a single-isomer sulfated γ-CD that was capable of resolving many individual amino acid enantiomer pairs at low pH with high resolution. High resolution was possible because the amino acids were positively charged at low pH causing them to migrate in the opposite direction of the sulfated CD, which enlarges the enantiomeric separation window. Hydroxypropyl-substituted CDs (20–22), methylated CDs (22–25), an L-ala-crown-L-ala capped β-CD (26), and cationic CDs (27–29) have all been employed for the separation of amino acids.

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Native CDs (used individually) have been employed for amino acid separations (30–34). Finally, buffers with dual CDs (anionic and neutral) have been prepared for enantioseparations (35–38). The CE multiple-CD work described in this study differs in the use of Simplex methods and molecular modeling in order to better optimize and understand these enantioseparations.

The identification of the optimal separation conditions for a complicated mixture of enantiomeric pairs can be time consuming and difficult because of the combination of chiral and achiral separation elements. Separation of the enantiomers is desired, but excess resolution is a problem because of the possibility of the comigration of the enantiomers of different compounds in the mixture. In this report, the Simplex method has been utilized for the rapid determination of the optimum separation conditions prior to engaging in lab work for the separation of mixtures of five amino acid enantiomeric pairs with dual-CD systems. The five amino acids were chosen because they represented different classes of amino acids such as nonpolar, polar, and acidic substituent groups. The representatives of the nonpolar group were dansyl (Dns)-leucine (Leu) and Dns-valine (Val). Dns-serine (Ser) was the polar group representative, and Dns-glutamic acid (Glu) and Dns-aspartic acid (Asp) represented the acidic substituent group. This set can present a complicated separation, because up to ten peaks can be obtained and three classes of amino acids are represented. Single-isomer substituted CDs have been synthesized and were evaluated for Dns-amino acid separations. The molecular mechanics modeling of amino acid–CD interactions was performed to assist in the understanding of the experimental results.

Experimental

Materials

Dns, Leu, Val, Ser, Glu, and Asp were purchased from Sigma Chemical Co. (St. Louis, MO). β-CD, sodium phosphate, and sodium borate were also purchased from Sigma. γ-CD was purchased from Cyclodextrin Technologies Development, Inc. (Gainesville, FL). Carboxymethyl β-CD degree of substitution 1 (1-CM-β-CD) was purchased from Advanced Separation Technologies Inc. (Whippany, NJ). Heptakis-(2,3-dimethyl-6-carboxymethyl)-β-CD (HDMCM-β-CD) and octakis-(2,3-dimethyl-6 hydroxy)-β-CD (16-me-γ-CD) were synthesized in-house (39). The running buffer (pH 8.1) used for all separations was 0.01M sodium phosphate and 0.006M sodium borate with the appropriate amount and type of CD in the buffer.

Apparatus and methods

CE experiments were performed using a Hewlett Packard automated CE system (HP^{3D} CE) interfaced to an HP Pentium I personal computer. An applied voltage of l6 kV and temperature control (24°C) were used in all experiments. Detection was accomplished on-column by monitoring UV absorbance at 214 nm. The migration order was established by injecting the amino acids individually. Fused-silica capillaries (50-µm i.d. × 360-µm o.d.) were obtained from Polymicro Technologies (Phoenix, AZ). The capillaries were cut to a total length of 60 cm, and a 1-cm section of the capillary coating was removed at 51 cm for on-column detection. New capillaries were conditioned for 30 min with 0.1N sodium hydroxide and 30 min with deionized water. At the beginning of each day, the capillary was first rinsed for 5 min with deionized water, 5 min with 0.1N sodium hydroxide, another 5 min with deionized water, and then 5 min with running buffer. The flush procedure between runs was for 2 min with 0.1N sodium hydroxide, 4 min with deionized water, 3 min with running buffer, and a 90-s application of 15 kV between the inlet and outlet. Column void time (t_0) was marked by a baseline solvent disturbance resulting from the inclusion of mesityl oxide in most of the sample solutions. The mobilities of the anionic Dns-amino acids were calculated from migration times after adjusting for electro-osmotic flow (EOF). The mobility was expressed in meters squared per minutes and voltage (m^2/min•V). For the sake of simplicity they are given as absolute values.

Molecular modeling

The modeling experiments were performed using Sybyl 6.6 molecular modeling software (40) and run on a Silicon Graphics Octane workstation with dual 270 MHz MIPS processors and 768 MB RAM. The Dns-amino acids were constructed with the SKETCH feature in Sybyl. The CD molecules were built by using SKETCH to modify β-CD and γ-CD structures obtained from the Cambridge Crystallographic Data Center's structural database. The molecules were then structurally minimized in a water environment using the Sybyl MAXIMIN2 function with the Tripos forcefield and the Geistiger–Huckel charge calculation method.

