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Deracemization of Racemic Amino Acids Using (*R*)- and (*S*)-Alanine Racemase Chiral Analogues as Chiral Converters

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Deracemization is the stereoselective process of converting a racemate into either a pure enantiomer or a mixture in which one enantiomer is present in excess.[1] Previous studies have shown that (*S*)-alanine racemase chiral analogue (ARCA) [(*S*)-2-hydroxy-2'-(3-phenyluryl-benzyl)-1,1'-binaphthyl-3-carboxaldehyde], developed as a chiral convertor compound that imitates the function of alanine racemase, plays an essential role in the stereoselective conversion of amino acid (Figure 1).[2-5] Since (*S*)-ARCA showed a higher stability with D-amino acids than with L-amino acids, several L-amino acids were preferentially converted to D-amino acids *via* (*S*)-ARCA/D-amino acid imine diastereomer formation. For the deracemization process undertaken in this study, we utilized both (*R*)–ARCA and (*S*)-ARCA as chiral converters, which were expected to generate L- and D-amino acids, respectively, from the starting racemic mixtures.

The stereoselective conversion of amino acids using (*S*)-ARCA has been monitored previously by ^{1}H NMR spectroscopy.[2-5] Although ^{1}H NMR spectroscopy is a useful analytical method for determining stereoselectivity, only the diastereomers of the (*S*)-ARCA-derived Schiff base imines have been monitored during reactions. Also, the analytical technique employed previously has intrinsic drawbacks, since typically the solvent choice for the reaction monitoring is limited to deuterated NMR solvents. In this study, instead of using NMR spectroscopy, we utilized HPLC to perform chiral analysis of the stereoselective conversion reaction. Compared to the NMR method for determining diastereomeric purity, especially, enantiomer separation using chiral HPLC columns would be more accurate and convenient analytical method to ascertain the enantiomeric purity of the final amino acids obtained after acid hydrolysis of the imine diastereomers.[6-10]

Table 1 shows the effect of the solvent in the stereoselective reaction involving racemic phenylalanine and (*S*)-ARCA. After acid hydrolysis of the Schiff base imine diastereomer, chiral HPLC was employed to determine the stereoselectivity of deracemized phenylalanine obtained using (*S*)-ARCA in several solvents. For these experiments, the crown ether type ChiroSil SCA column was used for the enantiomer purity.[10,11] Out of the solvents selected for the stereoselective reactions performed in this work, DMSO and methanol were the most and least effective, respectively. It is considered that the dielectric constant and solubility of the solvent are responsible for the stereoselectivities observed. On the other hand, alcohols gave the worst stereoselectivities, presumably because hydrogen bonding interaction formed by solvation of alcohols might interrupt chiral discrimination for stereoselectivity.

Table 2 shows the stereoselectivity results for several

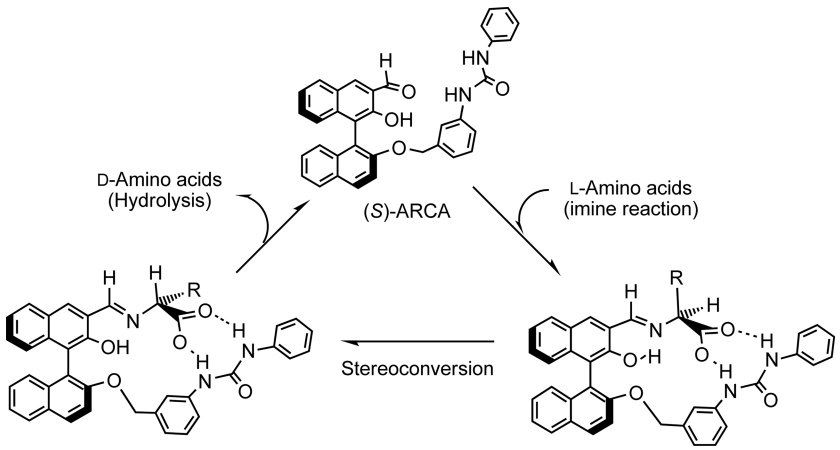

Figure 1. Stereoselective conversion of L-amino acids to D-amino acids *via* imine formation of (*S*)-ARCA used as a chiral converter.

