Theoretical and Experimental Studies of Chiral Recognition in Charged Pirkle Phases

One-Sun Lee, Yun Hee Jang, Young Gi Cho,† Myung Ho Hyun,† Hie-Joon Kim, and Doo Soo Chung*
School of Chemistry, Seoul National University, Seoul 151-747, Korea
†*Department of Chemistry, Pusan National University, Pusan 609-753, Korea*

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The role of electrostatic interactions in enantioselective separation was demonstrated. Enantioselective separation of *N*-(3,5-dinitrobenzoyl)leucine and its esterified analogue was investigated by (*S*)-phenylglycine-based HPLC under an intermediate pH, and examined with a relaxed scan calculation combined with a Monte Carlo conformation search.

Enantioselective separation has become increasingly important in many areas of chemistry including pharmacology, agrochemistry, and catalysis.[1,2] One of the most successful methods of enantioselective separation was developed by Pirkle and collaborators.[3] However, an important aspect that has not been addressed yet in the Pirkle-type host–guest interaction is the electrostatic interaction between charged species. If a separation is attempted under a pH where the carboxylic acid group in the analyte is deprotonated and the amine group in the chiral stationary phase (CSP) is protonated, the structure of the complex would be determined primarily by electrostatic interactions between these oppositely charged groups and secondarily by the π–π interaction or hydrogen bonding. Optimization of conditions affecting the electrostatic interaction could improve enantioselectivity.[4]

In this letter, we present a case where the role of electrostatic interaction in enantioselective separation can be demonstrated experimentally and theoretically: Enantioselective high performance liquid chromatography (HPLC) separation of *N*-(3,5-dinitrobenzoyl)leucine enantiomers (abbreviated as (*R*)-G and (*S*)-G) using an (*S*)-phenylglycine derivative as the CSP under an intermediate pH. Since there would be a stronger influence of the solvent on charged molecules than neutral molecules, simulations were performed both in the gas phase and in solvent, and the results were compared.

HPLC experiments. (*S*)-Phenylglycine and *N*-(3,5-dinitrobenzoyl)leucine were purchased from Aldrich (Milwaukee, WI, U.S.A.). (*S*)-Phenylglycine was endowed with a linker, which was subsequently used to covalently attach the host molecule to the silica stationary phase.[5] All organic solvents used as eluents were of HPLC grade.

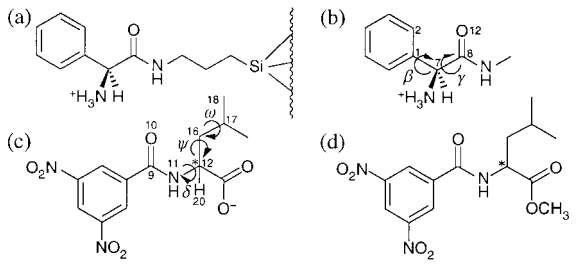

Figure 1. (a) The host used in experiments, (b) the host used in modeling, (c) the guest, (d) the esterified guest used in experiments. Torsion angles β and γ of the host are defined as C_2-C_1-C_7-C_8 and C_1-C_7-C_8-O_{12}, respectively. Torsion angles δ, ψ and ω of the guest are defined as H_{20}-C_{12}-N_{11}-C_9, N_{11}-C_{12}-C_{16}-C_{17}, and C_{12}-C_{16}-C_{17}-C_{18}, respectively. The symbol * indicates asymmetric carbons.

Results obtained using five different mobile phases are summarized in Table 1. Enantioselectivity was lost in the presence of water, probably because water screens electrostatic interactions. In less polar eluents, (*S*)-G was eluted prior to (*R*)-G. It is speculated that stronger electrostatic interactions between $-COO^-$ and $-NH_3^+$ primarily determined the configuration of the complex. With this constraint, the rest of the complex, particularly the two benzene rings, would be configured in a different manner in the two diastereomeric complexes, allowing different extents of secondary interactions, particularly π–π interactions. Among those solvents, the highest resolution (*Rs*) was obtained with dichloromethane. The effect of acetic acid concentration on enantioselective separation was investigated further in dichloromethane (Table 1), and 0.3 vol% acetic acid (pH ≅ 6.5) gave the best result. At higher acid concentrations, the carboxylate group would become more protonated and the charge interaction would decrease. Under the optimized condition, the enantioselectivity factor (α) was 1.20 and *Rs* 2.15 (Figure 2).

