Atmospheric Pressure Spray Mass Spectra of Thermolabile and Nonvolatile Compounds

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Atmospheric pressure spray (APS) mass spectra of thermolabile and nonvolatile compounds such as saccharides, glycosides, peptides, phospholipids and glycolipids are presented. The APS method can produce intense protonated and/or cationized molecules such as $(M+H)^+$, $(M+Na)^+$ and $(M+K)^+$ for these compounds without any buffers or external ion sources, unlike other ionization methods. The APS method combined with a collision-induced dissociation technique can provide significant fragment ions, which are very useful for structural elucidation.

1. Introduction

Mixture analyses of thermolabile and nonvolatile compounds by liquid chromatography/mass spectrometry (LC/MS) are important targets in mass spectrometry. Several ionization methods including thermospray (TSP)¹, ion spray (IS)², laser desorption (LD)³, fast atom bombardment (FAB)⁴ and atmospheric pressure ionization (API)⁵ are used for LC/MS and their systems are applied to several analytical fields. However, studies on ionization methods suitable for LC/MS have been continued because analysts using LC/MS desire milder and more sensitive methods for nonvolatile compounds.

The authors have developed a new ioniza-

tion method, that is, an atmospheric pressure spray (APS) ionization method⁶. This method can produce intense protonated and/or cationized molecules of nonvolatile compounds only by nebulizing sample solutions at a flow rate of 1 ml/min under atmospheric pressure. This method seems to be suitable for an ionization method for LC/MS. The purpose of this paper is to demonstrate that the APS ionization is very promising for the mass spectrometric study of thermolabile and nonvolatile compounds.

2. Experimental Section

2.1 Apparatus

A schematic diagram of the liquid chromatograph/atmospheric pressure spray mass spectrometer system presently in use is

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shown in Figure 1⁶. In this method, sample solutions are nebulized with a nebulizer consisting of a heated capillary, and produced cluster ions are introduced into a collision-induced dissociation (CID) region to produce protonated and/or cationized molecules.

2.1.1 LC

A Hitachi 655A high-performance liquid chromatograph (HPLC) with a loop injector was used. The LC was connected with a nebulizer through a Teflon tube because a high voltage was applied to the nebulizer.

2.1.2 Nebulizer.

The nebulizer is consisted of a stainless steel capillary (0.1 mm inner diameter) brazed to a stainless steel block, which could be heated to 550°C with temperature-controlled cartridge heaters. The capillary head of the nebulizer was set 5mm away

from the aperture of the first electrode. The capillary axis and first electrode axis were joined in alignment, in order to realize an efficient introduction of nebulized small droplets into the intermediate region.

2.1.3 Mass Spectrometer

Small droplets produced by the nebulizer were introduced into the ion source housing and the mass analyzing region through the intermediate region located between the first electrode (with a 250- μm aperture) and the second electrode (with a 400- μm aperture).

A drift voltage was applied to the intermediate region to dissociate cluster ions into cationized and/or protonated molecules. When a voltage was applied to the intermediate region, cluster ions were accelerated and collided with neutral molecules in the intermediate region. As a result, the kinetic

Fig. 1. Schematic diagram of the APS ion source. (1)liquid chromatograph, (2)stainless steel capillary, (3)stainless steel block, (4)first electrode with aperture, (5)second electrode with aperture, (6)lens, (8)intermediate region.

energy of the cluster ions was partly converted into the internal energy of the cluster ions. Cluster bonds are dissociated if the internal energy of the cluster ions is sufficient. This collision induced dissociation (CID) technique was very useful for breaking cluster ions. Normally the drift voltage was set to 100 V.

The intermediate region was evacuated with a 1500 L/min mechanical booster pump and a 500L/min rotary pump to about 60 Pa. The mass analyzing region were evacuated with a 700 L/s diffusion pump to about 1×10^{-4} Pa. Ion acceleration energy was determined to be 3 keV by the second electrode potential. A Hitachi M-80B double focusing mass spectrometer was used. The scan range was 1-1500 amu and scan speed was 1500 amu/4s under typical experimental conditions.

2.2 Operation

The temperature of the stainless steel block in the nebulizer was set at 480°C because a maximum cationized molecule intensity of sucrose ($m/z=365$ for $(M+Na)^+$) could be obtained. In the following experiments, the temperature of the stainless steel block was set to this level.

