Computational Chemical Analysis of Enantiomer Separations of Derivatized Amino Acids in Reversed-phase Liquid Chromatography

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Abstract

A fast quantitative structure-retention relationship method is required in chromatography for rapid optimization of chromatographic separation conditions. Chromatographic data of amino acid enantiomers were analyzed using a computational chemical method to simulate chromatographic separation. Using computational chemical calculations, the direct interaction between a model-phase and an enantiomer was calculated as an energy value using the MM2 calculation. Computational chemistry using a model adsorbent is a new method for quantitative analysis of retention in reversed-phase liquid chromatography. The correlation coefficient was 0.938 (n = 14) between the retention factors of derivatized amino acids and interaction energy values of the final structure (ΔFS) between an analyte and a model pentyl-bonded phase.

Keywords. QSRR, Liquid chromatography, Computational chemistry, Derivatized amino acid enantiomers

Abbreviations and notations

FS, final structure energy	VW, van der Waals
HB, hydrogen bonding energy	MM, molecular mechanics
ES, electrostatic energy	FEF, (+)-1-(9-fluorenyl)ethyl chloroformate

1 INTRODUCTION

A variety of chiral recognition phases have been synthesized and their chiral selectivity has been analyzed using a computational chemical method, especially for Pircle type phases [1-9]. The calculated energy values predicted the elution order of enantiomers but did not reflect the separation factor (α) of the enantiomers. One reason for this is that the density of bonded chiral phases is not consistent compared to alkylsilane-bonded phases, whose carbon contents and reproducibility in chromatography are consistent [10]. Thus, the reproducibility of retention time is not as reliable for chiral phases, and it is difficult to develop a model phase for computational chemical analysis. Untreated silanols affect the retention of analytes because hydrogen bonding is the predominant retention force for enantiomer separations in normal-phase liquid chromatography.

On the other hand, analytes derivatized with a chiral derivatization reagent have been separated in reversed-phase liquid chromatography [11-18]. A computational chemical simulation of the retention of phenolic compounds in reversed-phase liquid chromatography was achieved using a molecular mechanics calculation with a model phase where the retention factors of phenolic compounds, even partially ionized phenolic compounds, were related to calculated energy values

with high precision [19]. In this present paper, reversed-phase liquid chromatography of derivatized amino acids was analyzed using a molecular mechanics calculation, and the retention factors and separation factors were measured in isocratic elution related to energy value changes between an analyte and a model-phase.

2 COMPUTATIONAL CHEMICAL ANALYSIS

A Power Macintosh G3 computer equipped with a 450-MHz processor and 512-MB memory, and a Dell model Latitude C840 equipped with a 2-GHz processor and 1024- MB memory were used. The molecular properties of analytes and model phases and molecular interactions were calculated by molecular mechanics (MM2) using version 5 of the CAChe™ program from Fujitsu, Tokyo, Japan. The standard parameters used were bond stretch, bond angle, dihedral angle, improper torsion, van der Waals force, hydrogen bond, and electrostatic energy (MM2 bond dipoles). The van der Waals force cut-off distance was 9Å. The energy unit was kcal/mol (1 kj/mol = 4.18 kcal/mol). The Cricket-Graph™ program from Computer Associates (San Diego, CA, USA) and Project Reader of CAChe™ program were used for data analysis.

3 RESULTS AND DISCUSSION

A model phase was constructed based on dimethylpentylsilane, consisting of 991 atoms, 1051 bonds, and 15193 connectors, containing 171 silicones, 328 oxygens, 143 carbons, and 349 hydrogens. Dimethylpentylsilane (20) and trimethylsilane (1) were bonded within 900 $Å^2$ in the polysilicone dioxide phase. The trimethylsilane was considered to be an end-capped molecule. The optimized energy value change was less than 0.00001 kcal/mol. The molecular size and alkyl-chain length were determined by the calculation capacity of the computer used and the alkyl-chain length effect for the hydrophobicity [10]. An optimized structure of the model phase and a complex between this model phase and an enantiomer is shown in Figs. 1 and 2, respectively. Dimethylpentyl groups are in close proximity due to their steric hindrance. Some of them lie in free space after the optimized a molecular interaction.