A macro program was written in Sybyl Programming Language to achieve grid search docking for each amino acid with the different CDs. This program systematically translated and rotated the amino acid into the cavity of the CD as described in a previous work (41). Each CD was minimized 1000 times in a water environment. The centers of mass were used to aid in the alignment of the amino acid and CD prior to docking. Each Dns-amino acid was translated from –10.0 Å out of the CD cavity to 0 Å in increments of –0.1 Å. At each of these translational positions, the solute was rotated in increments of 5 degrees to 360 degrees. The solute–CD complex configuration was energy minimized using 250 iterations at each step with the dielectric constant set at 20.

Computational methods

The plots of mobility versus the CD concentration that appear in this study were generated using the TableCurve 3.0 plotting program developed by Jandel Scientific (San Rafael, CA). TableCurve substituted the data into many equations in its database and ranked the equations by wellness of fit, and the selected values for each of its constants were based on the best fit for the curve of observed mobility versus CD concentration. The MultiSimplex Lite program used for the Simplex procedure was purchased from MultiSimplex AB (Karlskrona, Sweden). This software used an optimization algorithm

patterned after that developed by Betteridge (42). The mobility, resolution, and a chromatographic response function (CRF) were calculated using Microsoft Excel programs.

CRF values were calculated after each optimization step and served as a measure of the success of the separation. The CRF was defined by:

$$CRF = a\Sigma \ln R_{so}/R_s + b\Sigma \ln R_s/R_{so} \qquad \text{Eq. 1}$$

where a and b are operator selectable numbers, R_{so} is the optimal resolution (1.5 used in this work), and R_s is the resolution between any two neighboring peaks. The operator selectable numbers can play an important role in the separation by placing more emphasis on a certain term in the CRF equation. The CRF values computed by Equation 1 are always negative and approach zero when each pair of adjacent peaks is optimally resolved. The excess resolution factor (a) weights those peaks with $R_s > R_{so}$ (1.5) and determines the penalty for excess resolution. The bounds of the a parameter are set on the low end by long analysis times and at the high end by the complexity of the sample. A value of 5 worked well in this application. The overlap degradation factor (b) was the selectable number that determined the CRF penalty for inadequately resolved peaks, which in most cases will involve enantiomer resolution. We have found that higher numbers for the overlap degradation factor (50 used in this study) are better because insufficient resolution can dramatically affect a separation, but the a and b terms in Equation 1 can be adjusted to suit the needs of the operator. Simplex methods can be employed for optimizing separation conditions (43). The Simplex program supplied three sets of CD concentrations as possible conditions for the separation. Excel programs generated the mobility and resolution for the amino acid enantiomers, and a CRF value was generated from equation 1 for each of the first three sets of CD concentrations. Typical plate counts per meter were 350,000, and this value was used when calculating resolution. The program generated the next set of conditions for β- and γ-CD concentrations using Simplex optimization. If the conditions were within experimental boundaries, a new set of calculations from these conditions were generated along with a new CRF value. This process continued on until an optimum was obtained.

Results and Discussion

Mobility and complexation data

The observed mobility of an analyte (μ_{obs}) depends on its distribution between the free and complexed forms, as seen in the following equation (44):

$$\mu_{obs} = \frac{\mu_f + \mu_c K_{CD}[CD]}{1 + K_{CD}[CD]} \qquad \text{Eq. 2}$$

where μ_f is the apparent free mobility of the analyte and μ_c is the apparent mobility of the analyte–CD complex. K_{CD}, the inclusion constant, is defined as:

$$K_{CD} = \frac{[CD - analyte]}{[CD]\,[analyte]} \qquad \text{Eq. 3}$$

It is generally expected that μ_f and μ_c will be equal for pairs of enantiomers because these analytes are mirror images. However, results obtained in our laboratory indicated that μ_c is not always the same for the D- and L-enantiomers of certain amino acids (the diastereomer complexes can have slightly different mobilities). Nevertheless, the difference in mobility for enantiomer pairs ($\Delta\mu_{obs}$) is primarily influenced by differences in K_{CD} values.

Separation performance was evaluated over a range of β-CD and γ-CD concentrations. At high CD concentrations many enantiomers comigrated, which was most likely because of the enantiomers becoming fully complexed at such high CD concentrations and thus their mobilities ($\mu_{c,L}$ and $\mu_{c,D}$) were equal or nearly equal. In some cases, the enantiomers of two different amino acids will comigrate (34,45). An example of the difficulty of the enantiomeric separation of all the amino acid

