Pergamon

Tetrahedron Letters 39 (1998) 2579–2582

TETRAHEDRON LETTERS

Further Application of Advanced Marfey's Method for Determination of Absolute Configuration of Primary Amino Compound

Kiyonaga Fujii[a], Tomoe Shimoya[a], Yoshitomo Ikai[b], Hisao Oka[b] and Ken-ichi Harada[a]*

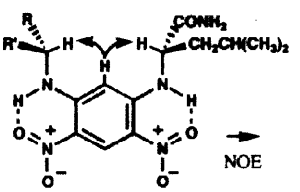
[a]*Faculty of Pharmacy, Meijo University, Tempaku, Nagoya 468, Japan*
[b]*Aichi Prefectural Institute of Public Health, Tsuji-machi, Kita, Nagoya 462, Japan*

Received 11 December 1997; revised 23 January 1998; accepted 26 January 1998

Abstract: The advanced Marfey's method was applied to the determination of the absolute configuration of primary amino compounds and its utility was extended from amino acids to primary amino compounds. This method was successfully applied to the characterization of not only constituent amino acids but also amino compounds in microginin and nostophycin produced by cyanobacteria.

The "advanced Marfey's method" has been developed to non-empirically determine the absolute configuration of constituent amino acids in a peptide using LC/MS and has been sucessfully applied to naturally occurring peptides.[1-5] This method consists of Marfey's method as a chromatographic technique for the separation of amino acids into each enantiomer, the detection of an amino acid by mass spectrometry and a procedure for obtaining the corresponding enantioner from either the L- or D-amino acid. We introduced the "DL-FDLA derivatization" as a procedure for obtaining the corresponding enantioner instead of the conventional chemical racemization.[3] The derivatization with an equal mixture of D- and L-FDLA (1-fluoro-2,4-dinitrophenyl-5-D-leucinamide and -L-leucinamide) was able to give the desired enantiomer from its L- or D-amino acid on the HPLC chromatogram, suggesting that the "advanced Marfey's method" including "DL-FDLA derivatization" is also applicable to the determination of the absolute configuration of primary amino compounds on the basis of the proposed separation mechanism for this method.[6,7] Therefore, we carried out the fundamental approaches of this method on primary amino compounds.

In this method, the resolution between the L- and D-amino acids derivatized with FDLA is due to the difference in their hydrophobicity, which is derived from the cis- or trans-type arrangement of two more hydrophobic substituents at both α-carbons of an amino acid and leucinamide in FDLA, indicating that the α-carboxyl group of an amino acid is not always essential for the resolution (Fig. 1).[6] This consideration suggested that this method is also applicable to compounds possessing an amino group at the asymmetric carbon, primary amino compounds, in addition to an amino acid. In order to confirm the conformation of the FDLA derivative of a primary amino compound, we



Primary amino compound-L-FDLA

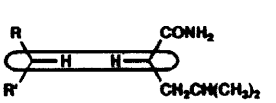


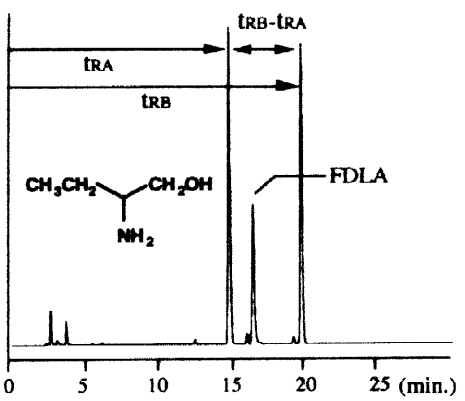
Fig. 1

*Corresponding author. Fax: 81-52-834-8780. E-mail: kiharada@meijo-u.ac.jp.

0040-4039/98/$19.00
PII: S0040-4039(98)00273-1

carried out the conformation analysis of the derivatives of the DL-1-phenylethylamine using UV and NMR spectral methods. As the results, the UV spectra and NOE correlations of the 1-phenylethylamine derivatives were quite similar to those of the amino acid derivatives, indicating that the derivatives of a primary amino compound have a similar conformation to that of an amino acid and the proposed separation mechanism for this method is also applicable to a primary amino acid (Fig. 1).[6]