Mass spectra of nonvolatile compounds were measured by flow injection without a column, using a sample with an amount of 5 μ g. One hundred percent distilled water was used for saccharides and glycosides, and 50%/50% water/methanol was used for peptides and phospholipids as mobile phases, at a flow rate of 1 mL/min. For glycolipids, 70%/30% methanol/chloroform was used at a flow rate of 1 mL/min.

2.3 Samples and Reagents

Samples of saccharides, glycosides and

phospholipids were purchased from Sigma Chemical Co. (St.Lewis, USA). Peptides were purchased from the Institute of Peptides (Osaka, Japan). Glycolipids were kindly supplied from Mitsubishi-Kasei Institute of Life Sciences (Tokyo, Japan).

3. Results and Discussion

3.1 Saccharides and glycosides.

γ -Cyclodextrin (m.w.1296) is one of the test compounds to evaluate the mildness of an ionization method because this compound is very thermolabile and nonvolatile and so it is difficult to obtain the molecular ion species. The APS mass spectrum of γ -cyclodextrin in water as a mobile phase is shown in Figure 2. This mass spectrum shows several APS ionization characteristics: (1) Only ion species of Na^+ , K^+ and H_3O^+ are observed as main background ions; (2) Intense cationized molecules of $(M+Na)^+$ ($m/z=1319$) and $(M+K)^+$ ($m/z=1335$) with very few fragment ions are observed. The low background noise levels are very convenient for observing molecular ions or fragment ions with low masses, unlike FAB or SIMS method. The easy observation of cationized molecules leads to high sensitive detection of saccharides and their related compounds with high alkali metal ion affinities due to strong bases of oxygen atoms. This feature of the APS ionization is similar to that of an LD method³. In addition, the observation of intense cationized molecules of saccharides in water is very convenient because saccharide analyses by a reversed phase chromatography mode are generally performed using water as mobile phases.

As examples of glycosides, APS mass spectra of digitoxin (m.w 764) and digitonin

(m.w. 1229) are shown in Figure 3 and 4, respectively. The mass spectra of these compounds by an LD method were reported by Posthumus et al³. Intense cationized molecules of $(M+Na)^+$ (digitoxin:787, digitonin:1252) and $(M+K)^+$ (digitoxin:803, digitonin:1268) are observed by both methods. However, in an LD method, fragment ion intensities are much higher than those of cationized molecules, while in the APS mass spectra there are few fragment ions. This means that the APS method is milder than an LD method as for saccharides and their related compounds.

Reduced fragmentation resulting from the mild ionization of APS method provides insufficient structural information for identi-

fication of unknown compounds. This difficulty was overcome by a collision-induced dissociation (CID) method in the intermediate region. This technique was very easy to operate compared with an MS/MS method². Figure 5 shows the mass spectra of sucrose, raffinose and stachyose under the drift voltage of 100V and 200V. Although no fragment ions are observed except for cationized molecules in the mass spectra at a drift voltage of 100V, the mass spectra at a drift voltage of 200V provide fragment ions which can be interpreted as glycosidic cleavages accompanied by cationization. It is noteworthy that the mass spectrum of raffinose at 200V shows fragment ions ($m/z=185, 203, 365$) corresponding to sucrose moiety, and

Fig. 2. APS mass spectrum of γ -cyclodextrin.

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Fig. 3. APS mass spectrum of digitoxin.

Fig. 4. APS mass spectrum of digitonin.

the mass spectrum of stachyose at 200 V shows fragment ions ($m/z=203$, 365 , 527) corresponding to sucrose and raffinose moieties. These fragment ions are very useful for structural elucidation of raffinose or stachyose. As demonstrated here, the APS method can easily generate alternate high and low fragmentation mass spectra.

3.2 Peptides.

(Leu⁶)enkephalin (m.w.555) has been investigated by several ionization methods. In the SIMS or FAB method, this peptide is characterized by observation of $(M+Na)^+$ and $(M+K)^+$ in addition to $(M+H)^{+7}$. However, cationized molecule intensities in the SIMS method are much lower than protonated

molecule intensity. In the APS mass spectrum shown in Figure 6, the intensities of $(M+Na)^+$ ($m/z=578$) and $(M+K)^+$ ($m/z=594$) are higher than that of $(M+H)^+$ ($m/z=556$), and even $(M+2Na-H)^+$ ($m/z=600$) is observed. This spectrum clearly shows that cationized molecules are easily produced by the APS method.