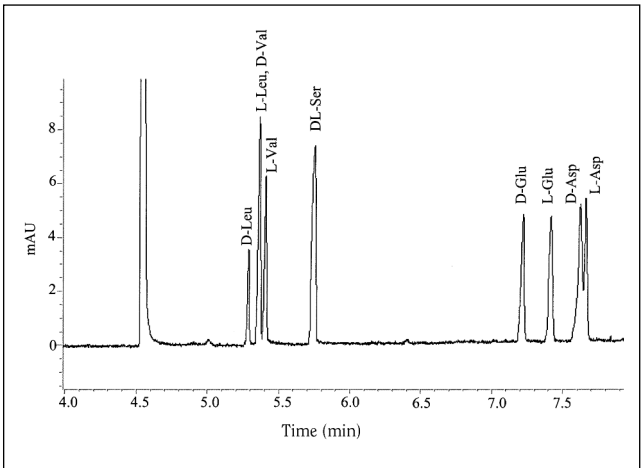

Figure 1. Separation of five Dns-amino acid enantiomers (separation conditions: 60-cm capillary, 16 kV, 8.5mM γ-CD in phosphate–borate buffer, and injection for 4 s at 15 mbar).

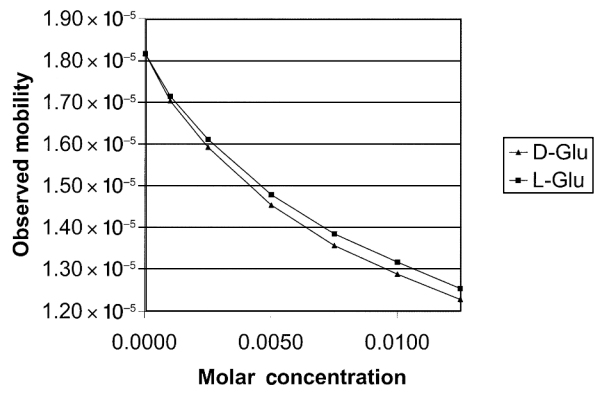

Figure 2. Plot of the observed mobility of glutamic acid enantiomers versus the molar concentration of β-CD (experimental conditions are the same as in Figure 1). Curves plotted based on equation 2 yielding correlation coefficients of 0.9995.

pairs with only γ-CD can be seen in Figure 1 in which the L-enantiomer of Dns-Leu coeluted with the D-enantiomer of Dns-Val. The one amino acid that is generally problematic is Dns-Ser; no enantiomeric resolution is possible when γ-CD is used, very little when both γ-CD and β-CD are used, and only partial resolution is observed with β-CD alone.

Figure 2 and Table I contain data from studies on the effect of β-CD and γ-CD concentration on the mobility of the Dns-amino acids. The figure illustrates the μ_{obs} for the Dns-Glu enantiomers over the range of 0 to l5mM β-CD. The value of μ_{obs} decreases as the degree of complexation with the large molecular weight neutral CD increases, and the mobility difference between the enantiomers increased in the range that we employed for β-CD. The table shows mobility and complexation-constant (K_c) data over the range of 0 to 15mM for β-CD and 0 to 100mM for γ-CD. A good fit was obtained by the plotting program that employed an expression of the form: $y = (a + cx)/(1 + bx)$ (see equation 2). The values of μ_f, μ_c, and K_c were generated from the constants supplied by the TableCurve fitting program from mobility data covering the full range of β-CD and γ-CD concentrations. Values of μ_f obtained with runs without CD present agreed with curve fit values within 1% for β-CD, but had a discrepancy of 10% for γ-CD. The last two columns in the table illustrate the analyte–CD complex mobility and complexed mobility ratios for the enantiomers. It is obvious in the β-CD data that the K_c values for the D-enantiomers were larger than for the L-enantiomers and that the K_c ratio played a more important role than the complexed mobility ratio for enantiomeric separation. However, in the case of the γ-CD data it seems that there was not much difference in K_c values for each enantiomeric pair except for Dns-Glu. It is also important to note that the K_c values for the γ-CD complexes were much smaller than the K_c values for the β-CD complexes. This suggests that the amino acid fits more sloppily into the larger cavity provided by the γ-CD. Therefore, the intermolecular interactions were weaker. This may also contribute to poorer selectivity, as reflected in K_c ratios near 1.0.

