Multi-dimensional HPLC with fluorescence detection as a promising method for trace analysis of chiral amino acids and hydroxy acids in clinical, food and extraterrestrial samples

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Introduction

Amino acids and hydroxy acids are one of the main components of living organisms on the Earth. Most of the amino acids and hydroxy acids have the "asymmetric carbon" and enantiomers are present chemically. However, due to the homochirality of the terrestrial molecules, one of the enantiomers are usually predominant and are playing essential roles in the life systems (e.g. L-forms for amino acids). On the other hand, the minor forms of the enantiomers like D-amino acids are considered to be "not present/not significant" especially in higher animals. However, along with the progress of sensitive and enantioselective analytical technologies, various minor enantiomers have been found in animals, plants and even in human beings. These minor enantiomers are increasingly recognized as new biomarkers in clinical samples and functional molecules in foods/beverages. In the extraterrestrial samples, these organic molecules are considered as origin compounds of life on the Earth. Therefore, enantioselective and quantitative analysis of amino acids, hydroxy acids and related compounds is useful for wade range of research areas. However, the determination of minor enantiomers is frequently interfered with various known/unknown intrinsic substances, and the highly selective analytical method is essential. In the present study, multi-dimensional (2D and 3D) chiral HPLC systems with fluorescence detectors have been designed/developed for the quantitative analysis of these chiral molecules.

Materials and Methods

The biological samples (tissues and physiological fluids) were homogenized with MeOH and centrifuged. The obtained supernatant was dried and amino acids, hydroxy acids were derivatized with 4-fluoro-7-nitro-2,1,3-benzoxadiazole (NBD-F) or 4-nitro-7-piperazino-2,1,3-benzoxadiazole (NBD-PZ). Food samples were also homogenized with MeOH, while the beverage samples were normally diluted with H_2O before derivatization. Extraterrestrial samples were hydrolysed with HCl and were subjected to the derivatization procedure. These reaction mixtures were injected into the 2D- and 3D-HPLC systems combining reversed-phase, anion-exchange/mixed-mode and enantioselective columns. Fluorescence detection of the NBD-derivatives was carried out at 530 nm with excitation at 470 nm.

Results and Discussion

In the first dimension of the multi-dimensional HPLC, a reversed-phase column was used and the target analytes were separated by their hydrophobicity. Because this step is "achiral", target chiral compounds were separated and fractionated as their scalemic D plus L mixtures. These fractions were on-line collected into the "multi-loop" device independently, and injected into the second dimension successively. As the second dimension, an "achiral" anion-exchange mode or a mixed-mode column was used and the target compounds were again separated and fractionated as their D plus L mixtures. The target fractions were on-line collected again to the loop and injected to the third dimension, where the final chiral separations were carried out. For the third dimension, a variety of enantioselective columns including Pirkle-type, cinchona alkaloid type and polysaccharide type columns could be used. The second dimension can be skipped when the sample matrices are not extremely complicated. The present 2D and 3D HPLC systems enable the highly selective analysis of target compounds without losing the sensitivity, because the whole fraction transfer concept is adopted both from 1D to 2D and from 2D to 3D separations. The present systems also enable the highly reproducible and quantitative analysis because the determination is carried out by the fluorescence detectors. By using these multi-dimensional HPLC systems, various D-amino acids, especially, D-Ala, D-Asn, D-Asp, D-Leu, D-Pro and D-Ser were observed in human tissues and physiological fluids, and the amounts were associated with diseases such as chronic kidney diseases. Chiral hydroxy acids (lactate and 3-hydroxybutyrate), and chiral dipeptides (especially containing D-Ser, Ser-Gly and Gly-Ser) were also found in various clinical, food/beverage and extraterrestrial samples, and further studies focusing on their origins and functions are in progress.