Several primary amino compounds were analyzed by HPLC after the derivatization using L- and DL-FDLA. As shown in Table 1, these derivatives showed good resolutions between their enantiomers and these results suggested that their resolutions are also reflected by the difference in the hydrophobicity of the two functional groups at the asymmetric carbon with a primary amino group. Therefore, if their hydrophobicity in a primary amino compound is estimated, the elution order of the derivatives can be elucidated and the absolute configuration can be non-empirically determined by this method. Fig. 2 shows an application guideline of the advanced Marfey's method for primary amino compounds, and the absolute configuration can be determinated according to the following steps: (1) derivatization with DL- and L-FDLA; (2) analysis of the DL-FDLA derivative using LC/MS with photodiode array UV detection; (3) confirmation of their conformation based on the UV spectra[6] and of the presence of two peaks of the DL-FDLA derivative on the mass chromatograms monitored at the *m/z* value of the molecular ion species of the derivative; (4) analysis of the L-FDLA derivative giving the chromatogram of either (a) or (b) in Fig. 2; (5) arrangement of more hydrophobic



(R)-2-Amino-1-butanol- DL-FDLA

Table 1. Analysis of primary amino compounds derivatized with FDLA.

Primary amino compounds	t_{RA}		t_{RB}		t_{RB}-t_{RA}
Alaninol	13.2	S	16.8	R	3.6
2-Amino-1-butanol	15.2	S	20.3	R	5.1
Leucinol	20.7	S	27.8	R	7.1
Phenylalaninol	21.4	S	25.9	R	4.5
Phenylglycinol	18.2	S	23.6	R	5.4
APP	17.0	R	20.6	S	3.6
NE	23.7	S	27.6	R	3.9
1-Phenylethylamine	29.5	R	31.4	S	1.9

(min.)

APP : 2-Amino-1-phenyl-1,3-propanediol (*threo*)
NE : Norephrdrine = 2-Amino-1-phenyl-1-propanol (*erythro*)
HPLC conditions: column, TSK gel ODS 80Ts (150 x 4.6 mm I.D.); mobile phase, CH3CN:0.01 M TFA aq.; gradient rate, CH 3CN 30 --> 90% (60 min); flow rate, 1.0 mL/min; detection, UV 340 nm.

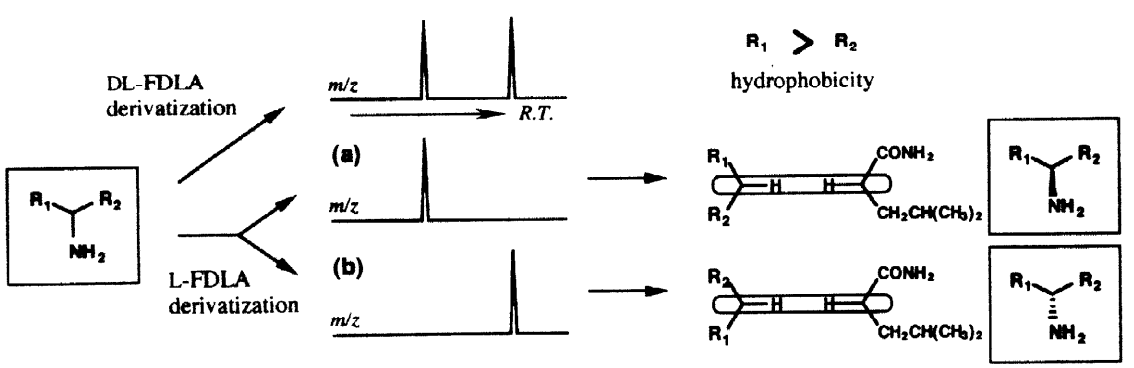


Fig. 2

functional groups on the left side and elucidation of its absolute configuration as shown in Fig. 2. In this method, the estimation of the hydrophobicity of two functional groups (R_1 and R_2) is essential, but no suitable method has been reported so far. We are developing an estimation method based on the separation behavior of amino acid derivatives and it will be reported elsewhere.[8]