Table 1. Effect of solvent in the stereoselective reaction of (*S*)-ARCA with racemic phenylalanine

Entry	Solvent used	D:L ratio measured by chiral HPLC[a]	RSD (%)[b]
1	DMSO	92.3 : 7.7	1.1
2	DMF	89.5 : 10.5	0.5
3	Tetrahydrofuran	88.9 : 11.1	1.2
4	Dioxane	85.9 : 14.1	0.6
5	Acetone	81.4 : 18.6	0.6
6	Ethanol	63.0 : 37.0	0.7
7	Methanol	59.3 : 40.7	0.6

[a]Chromatographic conditions: Column, ChiroSil SCA; Mobile phase, 90% ethanol/water (v/v) with 10 mM H_2SO_4; Flow rate, 0.8 mL/min; UV detection wavelength, 210 nm. [b]Value is an average of at least three experiments.

racemic amino acids when using (*S*)-and (*R*)-ARCA in DMSO. As noted earlier, starting from racemic amino acids, (*R*)-ARCA and (*S*)-ARCA selectively produce the corresponding L-and D-amino acids respectively. Furthermore, the degree of selectivity of (*S*)-ARCA and (*R*)-ARCA was rather similar; in general, the overall stereoselectivities were greater than 90%. However, amino acid methyl esters showed significantly low stereoselectivities (6–9%) compared to the corresponding amino acids. The reasons for these low stereoselectivities might be related to inefficient hydrogen bonding interactions between the urea moiety of ARCA and the carboxyl group of the amino acid ester (Figure 1).[2,3] Table 2 further shows the stereoselectivities of the amino acids examined using (*R*)- and (*S*)-ARCA, as determined by both chiral and achiral HPLC. The enantiomer purity was measured by chiral HPLC after acid hydrolysis, as described in Table 1. The diastereomer purity was measured using achiral HPLC before acid work-up (Table 2). The stereoselectivity (the diastereomer ratio) obtained by achiral HPLC was slightly higher than that (the enantiomer ratio) determined by chiral HPLC. Caution should be given, because the UV absorption of the diastereomers (D-amino acid/ARCA complex and L-amino acid/ ARCA complex) in the achiral HPLC analysis may not be identical.

Figure 2 shows typical chromatograms showing the resolved enantiomerically enriched thyronines, as well as racemic thyronine on ChiroSil SCA. After acid hydrolysis, chiral separation of the enantiomerically enriched thyronines (D:L = 93.2:6.8 and D:L = 6.9:93.1) obtained using (*S*)- and (*R*)-ARCA, respectively, was performed. Also, Figure 3 presents a typical chromatogram that shows the resolved diastereomers of the 4-fluorophenylalanine (D:L = 92.2:7.8)/(*S*)-ARCA complex by means of achiral HPLC. In addition, Table 2 shows some comparative stereoselective results determined by both the achiral HPLC and NMR methods (entry 7, 9, 10, and 14).[2,5] Comparison of the achiral HPLC and NMR analysis shows somewhat different stereoselectivities, which might be due to the intrinsic features of the relatively less accurate NMR analytical method. Since only deuterated NMR solvents should be used in this technique for the reaction and no overlapped NMR peaks for the diastereomer ratio should exist in resulting NMR spectra, we find that the HPLC method is much more accurate and convenient than NMR for this analysis.[6-8]

In conclusion, our findings show that both (*R*)- and (*S*)-

Table 2. Stereoselectivity results for racemic amino acids using (*S*)-ARCA or (*R*)-ARCA in DMSO

Entry	Racemic amino acids	D:L enantiomer ratio measured using chiral HPLC for (*S*)-ARCA reaction[a]	D:L amino acid/ (*S*)-ARCA diastereomer ratio measured using achiral HPLC for (*S*)-ARCA reaction[b]	D:L amino acid/ (*S*)-ARCA diastereomer ratio measured using NMR for (*S*)-ARCA reaction[c]	D:L enantiomer ratio measured using chiral HPLC for (*R*)-ARCA reaction[a]
1	4-Bromophenylalanine	92.6 : 7.4	95.0 : 5.0	–	7.2 : 92.8
2	4-Chlorophenylalanine	91.4 : 8.6	96.8 : 3.2	–	7.0 : 93.0
3	DOPA	93.9 : 6.1	94.6 : 5.4	–	5.5 : 94.5
4	2-Fluorophenylalanine	93.7 : 6.3	94.7 : 5.3	–	6.6 : 93.4
5	3-Fluorophenylalanine	92.7 : 7.3	93.0 : 7.0	–	7.2 : 92.8
6	4-Fluorophenylalanine	92.0 : 8.0	92.2 : 7.8	–	7.9 : 92.1
7	Leucine	84.7 : 15.3	87.9 : 12.1	90.0 : 10.0	14.5 : 85.5
8	Methionine	92.5 : 7.5	94.2 : 5.8	–	7.5 : 92.5
9	4-Nitrophenylalanine	92.5 : 7.5	92.8 : 7.2	93.3 : 6.7	7.3 : 92.7
10	Phenylalanine	92.3 : 7.7	94.5 : 5.5	91.7 : 8.3	7.7 : 92.3
11	Thyronine	93.2 : 6.8	93.5 : 6.5	–	6.9 : 93.1
12	Tryptophan	91.9 : 8.1	94.6 : 5.4	–	7.4 : 92.6
13	*m*-Tyrosine	93.1 : 6.9	93.3 : 6.7	–	6.9 : 93.1
14	Tyrosine	91.8 : 8.2	92.0 : 8.0	92.3 : 7.7	8.5 : 91.5