The APS mass spectra of Met-Lys-bradykinin (m.w.1318) and (Tyr-8) substance P (m.w.1362) are shown in Figure 7 and 8, respectively. In these spectra, protonated molecules of $(M+H)^+$ (Met-Lys-bradykinin: 1319, (Tyr-8) substance P:1363) are more abundant than cationized molecules of $(M+Na)^+$ (Met-Lys-bradykinin: 1341, (Tyr-8) sub-

Fig. 5. APS mass spectra of sucrose, raffinose and stachyose at the drift voltage of 100V (left) and 200V (right).

NC(=O)CC1CCC(CC1)C(=O)NCC2CCCC2C(=O)NCC3CCCC3C(=O)NCC4CCCC4C(=O)NCC5CCCC5C(=O)NCC6CCCC6C(=O)NCC7CCCC7C(=O)NCC8CCCC8C(=O)NCC9CCCC9C(=O)NCC10CCCC10C(=O)NCC11CCCC11C(=O)NCC12CCCC12C(=O)NCC13CCCC13C(=O)NCC14CCCC14C(=O)NCC15CCCC15C(=O)NCC16CCCC16C(=O)NCC17CCCC17C(=O)NCC18CCCC18C(=O)NCC19CCCC19C(=O)NCC20CCCC20C(=O)NCC21CCCC21C(=O)NCC22CCCC22C(=O)NCC23CCCC23C(=O)NCC24CCCC24C(=O)NCC25CCCC25C(=O)NCC26CCCC26C(=O)NCC27CCCC27C(=O)NCC28CCCC28C(=O)NCC29CCCC29C(=O)NCC30CCCC30C(=O)NCC31CCCC31C(=O)NCC32CCCC32C(=O)NCC33CCCC33C(=O)NCC34CCCC34C(=O)NCC35CCCC35C(=O)NCC36CCCC36C(=O)NCC37CCCC37C(=O)NCC38CCCC38C(=O)NCC39CCCC39C(=O)NCC40CCCC40C(=O)NCC41CCCC41C(=O)NCC42CCCC42C(=O)NCC43CCCC43C(=O)NCC44CCCC44C(=O)NCC45CCCC45C(=O)NCC46CCCC46C(=O)NCC47CCCC47C(=O)NCC48CCCC48C(=O)NCC49CCCC49C(=O)NCC50CCCC50C(=O)NCC51CCCC51C(=O)NCC52CCCC52C(=O)NCC53CCCC53C(=O)NCC54CCCC54C(=O)NCC55CCCC55C(=O)NCC56CCCC56C(=O)NCC57CCCC57C(=O)NCC58CCCC58C(=O)NCC59CCCC59C(=O)NCC60CCCC60C(=O)NCC61CCCC61C(=O)NCC62CCCC62C(=O)NCC63CCCC63C(=O)NCC64CCCC64C(=O)NCC65CCCC65C(=O)NCC66CCCC66C(=O)NCC67CCCC67C(=O)NCC68CCCC68C(=O)NCC69CCCC69C(=O)NCC70CCCC70C(=O)NCC71CCCC71C(=O)NCC72CCCC72C(=O)NCC73CCCC73C(=O)NCC74CCCC74C(=O)NCC75CCCC75C(=O)NCC76CCCC76C(=O)NCC77CCCC77C(=O)NCC78CCCC78C(=O)NCC79CCCC79C(=O)NCC80CCCC80C(=O)NCC81CCCC81C(=O)NCC82CCCC82C(=O)NCC83CCCC83C(=O)NCC84CCCC84C(=O)NCC85CCCC85C(=O)NCC86CCCC86C(=O)NCC87CCCC87C(=O)NCC88CCCC88C(=O)NCC89CCCC89C(=O)NCC90CCCC90C(=O)NCC91CCCC91C(=O)NCC92CCCC92C(=O)NCC93CCCC93C(=O)NCC94CCCC94C(=O)NCC95CCCC95C(=O)NCC96CCCC96C(=O)NCC97CCCC97C(=O)NCC98CCCC98C(=O)NCC99CCCC99C(=O)NCC100CCCC100C(=O)NCC101CCCC101C(=O)NCC102CCCC102C(=O)NCC103CCCC103C(=O)NCC104CCCC104C(=O)NCC105CCCC105C(=O)NCC106CCCC106C(=O)NCC107CCCC107C(=O)NCC108CCCC108C(=O)NCC109CCCC109C(=O)NCC110CCCC110C(=O)NCC111CCCC111C(=O)NCC112CCCC112C(=O)NCC113CCCC113C(=O)NCC114CCCC114C(=O)NCC115CCCC115C(=O)NCC116CCCC116C(=O)NCC117CCCC117C(=O)NCC118CCCC118C(=O)NCC119CCCC119C(=O)NCC120CCCC120C(=O)NCC121CCCC121C(=O)NCC122CCCC122C(=O)NCC123CCCC123C(=O)NCC124CCCC124C(=O)NCC125CCCC125C(=O)NCC126CCCC126C(=O)NCC127CCCC127C(=O)NCC128CCCC128C(=O)NCC129CCCC129C(=O)NCC130CCCC130C(=O)NCC131CCCC131C(=O)NCC132CCCC132C(=O)NCC133CCCC133C(=O)NCC134CCCC134C(=O)NCC135CCCC135C(=O)NCC136CCCC136C(=O)NCC137CCCC137C(=O)NCC138CCCC138C(=O)NCC139CCCC139C(=O)NCC140CCCC140C(=O)NCC141CCCC141C(=O)NCC142