In order to demonstrate the applicability of the advanced Marfey's method, we tried to confirm and determine the absolute configurations of constituent primary amino compounds together with amino acids in microginin and nostophycin produced by cyanobacteria. Microginin is a linear peptide containing an 3-amino-2-hydroxydecanoic acid (Adha) unit and the absolute configuration at the C-3 of Ahda had not been elucidated in the first report (Fig. 3).[9] The hydrolysate (6 M HCl, 110 °C, 16 hrs) of microginin (200 μg) was divided into two portions and each portion was derivatized with L- or D-FDLA. The L-FDLA derivatives alone and the equal mixture of D- and L-FDLA derivatives, the DL-FDLA derivatives, were analyzed using ESI LC/MS in the negative ion mode.[10] The DL-FDLA dervatives of Ahda were detected as two peaks (retention time: 27.2 and 36.2 min) and its L-FDLA derivative was detected at 36.2 min on the mass chromatograms monitored at *m/z* 496 $[M-H]^-$. According to the application guideline in Fig. 2, the absolute configuration at the C-3 of Ahda was determined as *R*, because the heptyl side chain can be estimated to be more hydrohobic than the other side. Indeed, the total synthesis of microginin was achieved and the absolute configuration was determined as 3*R*.[11] Nostophycin isolated from *Nostoc* sp. 152 is a cyclic peptide possessing an 3-amino-2,5-dihydroxy-8-phenyloctanoic acid (Ahoa) unit (Fig. 3).[12] The hydrolysate (1.5 M HCl, 110 °C, 14 hrs) of nostophycin (100 μg) was treated in the same way. Figs. 4 (a) and (b) show the mass chromatograms monitored at the *m/z* value of the deprotonated ion of the DL- (a) and L-FDLA derivatives (b) of constituent amino acids and Ahoa. As a result, we can conclude that Pro and Phe have L-configurations and that *allo*-Ile and Glu (from Gln) have D-configurations. Additionally, the absolute configuration at the C-3 of Ahoa can be determined as *R* according to the proposed application guideline.

Thus, the "advanced Marfey's method" was successfully applied to the determination of the absolute configuration of primary amino compounds and its utility was extended from amino acids to primary amino compounds. Although the modified Mosher's method using an NMR technique is effective as a non-empirical method for the determination of the absolute configurations of a primary amino compound, the desired derivatives of the primary amino compounds have to be isolated and a large amount of the derivative is required for this method.[13]