[a]Chiral chromatographic conditions: Column, ChiroSil SCA; Mobile phase, 90% ethanol/water (v/v) with 10 mM H_2SO_4; Flow rate, 0.8 mL/min; UV detection wavelength, 210 nm. [b]Achiral chromatographic conditions: Column, Capcell Pak C18; Mobile phase solvent A consisted of 4 mM dipotassium phosphate (K_2HPO_4) solution and solvent B was acetonitrile. The gradient was run as follows: (a) 0–5 min, 50% B in A, (b) 5–20 min, 50–100% B in A and (c) 20–30 min, 100–50% B in A; Flow rate 0.8 mL/min; UV detection wavelength, 230 nm. [c]NMR data taken from previously published work.[2,5]

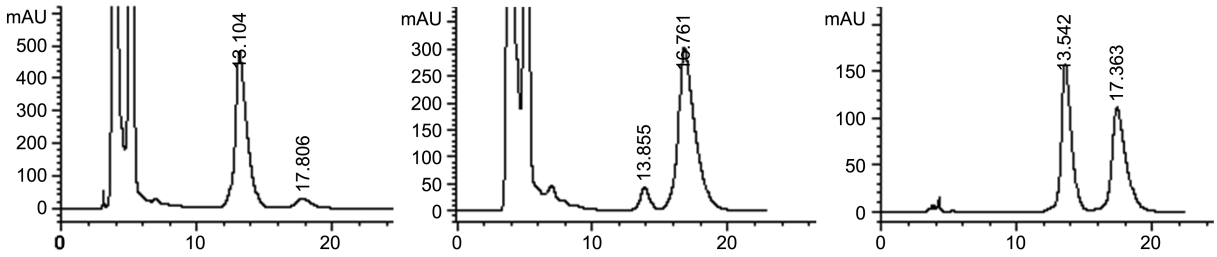

Figure 2. Typical chromatograms of the resolution of enantiomerically enriched thyronines [(D:L = 93.2:6.8) (left) and (D:L = 6.9:93.1) (middle)], as well as racemic thyronine (right) on ChiroSil SCA. The chromatographic conditions are described in Table 2.

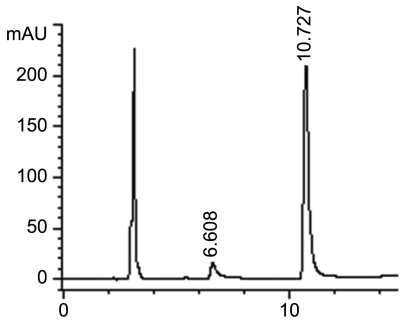

Figure 3. Typical chromatogram of the resolution of diastereomers [4-fluorophenylalanine (D:L = 92.2:7.8)/(*S*)-ARCA complex] on Capcell Pak C18 column. The peaks of retention times at 6.608 and 10.727 min. indicate the L- and D-4-fluorophenylalanine/(*S*)-ARCA complex diastereomers, respectively. The chromatographic conditions are described in Table 2.

ARCA can be practical chiral converters for L- and D-amino acids, respectively, in the deracemization of racemic amino acids. The overall stereoselectivities of both chiral converters are generally greater than 90%. In addition, we developed chiral and achiral HPLC methods for the analysis of stereoselectivity determination. This chromatographic method proved much more accurate and convenient at determining both enantiomer and diastereomer purity than did those previously reported.

Experimental

The chemicals used in this study were purchased from Aldrich (Milwaukee, WI) or Fluka (Switzerland) and were analytical-reagent grade. (*R*)- and (*S*)-ARCA were provided by Aminologics Company (Sungnam, Korea). The amino acid stereoconversion samples using (*R*)- and (*S*)-ARCA were prepared as described previously.[2-5] HPLC studies were carried out using a Breeze system (Waters, Milford, MA) equipped with a binary pump (Waters model 1525), an autosampler, and a dual absorbance detector (Waters 2487 detector). Capcell Pak C18 UG 120 (250 × 4.6 mm I.D., 5 μm) (Shiseido Company, Japan) for diastereomer analysis and ChiroSil SCA (250 × 4.6 mm I.D., 5 μm) (RS Tech, Daejon, Korea) based on (−)-18-C-6-TA for enantiomer analysis were used.[10,11]

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