Table I. Mobility and K_c Data for Amino Acids with β- and γ-CD

	β-CD data for Dns-amino acids				
	μ_f	K_c	K_{c_D}/K_{c_L}	μ_c	μ_{c_D}/μ_{c_L}
D-Leu	9.70×10^{-7}	167	1.12	3.65×10^{-7}	0.978
L-Leu	9.67×10^{-7}	150		3.74×10^{-7}	
D-Val	9.83×10^{-7}	202	1.28	4.54×10^{-7}	1.05
L-Val	9.87×10^{-7}	158		4.34×10^{-7}	
D-Ser	1.05×10^{-6}	146	1.15	4.50×10^{-7}	1.04
L-Ser	1.05×10^{-6}	127		4.35×10^{-7}	
D-Glu	1.81×10^{-6}	120	1.15	8.49×10^{-7}	1.02
L-Glu	1.81×10^{-6}	104		8.35×10^{-7}	
D-Asp	1.88×10^{-6}	124	1.17	8.57×10^{-7}	0.997
L-Asp	1.88×10^{-6}	105		8.59×10^{-7}	
	γ-CD data for Dns-amino acids				
	μ_f	K_c	K_{c_D}/K_{c_L}	μ_c	μ_{c_D}/μ_{c_L}
D-Leu	7.33×10^{-7}	51.0	0.995	2.38×10^{-7}	1.07
L-Leu	8.02×10^{-7}	51.3		2.24×10^{-7}	
D-Val	8.06×10^{-7}	56.6	1.01	2.52×10^{-7}	1.03
L-Val	8.39×10^{-7}	56.2		2.45×10^{-7}	
D-Ser	1.01×10^{-6}	36.1	1.00	1.99×10^{-7}	1.00
L-Ser	1.01×10^{-6}	36.1		1.99×10^{-7}	
D-Glu	1.74×10^{-6}	31.4	1.22	3.59×10^{-7}	1.17
L-Glu	1.77×10^{-6}	25.7		3.06×10^{-7}	
D-Asp	1.83×10^{-6}	24.4	1.05	2.81×10^{-7}	1.04
L-Asp	1.83×10^{-6}	23.4		2.71×10^{-7}	

Use of the Simplex method and CRF values

Trial-and-error approaches of finding the appropriate running buffer concentration for enantiomeric separations can be very time consuming. Furthermore, the challenge is increased in proportion to the number of enantiomeric pairs present in the sample. Simplex methods can locate the true optimum of a response with fewer trials than nonsystematic approaches or one-variable-at-a-time methods (46). The Simplex method has been used for previous applications such as the separation of DNA fragments (47), the achiral separation of amino acids by multidimensional thin-layer chromatography (48), and the achiral separation of phenylthiohydantoin amino

Table II. Selected Data Points from the Simplex Method with CD Concentrations in Millimolars

Trial number	β-CD	γ-CD	CRF
1	0	0	undefined
2	9.7	7.8	–253.7
3	2.6	29.0	–491.1
4	12.3	36.8	–509.6
5	9.2	27.6	–545.4
8	11.1	17.4	–444.7
9	7.45	18.9	–425.5
10	13.3	9.5	–300.8
13	7.7	11.3	–279.8
15	3.5	2.7	–236.0
23	6.7	2.5	–205.2
26	8.1	4.4	–220.7
28	4.6	2.1	–208.0
30	5.7	1.4	–200.6
33	5.8	1.8	–201.3
34	6.0	0.7	–200.3
39	5.8	0.8	–200.2
45*	5.8	0.9	–200.2
46	5.7	0.9	–200.3
48*	5.8	0.9	–200.2

* Denotes optimum separation conditions according to the Simplex.

acids by MEKC (49). The use of the Simplex method with amino acids as a test case is appropriate for quickly determining the optimum concentrations of CDs needed for a complex mixture enantiomeric separation. These separations can be complex because of the need for chiral resolution between individual enantiomers and achiral separations of the different compounds in the mixture.

An equation was generated for the calculation of analyte mobility whenever two CDs are present in the running buffer. In the case of a β- and γ-CD system, the observed mobility of an analyte is given by the following equation:

$$\mu_{obs} = f_{rb}\mu_f + f_{\beta\text{-CD}}\mu_{c_{\beta\text{-CD}}} + f_{\gamma\text{-CD}}\mu_{c_{\gamma\text{-CD}}} \qquad \text{Eq. 4}$$

where the f variables are the fractional amounts of the analyte associated with the phases involved (running buffer or CDs) and the μ variables are the apparent mobilities of the analyte when fully associated with those phases. The final expression for analyte mobility with our two-CD system is given by the following equation:

$$\mu_{obs} = \frac{\mu_f + K_{\beta\text{-CD}}[\beta\text{-CD}]\mu_{c_{\beta\text{-CD}}} + K_{\gamma\text{-CD}}[\gamma\text{-CD}]\mu_{c_{\gamma\text{-CD}}}}{1 + K_{\beta\text{-CD}}[\beta\text{-CD}] + K_{\gamma\text{-CD}}[\gamma\text{-CD}]} \qquad \text{Eq. 5}$$

In order to apply this equation with other CDs, it is only necessary to determine the appropriate K_c values and complex mobilities. Furthermore, an expression could be developed for three or more CDs in the running buffer. When using multiple CDs it is assumed that the effects of the CDs on mobilities are additive and the CDs do not interact with each other. The ranges employed in the Simplex were 0 to 15mM for β-CD because of its limited solubility and 0–30mM for γ-CD because of poor CRF values at high concentrations of γ-CD.

