APPLICATION OF THERMOSPRAY LIQUID CHROMATOGRAPHY/MASS SPECTROMETRY TO ANALYSIS OF GLYCOSIDES

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Abstract Thermospray liquid chromatography/mass spectrometry (TSP LC/MS) was applied to the analysis of glycosides. Mono-, di-, trisaccharide glycosides belonging to iridoid and flavonoid glycoside and saponin were used. It was found that the ion source block temperature was an influential factor on sensitivities and/or peak shapes and it was suggested that the optimum temperatures were affected by the saccharide number rather than the structures of aglycon moieties. The optimum temperature for monosaccharide glycosides was ca. 220°C and that for disaccharide glycosides was ca. 300°C. The best sensitivity and peak shape for saikosaponin C, a trisaccharide glycoside, was given at a temperature of 340°C. The mass spectrum of each compound gave diagnostic ions indicating molecular weight as well as sugar and aglycon moieties.

Key words thermospray, LC/MS, glycoside

Many glycosides play important roles as drugs and dyes. They are highly polar and thermally labile and their analyses by mass spectrometry have been carried out with soft ionization techniques. We have already reported results of the application of thermospray liquid chromatography/mass spectrometry (TSP LC/MS) to the identification of iridoid glycosides in extracts from plants. [1, 2] These are the first examples of application of TSP to the analysis of glycosides, as far as we know. During these investigations, it was found that the ion source block temperature which gave the best sensitivity to monosaccharide iridoid glycosides and to disaccharide iridoid glycosides differed. We now report the results of the application of the method to other kinds of glycosides, i.e., flavonoid glycosides and saponins. Sensitivity dependence on ion source block temperature for these substances has been investigated and compared with that of the iridoid glycosides.

EXPERIMENTAL

Iridoid glycosides such as geniposide and genipin gentiobioside were donated by the faculty of pharmaceutical sciences of Kyoto university. Rutin, saikosaponin A and saikosaponin C were purchased from Wako Chemicals (Osaka, Japan). The instrument used was LCMS-QP1000EX (Shimadzu) equipped with a thermospray interface (Vestech, U.S.A.). The mobile phase was 0.1M ammonium acetate and methanol (70:30, v/v). The mobile phase was degassed prior to use and was delivered at 1.0ml/min with LC-6A pump (Shimadzu). Each compound was dissolved with the mobile phase and introduced to the mass spectrometer directly, without a column. The injection amounts were: monosaccharide glycosides; 2.5µg, disaccharide glycosides; 5µg, trisaccharide glycoside; 10µg. The ion source temperature was changed by ca. 10°C from 190°C to 380°C. After the temperature had stabilized, each compound was injected three times. The ion source TH temperatures (The ion source entrance temperature) were kept ca. 20°C higher than the ion source block temperature. [3] The investigation of the vaporizer temperature was carried out and optimum values were 2-5°C below takeoff, similar to most compounds amenable TSP LC/MS. [3] The ionization mode used was pure thermospray mode.

RESULTS AND DISCUSSION

In the table, the compounds used were classified according to the structure of aglycon and the number of saccharide residue, for convenience.

Table Compounds used for this study

type of glycoside	aglycon	sugar moiety		
		mono	di	tri
flavonoid glycoside	flavonoid		rutin	
saponin	triterpene		saikosaponin A	saikosaponin C
iridoid glycoside	iridoid	geniposide etc.	genipin gentiobioside	

Fig. 1 shows the change in peak height of TIC as a function of the ion source block temperature. Iridoid glycosides used for this study were from *Gardenia jasminoides* and the influence of the block temperature, on not only geniposide, but also on other monosaccharide glycosides, was examined. Because all of them gave similar results, only the case of geniposide is shown in Fig. 1. Although the peak shapes of monosaccharide glycosides were independent of the temperature, those of glycosides with more than two saccharides were influenced considerably. As an example, TICs of genipin gentiobioside at 220°C (the optimum for monosaccharides) and at 300°C (the optimum for genipin gentiobioside) are shown in Fig. 2. The optimum ion source block temperatures were 1) ca. 220°C for the monosaccharides, 2) ca. 300°C for the disaccharides, and 3) ca. 340°C for the trisaccharides. These optima in block temperature appear to be independent of the type of aglycon moiety, but seem to be related number of saccharide residues. As the number of saccharide residues increases, the optimum temperature becomes higher; a phenomenon which can not be explained by the proposed main ionization mechanism of TSP (gas phase ion-molecule reactions) [4, 5]. The ion-molecule reaction predicts that when increasing the number of the saccharide residues, lower block temperature is desirable because of increasing thermally instability. [6, 7] A clear explanation of the ionization mechanism of TSP has not been made. Vestal has advocated a mechanism including ion evaporation prior to ion-molecule reaction. He has mentioned that ion evaporation plays an important role for ionization of non-volatile compounds and high ion source block temperature is required for ionization of non-volatile compounds. [3] Although the mechanism of ion evaporation is still controversial [8], the idea that some kind of ionization step in liquid phase is involved in TSP ionization seems to be accepted. The observed fact here that a higher temperature was more suitable as the number of saccharide residues increased appears to indicate that reducing the volatilities of glycosides is more influential than increasing the thermal instability. This may support the contribution of ionization steps besides ion-molecule reaction, e.g. ion evaporation, to the ionization mechanism of TSP. The mass spectra of glycosides listed in table 1, acquired at each optimum ion source block temperature, are shown in Fig. 3(a) ~ (e). Diagnostic ions indicating sugar and aglycon moieties are clearly observed, along with the ions in molecular ion regions. From this study, it is suggested that the optimum ion source block temperature can be predicted by the saccharide number. Additionally, at the optima in block temperature, mass spectra of glycosides gave information satisfactory for identification.

Fig. 1 Influence of ion source block temperature on peak height of total ion current

Fig. 2 Total ion current of genipin-gentiobioside
(a) ion source block: 220°C
(b) ion source block: 300°C

(d)

(e)

Fig. 3 (a) Mass spectrum of geniposide
 (b) Mass spectrum of genipin-gentiobioside
 (c) Mass spectrum of rutin
 (d) Mass spectrum of saikosaponin A
 (e) Mass spectrum of saikosaponin C

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