In this bonded-phase, dimethylpentyl groups surrounded one trimethyl group. Silanol groups around the trimethylsilanol group were completely covered by alkyl groups. The silanol group effect might not be a factor. The first circle of the dimethylpentyl groups might not be pushed down by the presence of an analyte. The second circle of dimethylpentyl groups should support the first circle of dimethylpentyl groups. The calculated energy values of the amino acid enantiomers derivatized with (+)-1-(9-fluorenyl)ethyl chloroformate by MM2 are listed in Table 1 with chromatographic data measured in reversed-phase liquid chromatography from reference 14. The

calculated energy values are final (FS), hydrogen bonding (HB), electrostatic (ES), and van der Waals (VW) energy. The energy values of individual complexes between the model pentyl-phase and an enantiomer are listed in Table 2 as FS1, HB1, ES1, and VW1. For docking between an analyte and the model phase, the most hydrophobic site of the analyte was faced towards the hydrophobic model phase whose silanol activity was eliminated. The molecular interaction energy (Δenergy) was calculated from the following equation. Δenergy = energy value of an analyte + energy value of the model phase - energy value of a complex. The Δenergy values were related to retention factors of the amino acid derivatives. The correlation coefficient between ΔFS and log *k* is shown in Fig. 3.

$\Delta FS = 22.127(\log k) + 20.692$, r=0.938, n=14, ---(1)

A one-to-one molecular interaction model might be ideal for chromatography using a Pircle-type bonded phase, however this model can be applied to reversed-phase liquid chromatography of enantiomer separations, such as for phenolic compounds. Amino acids R- and S-aspargine and R- and S-glutamine do not fit in the above correlation.

Under these liquid chromatographic conditions, the elution order of amino acids was Asn < Gln < Ser < Asp < Thr < Glu < Arg < Ala < Pro. The interaction energy values of Asn and Gln, however, were high. The elution order of amino acids derivatized with orthophthaldialdehyde was Asp < Glu < Cys < Asn < Ser < Gln < Thr < Arg < Ala [20]. The difference in elution order depends on the organic modifiers used in the eluent. The former system [14] used tetrahydrofuran and the latter system used acetonitrile and methanol. Tetrahydrofuran is a selective organic modifier in reversed-phase liquid chromatography. The elution order of analytes did not have a linear relation with the octanol-water partition coefficient, log *P* relative hydrophobicity, in reversed-phase liquid chromatography using tetrahydrofuran as an organic modifier [21]. Acetonitrile is the most suitable organic modifier to obtain a high correlation coefficient between log *k* and log *P*. Therefore, if the purpose is to predict the elution order, the retention factors should be measured in reversed-phase liquid chromatography using acetonitrile as the organic modifier. In the chromatographic results, all R-amino acids eluted before the S-amino acids, however the interaction energy value of S-alanine was smaller than that of R-alanine (Table 1). More precise calculations obtained using a better model phase and solvent effect might resolve this discrepancy. Even though the solvent effect is not predicted by the above calculation, computational chemical simulation of reversed-phase liquid chromatography is useful for determining enantiomer separation.

4 CONCLUSION

The retention factors and the separation factors of amino acid enantiomers derivatized with (+)-1-(9-fluororenyl)ethyl chloroformate measured in isocratic elution on reversed-phase liquid chromatography were related to energy value changes between an analyte and a model-phase. The energy values of their final structures was correlated well to log *k* values of the amino acid derivatives.

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Biographies

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Figure captions

Figure 1. Model dimethylpentylsilane bonded-silica gel. small white ball, hydrogen; large white ball, carbon; small black ball, oxygen; large black ball, silicone.

Figure 2. Adsorption of FEF-S-arginine in the dimethylpentylsilane bonded-silica gel.