Some example data from the Simplex method can be seen in Table II with the corresponding CRF values. Three conditions from the Simplex table (poor, moderate, and good CRF values) were evaluated experimentally. It can be seen from Figure 3 that the experimental results concurred with the predictions from the Simplex. The separation in Figure 3A (Table II, trial 4) showed that the Dns-Val enantiomers were very poorly resolved and the Dns-Leu enantiomers comigrated. A poor CRF was the result for this condition because of the large penalty for insufficient resolution. In the second separation in Figure 3B (Table II, trial 9), it can be seen that at this point Dns-Leu was somewhat resolved and Dns-Val was resolved better than it was in Figure 3A. The CRF was calculated (Table II, trial 9) using experimentally observed resolution. The experimental (–395) and equation 5 (–425) CRF values agreed reasonably well considering the inaccuracy in graphically computing resolution from electropherograms such as these. It should also be noted that the correlation coefficients were not as good for the Dns-Leu and Dns-Val data with γ-CD in Table I. This could explain why there was a slight difference between the CRF values mentioned previously. The final separation (Figure 3C) illustrated that the best conditions predicted by the Simplex (Table II, trials 45 and 48) provided a superior separation of the amino acids because the enantiomers of Dns-Leu and Dns-Val were resolved and the enantiomers of Dns-Ser showed slight resolution. The conditions for Figure 3C demonstrated that a low concentration of γ-CD was beneficial for the separation of the early eluting enantiomers. This is consistent with the data in Table I because the K_c values were similar for the enantiomers of Dns-Leu and Dns-Val with γ-CD; therefore, high concentrations of γ-CD lead to the bunching up of the early eluting enantiomers. Finally, we observed that the use of two CDs in the running buffer does provide a better separation than when only one CD is used.

Studies with modified single-isomer CDs

Several CDs were used in preliminary studies to determine whether they could provide the resolution of Dns-amino acid enantiomers. The important factors in determining the resolution of enantiomers in these studies were efficiency (uniformly high in this work), selectivity, the ability to manipulate the CD-enantiomer equilibrium to produce a suitable balance between free and complexed forms (poor inclusion into the CD or low CD solubility can be limiting factors), and an appreciable difference in free and complexed mobilities (an elution window consideration). It is generally thought that inclusion complex

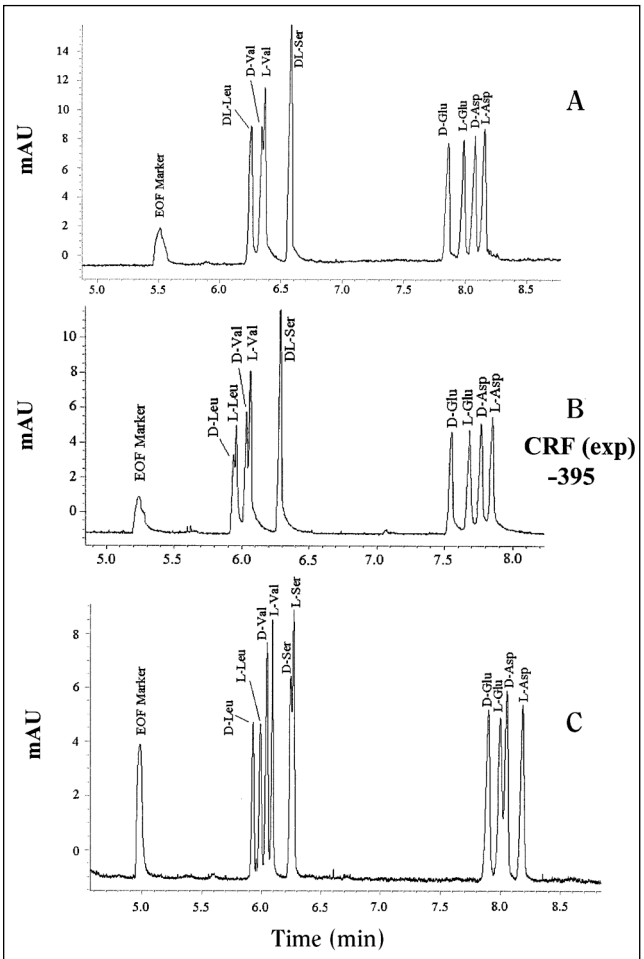

Figure 3. Examination of the Simplex predictions from Table II with the separation of amino acids at various CD concentrations (experimental conditions are the same as in Figure 1): (A) 9.2mM β-CD and 27.6mM γ-CD (trial 4 from Table II), (B) 7.45mM β-CD and 18.9mM γ-CD (trial 9 from Table II), and (C) 5.8mM β-CD and 0.9mM γ-CD (trials 45 and 48 from Table II). An experimental CRF value is shown in Figure 3B.