CCCC142C(=O)NCC143CCCC143C(=O)NCC144CCCC144C(=O)NCC145CCCC145C(=O)NCC146CCCC146C(=O)NCC147CCCC147C(=O)NCC148CCCC148C(=O)NCC149CCCC149C(=O)NCC150CCCC150C(=O)NCC151CCCC151C(=O)NCC152CCCC152C(=O)NCC153CCCC153C(=O)NCC154CCCC154C(=O)NCC155CCCC155C(=O)NCC156CCCC156C(=O)NCC157CCCC157C(=O)NCC158CCCC158C(=O)NCC159CCCC159C(=O)NCC160CCCC160C(=O)NCC161CCCC161C(=O)NCC162CCCC162C(=O)NCC163CCCC163C(=O)NCC164CCCC164C(=O)NCC165CCCC165C(=O)NCC166CCCC166C(=O)NCC167CCCC167C(=O)NCC168CCCC168C(=O)NCC169CCCC169C(=O)NCC170CCCC170C(=O)NCC171CCCC171C(=O)NCC172CCCC172C(=O)NCC173CCCC173C(=O)NCC174CCCC174C(=O)NCC175CCCC175C(=O)NCC176CCCC176C(=O)NCC177CCCC177C(=O)NCC178CCCC178C(=O)NCC179CCCC179C(=O)NCC180CCCC180C(=O)NCC181CCCC181C(=O)NCC182CCCC182C(=O)NCC183CCCC183C(=O)NCC184CCCC184C(=O)NCC185CCCC185C(=O)NCC186CCCC186C(=O)NCC187CCCC187C(=O)NCC188CCCC188C(=O)NCC189CCCC189C(=O)NCC190CCCC190C(=O)NCC191CCCC191C(=O)NCC192CCCC192C(=O)NCC193CCCC193C(=O)NCC194CCCC194C(=O)NCC195CCCC195C(=O)NCC196CCCC196C(=O)NCC197CCCC197C(=O)NCC198CCCC198C(=O)NCC199CCCC199C(=O)NCC200CCCC200C(=O)NCC201CCCC201C(=O)NCC202CCCC202C(=O)NCC203CCCC203C(=O)NCC204CCCC204C(=O)NCC205CCCC205C(=O)NCC206CCCC206C(=O)NCC207CCCC207C(=O)NCC208CCCC208C(=O)NCC209CCCC209C(=O)NCC210CCCC210C(=O)NCC211CCCC211C(=O)NCC212CCCC212C(=O)NCC213CCCC213C(=O)NCC214CCCC214C(=O)NCC215CCCC215C(=O)NCC216CCCC216C(=O)NCC217CCCC217C(=O)NCC218CCCC218C(=O)NCC219CCCC219C(=O)NCC220CCCC220C(=O)NCC221CCCC221C(=O)NCC222CCCC222C(=O)NCC223CCCC223C(=O)NCC224CCCC224C(=O)NCC225CCCC225C(=O)NCC226CCCC226C(=O)NCC227CCCC227C(=O)NCC228CCCC228C(=O)NCC229CCCC229C(=O)NCC230CCCC230C(=O)NCC231CCCC231C(=O)NCC232CCCC232C(=O)NCC233CCCC233C(=O)NCC234CCCC234C(=O)NCC235CCCC235C(=O)NCC236CCCC236C(=O)NCC237CCCC237C(=O)NCC238CCCC238C(=O)NCC239CCCC239C(=O)NCC240CCCC240C(=O)NCC241CCCC241C(=O)NCC242CCCC242C(=O)NCC243CCCC243C(=O)NCC244CCCC244C(=O)NCC245CCCC245C(=O)NCC246CCCC246C(=O)NCC247CCCC247C(=O)NCC248CCCC248C(=O)NCC249CCCC249C(=O)NCC250CCCC250C(=O)NCC251CCCC251C(=O)NCC252CCCC252C(=O)NCC253CCCC253C(=O)NCC254CCCC254C(=O)NCC255CCCC255C(=O)NCC256CCCC256C(=O)NCC257CCCC257C(=O)NCC258CCCC258C(=O)NCC259CCCC259C(=O)NCC260CCCC260C(=O)NCC261CCCC261C(=O)NCC262CCCC262C(=O)NCC263CCCC263C(=O)NCC264CCCC264C(=O)NCC265CCCC265C(=O)NCC266CCCC266C(=O)NCC267CCCC267C(=O)NCC268CCCC268C(=O)NCC269CCCC269C(=O)NCC270CCCC270C(=O)NCC271CCCC271C(=O)NCC272CCCC272C(=O)NCC273CCCC273C(=O)NCC274CCCC274C(=O)NCC275CCCC275C(=O)NCC276CCCC276C(=O)NCC277CCCC277C(=O)NCC278CCCC278C(=O)NCC279CCCC279C(=O)NCC280CCCC280C(=O)NCC281CCCC281C(=O)NCC282CCCC282C(=O)NCC283CCCC283C(=O)NCC284CCCC284C(=O)NCC285CCCC285C(=O)NCC286CCCC286C(=O)NCC287CCCC287C(=O)NCC288CCCC288C(=O)NCC289CCCC289C(=O)NCC290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stance P:1385). This tendency differs from that of (Leu⁵)enkephalin.