The role of the charge carried by the guest was investigated further by attempting the enantioselective separation of the esterified analogue (Figure 1d). The enantiomers of this neutral analogue were not separated under the same HPLC conditions, indicating the important role of the electrostatic interaction between charged groups in the enantioselective separation.

Gas-phase calculations. An approach suggested by Lipkowitz et al. has been used to obtain $\Delta\Delta G$ in Pirkle-type host–guest interactions.[6] In this approach, the Boltzmann distributions of the host and the guest structures are obtained by calculating their energies varying all the significant torsion angles incrementally. Significant structures are selected based on the Boltzmann factor and then treated as rigid bodies in calculating the Boltzmann-averaged energy of the host–guest complex by a Monte Carlo (MC) search.

The structure of the model host, (*S*)-phenylglycine (Figure 1b), was built and energy-minimized in MacroModel 6.0[7] with the

Table 1. Effect of the constituents of the mobile phase.[a]

Solvent[b]	k_S'	k_R'	α	*Rs*
80% CH_3OH in H_2O	8.82	8.82	1	0
80% CH_3CN in H_2O	2.66	2.66	1	0
40% IPA in *n*-hexane	16.36	18.55	1.13	1.18
40% IPA, 20% CH_2Cl_2 in *n*-hexane	5.44	6.10	1.12	0.72
CH_2Cl_2	4.92	5.58	1.13	1.65
CH_3COOH concentration[c]	k_S'	k_R'	α	*Rs*
0.1 vol %	4.92	5.58	1.13	1.65
0.2 vol %	6.01	7.13	1.19	1.85
0.3 vol %	6.51	7.81	1.20	2.15
0.4 vol %	6.82	8.14	1.19	2.00
0.5 vol %	6.66	7.94	1.19	1.28

[a] k': Capacity factor; α: Enantioselectivity factor; *Rs*: Resolution.
[b] 0.1 vol% CH_3COOH and 0.1 vol% Et_3N were added to all the solvents.
[c] CH_3COOH was added to CH_2Cl_2 containing 0.1 vol% Et_3N.

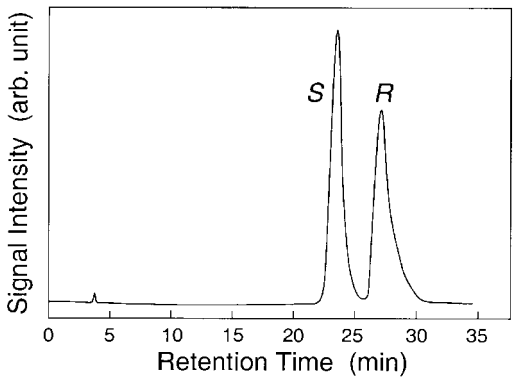

Figure 2. Chromatogram of *N*-(3,5-dinitrobenzoyl)leucine (eluent: 0.3 vol % CH_3COOH + 0.1 vol % Et_3N in CH_2Cl_2).

MM2 force field which is adequate for describing Pirkle-type host–guest interactions.[6] The potential energy surface was obtained by varying its torsion angles, β and γ, by 10° (Figure 3a). Only one minimum was located at (β, γ) = (130°, 130°) and further minimization led to a minimum at more refined angles (134°, 127°). A separate MC conformation search[7] led to the same result. Thus, only this conformer (H_g) was considered for the complex. The same relaxed scan minimization was performed for the guest in three independent variables (δ, ψ and ω) by a total of 42 875 calculations [(35 for δ) × (35 for ψ) × (35 for ω)]. Figure 3b shows a potential energy curve as a function of δ. There are two minima, one at δ = 20° (*syn*) and another at δ = 170° (*anti*). The *anti* conformer was 14 kJ/mol less stable than the *syn* conformer, probably due to larger repulsion between the two carbonyl oxygens. About 99% of the guest molecules should exist as *syn* and thus only the *syn* conformer was considered in the simulation. Figure 3c shows a contour diagram of potential energies of this *syn* conformer as a function of ψ and ω. Five stable conformers (G1–G5) were located. Since the energy differences were small and the energy barriers between them were low, all of these conformers were included in the calculations. Using these selected conformers, five host–guest complexes (H_g**:**G1, ⋯, H_g**:**G5) were built, assuming a 1:1 ratio of the host to the guest. The host and the guest were treated as rigid bodies.[6] Minimum-energy configurations of the complexes were identified by the MC search method by rotating and translating the guest relative to the host randomly. Ten thousand host–guest complex structures were tried. The Boltzmann-averaged $\Delta\Delta G$ was calculated[6] over the stable configurations of the complexes.