formation between CDs and CD derivatives and Dns-amino acids occurs by inclusion of the Dns moiety into the cavity. Differences in K_{CD} values are attributed to different interactions between the carboxylate and amino acid side chains, especially with the secondary hydroxyl groups at the C2 and C3 positions of the CD (25). This situation is depicted in Figure 4 and it aids in the visualization for the molecular modeling of amino acids with the CD. Figure 5A shows a separation without any CD present in the running buffer. β-CD used alone was able to provide some enantiomeric resolution of all the amino acids (although not as good as certain β- and γ-CD combinations), and good inclusion was seen based on the analyte mobility change upon the addition of this CD (Figure 5B). The derivatized CDs employed in our studies were all single isomer because their use facilitated reproducible separations and molecular modeling. It should be noted that most commercial CD derivatives consist of mixtures having different degrees of substitution. One exception is commercially available, single-substituted 1-CM-β-CD, which has been successfully employed for the separation of neutral solutes and seemed to be a reasonable candidate for testing in this work. This CD exhibited fair inclusion with the amino acids as well, but it did not provide enantiomeric resolution (as seen in Figure 5C). This could be because of the narrow elution window that was found with this –1-charged CD. That is to say, $\mu_c - \mu_f$ for β-CD and 1-CM-β-CD were both positive, but larger for β-CD. It is unlikely that pH adjustment would improve the situation. HDMCM-β-CD (–7 charge) as synthesized in our laboratory has been used for

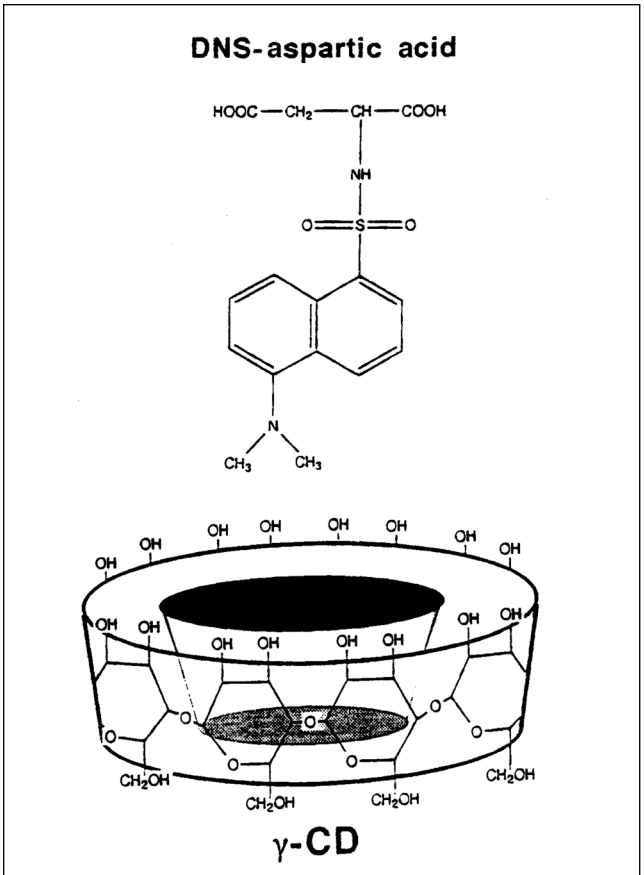

Figure 4. Depiction of Dns-Asp with the Dns group poised to insert into the cavity of γ-CD. Secondary hydroxyl groups (two per sugar) are shown on the top lip of the CD and primary hydroxyl groups (one per sugar) are shown on the truncated bottom of the CD.