Figure 3. Relationship between Δenergy and log *k*, No.1, R-Ser; 2, S-Ser; 3, R-Asp; 4, S-Asp; 5, R-Thr; 6, R-Glu; 7, S-Thr; 8, R-Arg; 9, S-Glu; 10, S-Arg; 11, R-Pro; 12, S-Pro; 13, S-Ala; 14, R-Ala; 15, R-Gln; 16, R-Asn; 17, S-Asn; 18, R-Gln; Δenergy = 22.127 (log *k*) + 20.692, r = 0.938.

Table 1. Chromatographic data and calculated energy values

Amino acid	log k^a	α^b	α^c
Serine R	0.303		
Serine S	0.320	1.04	1.09
Aspartic acid R	0.375		
Aspartic acid S	0.405	1.07	1.03
Threonine R	0.468		
Threonine S	0.520	1.13	1.00
Glutamic acid	0.513		
Glutamic acid S	0.567	1.14	1.07
Arginine R	0.549		
Arginine S	0.605	1.10	1.09
Alanine R	0.751		
Alanine S	0.792	1.00	0.95
Proline R	0.741		
Proline S	0.741	1.09	1.04
Aspargine R	0.127		
Aspargine S	0.164	1.09	1.04
Glutamine R	0.111		
Glutamine S	0.164	1.13	1.04

Table 1. (Continued)

Amino acid	FE	HB	ES	VW
Serine R	-35.288	-20.927	-7.579	6.052
Serine S	-33.205	-19.384	-7.165	5.786
Aspartic acid R	-60.000	-21.349	-26.546	7.460
Aspartic acid S	-59.709	-24.120	-26.975	6.120
Threonine R	-31.208	-11.589	-7.939	5.806
Threonine S	-38.323	-24.938	-8.073	6.657
Glutamic acid R	-49.242	-19.914	-19.216	4.161
Glutamic acid S	-48.433	-17.487	-18.687	6.944
Arginine R	-50.046	-14.696	-33.766	6.954
Arginine S	-52.529	-8.815	-34.164	5.952
Alanine R	-30.544	-10.952	-12.045	5.970
Alanine S	-35.001	-13.680	-11.973	6.923
Proline R	-15.360	-3.649	-10.543	7.054
Proline S	-15.683	-3.735	-10.490	6.595
Aspargine R	-65.290	-20.718	-41.294	7.529
Aspargine S	-66.426	-23.673	-40.734	7.351
Glutamine R	-55.929	-15.865	-30.937	6.201
Glutamine S	-54.911	-17.048	-30.485	6.926
Model phase	-648.713	0.000	-403.448	-400.646

Table 1. (Continued)

Amino acid	FS1	HB1	ES1	VW1
Serine R	-710.131	-18.668	-410.908	-421.484
Serine S	-710.505	-24.279	-409.569	-418.956
Aspartic acid R	-736.477	-18.916	-429.722	-419.922
Aspartic acid S	-737.038	-17.503	-430.095	-425.358
Threonine R	-712.766	-14.450	-411.557	-422.818
Threonine S	-719.963	-24.770	-411.591	-423.652
Glutamic acid R	-729.851	-11.721	-422.989	-426.524
Glutamic acid S	-731.292	-22.132	-422.767	-424.641
Arginine R	-731.092	-12.143	-438.115	-423.195
Arginine S	-736.621	-16.415	-437.406	-426.794
Alanine R	-716.373	-15.449	-414.971	-424.942
Alanine S	-718.915	-16.922	-415.852	-424.892
Proline R	-701.278	-3.615	-413.991	-429.290

Proline S	-703.224	-3.660	-413.679	-431.488
Aspargine R	-745.840	-25.170	-444.725	-421.302
Aspargine S	-748.306	-23.438	-444.369	-422.947
Glutamine R	-738.278	-21.341	-436.870	-424.949
Glutamine S	-738.620	-14.028	-434.048	-432.012

a, converted from retention factors in reference 14; b, separation factor (R/S) calculated from reference retention factos [14]; c, separation factor (R/S) calculated from molecular interaction energy ΔFS.