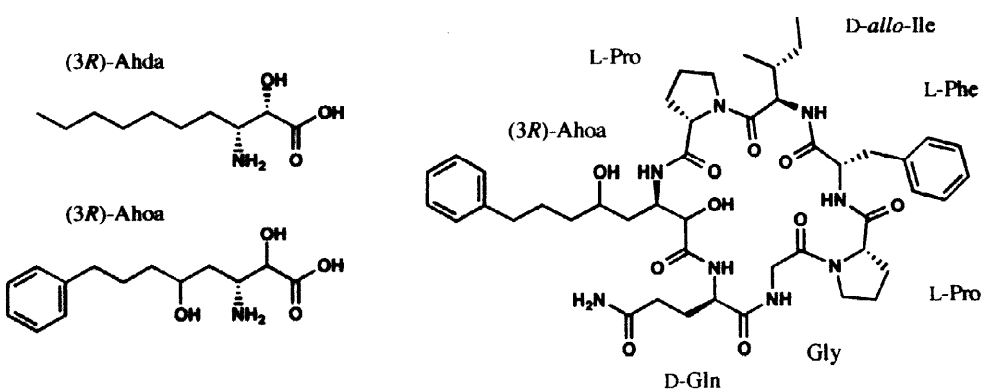


Fig. 3

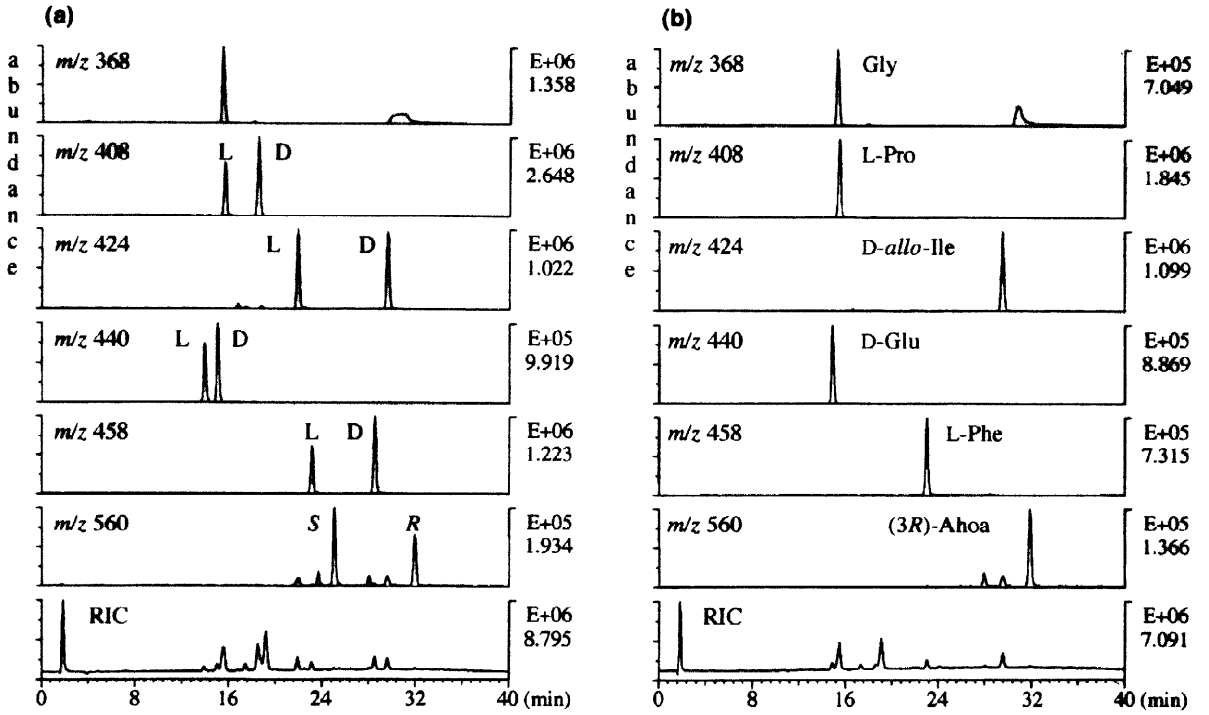


Fig. 4

Acknowledgment. The authors thank Drs. Kenji Matsuura and Hideo Takashina of the Santen Pharmaceutical Company for providing the ESI LC/MS spectra and Dr. Masahiro Murakami, Graduate School of Agricultural Life Sciences, The University of Tokyo, for the generous gift of microginin.

REFERENCES AND NOTES

1. Harada, K.-I.; Fujii, K.; Shimada, T.; Suzuki, M.; Sano, H.; Adachi, K.; Carmichael, W. W. *Tetrahedron Lett.* **1995**, *37*, 1511-1514.
2. Harada, K.-I.; Fujii, K.; Mayumi, T.; Hibino, Y.; Suzuki, M.; Ikai, Y.; Oka, H. *Tetrahedron Lett.*, **1995**, *36*, 1515-1518.
3. Harada, K.-I.; Fujii, K.; Hayashi, K.; Suzuki, M.; Ikai, Y.; Oka, H. *Tetrahedron Lett.* **1996**, *37*, 3001-3004.
4. Fujii, K.; Sivonen, K.; Adachi, K.; Noguchi, K.; Sano, H.; Hirayama, K.; Suzuki, M.; Harada, K.-I. *Tetrahedron Lett.* **1997**, *38*, 5525-5528.
5. Fujii, K.; Sivonen, K.; Adachi, K.; Noguchi, K.; Sano, H.; Hirayama, K.; Shimizu, Y.; Suzuki, M.; Harada, K.-I. *Tetrahedron Lett.* **1997**, *38*, 5529-5532.
6. Fujii, K.; Ikai, Y.; Mayumi, T.; Oka, H.; Suzuki, M.; Harada, K.-I. *Anal. Chem.* **1997**, *69*, 3346-3352.
7. Fujii, K.; Ikai, Y.; Oka, H.; Suzuki, M.; Harada, K.-I. *Anal. Chem.* **1997**, *69*, 5146-5151.
8. For example, in the case of 1-phenylethylamine, the hydrophobicity of the phenyl group is needed for comparison with that of the methyl group. For this purpose, the retention time difference of the FDLA derivatives of phenylglycine and alanine can be used, because it is reflected in the difference of the hydrophobicity between the phenyl and methyl groups.
9. Okino, T.; Matsuda, H.; Murakami, M.; Yamaguchi, K. *Tetrahedron Lett.* **1993**, *34*, 501-504.
10. The FDLA derivatives were analyzed by ESI LC/MS under the following conditions: LC, column, Develosil ODS-HG-5 (150 x 2.0 mm I.D., Nomura Chemical, Seto, Japan); mobile phase, A) H_2O containing 0.01 M TFA, B) CH_3CN; gradient rate, A) : B) = 7:3 --> 3:7 (40 min), linear; flow rate, 0.2 mL/min : MS, TSQ7000 (Finnigan MAT, California, U.S.A.).
11. Matsuura, F.; Hamada, Y.; Shioiri, T. *Tetrahedron* **1994**, *50*, 11303-11314.
12. Fujii, K.; Sivonen, K.; Kashiwagi, T.; Hirayama, K.; Harada, K.-I. *Chem. Commun.* to be submitted.
13. Kusumi, T.; Fukushima, T.; Ohtani, I.; Kakisawa, H. *Tetrahedron Lett.* **1991**, *32*, 2939-2942.