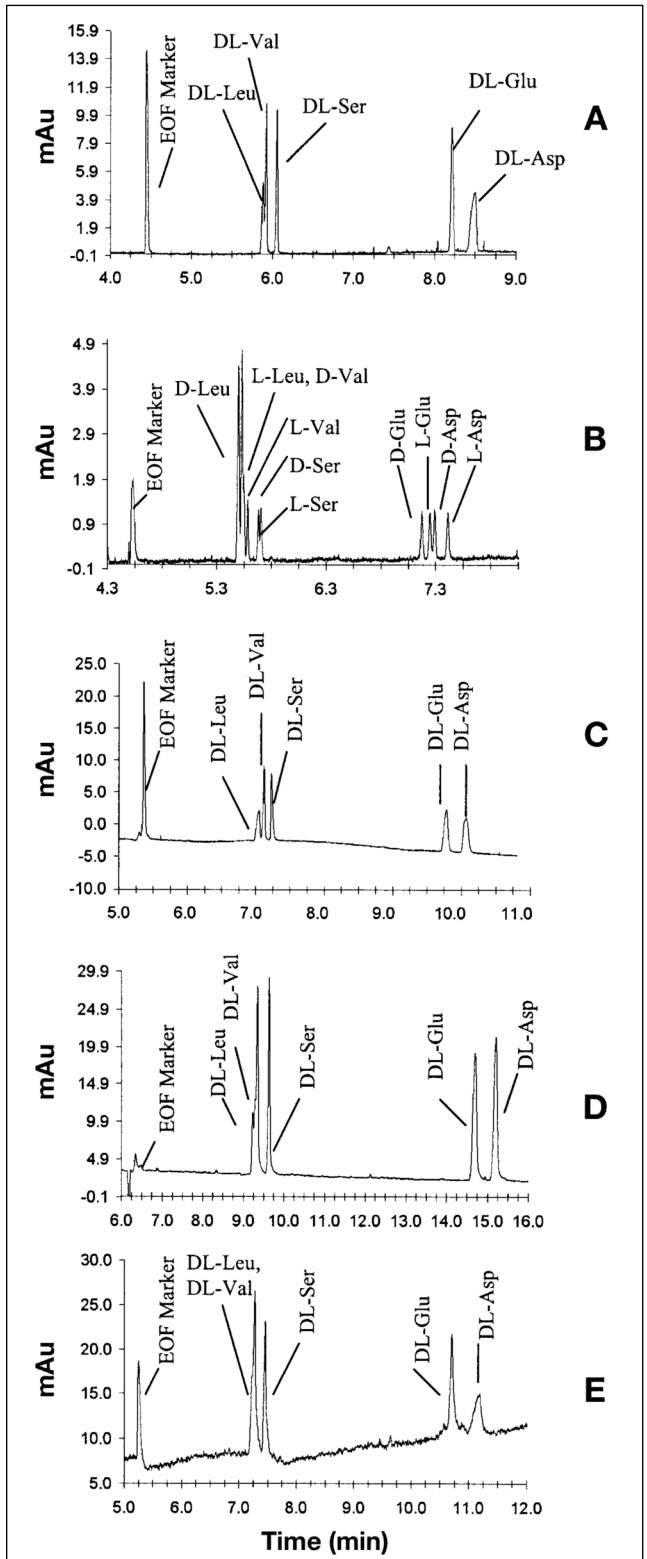

Figure 5. Separation of amino acids with various CDs (experimental conditions are the same as in Figure 1): (A) no CD present, (B) 5.0mM β-CD, (C) 5.0mM 1-CM-β-CD, (D) 5.0mM HDMCM-β-CD, and (E) 5.0mM 16-me-γ-CD.

the separation of naphthalene compounds (39). It was tested because of the presence of the naphthalene ring in the Dns group of the amino acids. This CD should provide a large separation window ($\mu_c - \mu_f$ is negative and large) because of its high negative charge. However, HDMCM-β-CD (Figure 5D) did not provide enantiomeric resolution, probably because of the electrostatic repulsion that occurs between the negatively charged Dns-amino acids and the highly negative-charged HDMCM-β-CD. When adjustments were made for EOF variability, the effect of HDMCM-β-CD on the observed mobilities of the amino acids was much smaller than for β-CD. Without a good level of inclusion there can be no enantiomeric resolution.

16-me-γ-CD (also synthesized in our laboratory) did not provide enantiomeric resolution and, surprisingly, also did not provide very good inclusion of the amino acids (Figure 5E). A methylated CD seemed to be a plausible buffer additive, because it was thought that the naphthalene ring of the amino acid would be favorably included into the nonpolar environment of the methylated CD. In regards to enantioselectivity, it has been found that methylation affects the steric or hydrogen-bonding interactions (or both) between the CD and the analyte. Yoshinaga et al. (25) found that there is a major participation of the secondary hydroxyl groups in the chiral recognition process. They found that the methylation of the C2 hydroxyl groups in β-CD resulted in the absence of enantiomeric resolution except for Dns-D,L-α-amino butyric acid. We anticipated that a similar absence in enantiomeric resolution might be observed for our fully methylated γ-CD product, especially given that native γ-CD was somewhat less effective than the smaller β-CD. However, the issue of enantioselectivity never came into play, because we found that the mobilities with 16-me-γ-CD present in the running buffer were similar to the mobilities in the absence of CD, thus implying only small inclusion with the amino acids and 16-me-γ-CD (as will be further discussed). Also, inclusion cannot be forced by employing a high CD concentration because of solubility limitations.