3.3 Lipids.

Analyses of lipids are important targets in LC. Generally, in these analyses, solvents extremely high in organic content are used as mobile phases for LC. In a TSP method, external ionization method is necessary for sufficient ionization, which results in obtaining CI type mass spectra of these compounds⁸. On the other hand, the APS method can produce intense cationized molecules of $(M+Na)^+$ and $(M+K)^+$ without external ion sources.

The APS mass spectrum of dipalmitoylphosphatidylcholine (m.w.733) is shown in Figure 9. The cationized molecules of $(M+Na)^+$ ($m/z=756$) are observed as a base peak in addition to $(M+K)^+$ ($m/z=772$) and $(M+H)^+$ ($m/z=734$), although only $(M+H)^+$ species are observed as quasi-molecular ions in the API⁵ and SIMS methods. This APS mass spectrum of the lipid also clearly

reveals the APS ionization characteristic. The fragment ion at m/z 551 is due to the cleavage as indicated in the figure.

The APS mass spectra of lactosyl ceramide (m.w.861) and globoside (m.w.1338) are shown in Figure 10 and 11, respectively. These figures show cationized molecules of (M+Na) (lactosyl ceramide:884, globoside:1361) and (M+K)⁺ (lactosyl ceramide:900, globoside:1377). The ions at m/z 882 in lactosyl ceramide and 1359 in globoside are due to impurities with unsaturated hydrocarbons of ceramides. It is noteworthy that these spectra are very similar to those obtained by the FD method⁹.

4. Conclusions.

As demonstrated here, the APS ionization method provides intense protonated and/or cationized molecules such as $(M+H)^+$, $(M+Na)^+$ and $(M+K)^+$ for thermolabile and nonvolatile compounds including saccharides, peptides and lipids with molecular

Fig. 8. APS mass spectrum of (Tyr-8) substance P.

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Fig. 9. APS mass spectrum of dipalmitoylphosphatidylcholine.

Fig. 10. APS mass spectrum of lactosyl ceramide.

Fig. 11. APS mass spectrum of globoside.

weights larger than 1000 daltons. The APS method can generate molecular ion species without any buffers or external ion sources. Since mobile phases without buffers are generally used in the analyses of saccharides and lipids by a reversed phase chromatography mode, the APS method is very useful for analyses of these compounds. The APS applicability seems complementary to the previously reported API. The APS method will provide a very powerful LC/MS system to combine LC/API-MS and LC/APS-MS.

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