The free energy of the (*S*)-H**:**(*R*)-G was 9.2 ± 0.7 kJ/mol lower than that of the (*S*)-H**:**(*S*)-G. This indicates that (*R*)-G would be retained longer than (*S*)-G. The relation

$$\Delta\Delta G = -RT \ln \alpha \qquad (1)$$

leads to a very high enantioselectivity factor α = 40 ± 10. The elution order was correctly predicted but the enantioselectivity was severely overestimated.

Solution-phase calculations. The same approach used in the gas-phase calculation was used in solvent. A continuum solvent model was used with a dielectric constant (ε) of 10 (for dichloromethane, ε = 8.93). Again, only one minimum was located for the host (β = 125° and γ = 121°; H_s) and this was used for the host–guest complex calculation in solvent. For the guest, the relative energy of the *anti* conformer with respect to the *syn* conformer and the barrier between them decrease as the dielectric constant increases (Figure 3b), probably because the solvent screens the

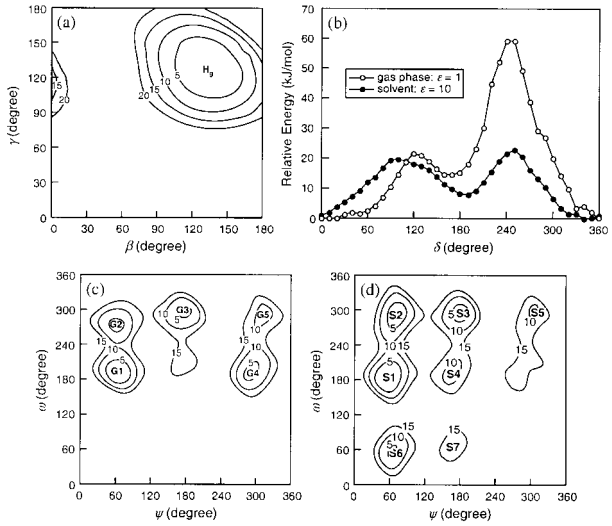

Figure 3. (a) Potential energy contour for the host as a function of β and γ (gas phase), (b) potential energy curves of the guest as a function of δ (gas and solution phases), potential energy contours of the guest as a function of ψ and ω at γ = 20° (c) in the gas phase, and (d) in solution.

repulsion between the two carbonyl oxygens. However, the *syn* conformer is still dominant (97% population) and was considered in the complex simulation in solvent. The potential energy surface of ψ and ω identified seven local-minima (S1–S7; Figure 3d) and all of them were used for the host–guest complex study in solvent. The global minimum energy structures are in Figure 4. The free energy of the (*S*)-H**:**(*R*)-G was 0.6 ± 0.4 kJ/mol lower than that of the (*S*)-H**:**(*S*)-G, leading to an enantioselectivity of 1.3 ± 0.2. Overestimation of $\Delta\Delta G$ in the gas phase calculation was largely resolved by including the solvent effect.

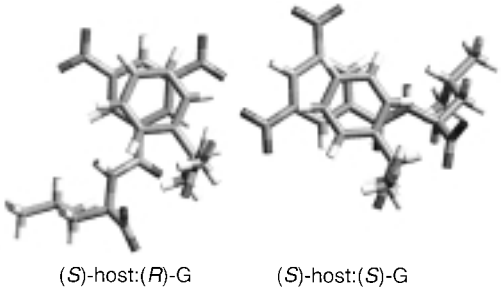

Figure 4. Global minimum structures of the host-guest complexes in solvent.

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