Molecular modeling of various CDs

An amino acid (valine) was docked into five different single-isomer CDs as described in the Experimental section. The interaction energies (kcal/mol, average of D and L forms) of the amino acid with the five CDs were as follows: –36.3 for β-CD, –35.0 for γ-CD, –29.3 for 1-CM-β-CD, –17.3 for HDMCM-β-CD, and –28.4 for 16-me-γ-CD. It should be stated that with quick grid search docking using only 250 minimizations at each grid position, the listed relative interaction energies were very approximate (41). Molecular modeling was not performed to study enantioselectivity in this work because the subtle differences in the interactions between CDs and enantiomer pairs would require extremely long, comprehensive modeling approaches (41). It can be seen that native β- and γ-CD had the best interactions with the amino acid, possibly in part because of the hydrogen bonding between the amino acids and the secondary hydroxyls. In agreement with the experimental data in Table I, the inclusion with β-CD was stronger. 1-CM-β-CD produced an interaction energy that was lower than β-CD, again consistent with experimental findings. Despite the expected substitution of the carboxymethyl group at the C6 primary hydroxyl site (confirmed by nuclear magnetic resonance spectrometry), electrostatic repulsion between the similarly charged CD and Dns-amino acid may have contributed to the less favorable interaction energy. The weaker interaction and possible changes in selectivity (relative to native β-CD) were probably less of an issue in terms of enantiomeric resolution with 1-CM-β-CD than the limited elution window (as previously mentioned). The poorest interaction energy of all was with HDMCM-β-CD (–7 charge). This partially explains the lack of enantiomeric separation observed experimentally with this CD, although enantioselectivity could also be compromised with the extensive functionalization of this CD.

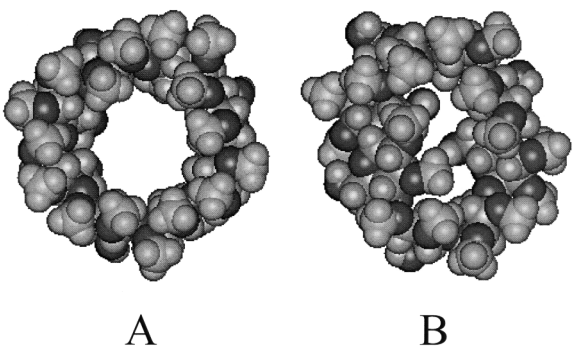

Figure 6. Space-filled model depictions of the (A) open and (B) closed cavity 16-me-γ-CD conformations used in modeling studies. The view is from the top of the CD (methylated at the C2 and C3 positions).

A comparison of the molecular modeling and experimental findings for 16-me-γ-CD revealed both the value and potential pitfalls of elucidating modes of molecular recognition using modeling approaches. The interaction energy listed previously for Dns-Val with this CD was more favorable than with 1-CM-β-CD, yet it exhibited a negligible effect on the mobility of the amino acid. This contradiction was resolved by searching for less inclusion-friendly conformations of the 16-me-γ-CD. The minimized open structure shown in Figure 6A was used in the initial work. By manipulation of the inward methyl groups, another conformer was achieved, as shown in Figure 6B. The minimized energy of this structure was approximately 1.6-kcal/mol more favorable than the initial Figure 6A structure and showed significant van der Waals stabilization, presumably because of intramolecular interactions between the various methyl groups located at the C2 and C3 positions. The local energy minimum structure in Figure 6A may have converted to the lower energy conformer in Figure 6B with more minimization steps (41). Simulated annealing may also be used to locate the lower energy conformer. The closed cavity of the conformation in Figure 6B did not appear to be conducive to inclusion. Also, under certain conditions we have noted inter-CD interactions that inhibit inclusion (39). One can envision in an aqueous environment, inter-CD van der Waals interactions between the lipophilic C2 and C3 methylated portions of two (or more) 16-me-γ-CDs, thereby limiting analyte access to CD cavities. Although none of the derivatized CDs that were

studied showed potential for Dns-amino acid enantiomeric separations, the correlation with modeling work illustrated its value.

Conclusion

This study presents the separation of mixtures of Dns-amino acid enantiomers with native CDs and the subsequent determination of mobility and K_c data. An equation was developed to predict the analyte mobility for a two-CD system in the running buffer. The best separation conditions were quickly located prior to setting foot in the laboratory with the use of a Simplex-optimization method. Various conditions from the Simplex data were tested to see if a CRF used in this work was an accurate measure of the quality of the separation. It was found that the predictions from the Simplex agreed with the experimental results obtained with the CE instrument. These studies should provide researchers with useful information when attempting to optimize separation conditions for complicated mixtures in a short period of time. A few additional commercial or synthesized single-isomer CDs were evaluated as to their potential for Dns-amino acid separations with disappointing results. Poor separations of enantiomers can be attributed to factors such as elution window reduction, poor inclusion, loss of enantioselectivity, or all three. Molecular modeling studies were informative with regard to poor inclusion and could provide insights into enantioselectivity in future studies.

Acknowledgments

This work was sponsored by the Division of Chemical Sciences, Office of Basic Energy Sciences, U.S. Department of Energy under Grant DE-FG02-96ER14609 with the University of Tennessee, Knoxville. The authors would like to thank Mustafa Culha for supplying the synthetic CDs and Marco De Jesus for assistance with the modeling work.

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Manuscript accepted July 17, 2001.