Journal of Chromatography, 542 (1991) 373–383
Elsevier Science Publishers B.V., Amsterdam

CHROM. 23 074

High-performance liquid chromatography–continuous-flow fast atom bombardment mass spectrometry of chlorophyll derivatives

RICHARD B. VAN BREEMEN*
Department of Chemistry, Box 8204, North Carolina State University, Raleigh, NC 27695 (U.S.A.)
and
FATIMA L. CANJURA and STEVEN J. SCHWARTZ
Department of Food Science, North Carolina State University, Raleigh, NC 27695 (U.S.A.)
(First received August 21st, 1990; revised manuscript received December 18th, 1990)

ABSTRACT

Nine chlorophyll derivatives from different spinach preparations were separated and identified using reversed-phase high-performance liquid chromatography–mass spectrometry (HPLC–MS). These pigments included chlorophylls *a* and *b*, chlorophyllides *a* and *b*, pheophorbide *a*, pheophytins *a* and *b*, and pyropheophytins *a* and *b*. HPLC–MS measurements were carried out using HPLC–frit-fast atom bombardment (FAB)-MS, which is a continuous-flow FAB-MS interface. The highly hydrophobic chlorophyll derivatives were eluted from a reversed-phase HPLC column using a gradient of increasing ethyl acetate concentration. Glycerol was included in the mobile phase to serve as the matrix for FAB ionization. During analysis by positive-ion HPLC–frit-FAB-MS, abundant protonated molecules, $[M + H]^+$, were detected for all nine chlorophyll derivatives. Fragment ions were observed in the mass spectra that were similar to those produced during standard probe FAB-MS. These HPLC–MS procedures were shown to be useful for the rapid separation and identification of a variety of chlorophyll derivatives from natural sources.

INTRODUCTION

The importance of chlorophyll pigments in photosynthesis and plant physiology has prompted enormous research efforts spanning almost 100 years that have resulted in the complete structural determination of chlorophyll [1]. Nevertheless, numerous questions remain regarding the catabolism of chlorophyll during senescence in plant tissues and the structures and functions of chlorophyll derivatives involved in ripening [2]. Previous studies of chlorophyll pigments have been hindered by the lack of sensitive techniques for the analysis and identification of small quantities of their metabolic intermediates in plant tissues and degradation products formed during heat processing of food products.

Since the development of desorption methods suitable for the ionization of non-volatile and thermally labile compounds, mass spectrometry (MS) has been ap-

0021-9673/91/$03.50

plied to the characterization and identification of a variety of chlorophyll derivatives. Mass spectra of chlorophylls have been obtained using laser desorption [3–7], field desorption [8,9], plasma desorption [10–12], fast atom bombardment (FAB) [13–18] and "in beam" (desorption) electron impact ionization [19]. Because FAB facilitates the continuous formation of chlorophyll ions, this ionization technique has been the method of choice for tandem mass spectrometric (MS–MS) studies of chlorophylls [15–18]. Among the ionization methods used to obtain mass spectra of chlorophylls, FAB is the only technique that has been coupled with high-performance liquid chromatography (HPLC) in a routine analytical instrument. However, no HPLC–MS studies of chlorophylls have yet been reported.

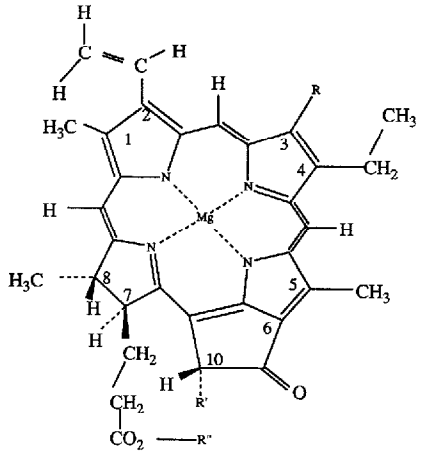
During FAB, sample ions are desorbed into the gas phase from a liquid matrix of low volatility (usually glycerol) as a result of bombardment by a beam of energetic atoms (usually xenon or argon at 3–10 keV) [13,20]. If fast ions are substituted for the fast atoms, then the technique is called liquid secondary-ion mass spectrometry. FAB-MS has been interfaced to HPLC in the HPLC–MS system called continuous-flow FAB-MS [21] or a variation of this technique known as HPLC–frit-FAB-MS [22]. In these HPLC–MS systems, the FAB matrix is typically mixed with the mobile phase prior to being pumped into the ion source of the mass spectrometer [22,23]. Small amounts (up to 10% by volume) of a matrix such as glycerol in the mobile phase have been shown to slightly increase band widths during reversed-phase (RP-) HPLC separations of peptides [24]. Instead of mixing the matrix with the mobile phase prior to chromatographic separation, coaxial flow of matrix and column effluent onto the FAB probe has been used to improve chromatographic resolution [24]. Alternatively, post-column addition of matrix has been carried out without significantly reducing chromatographic resolution [25].

During frit-FAB, the HPLC effluent is pumped through a fused-silica capillary and then through a stainless-steel frit located inside the ion source of the mass spectrometer [22]. The fast atom beam is focused onto the opposite side of the frit from the capillary, so that sample ions are desorbed into the gas phase as they flow through the frit. The HPLC solvent rapidly evaporates and is pumped away by the vacuum system of the mass spectrometer.

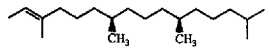
Our recent chlorophyll studies have involved the development of RP-HPLC procedures to separate and identify chlorophyll derivatives contained in complex mixtures extracted from spinach leaves. In one study, chlorophyll detection and identification was based on visible light absorption using an array detector [26]. Subsequently, structural confirmation of the HPLC-purified chlorophyll derivatives was obtained using FAB-MS combined with collisional activation and MS–MS analysis [18]. In the present investigation, HPLC separation of chlorophyll derivatives contained in extracts from spinach leaves was combined on-line with mass spectrometric detection using HPLC–frit-FAB-MS. The structures of the chlorophylls and chlorophyll derivatives discussed in this paper are shown in Fig. 1.

EXPERIMENTAL

Identification of chlorophyll derivatives in spinach leaf extracts was based on comparison to standards isolated from leaves and purified by RP-HPLC [26]. The identity of each standard compound was determined by its visible light absorbance



1a Chlorophyll *a*, R = CH_3, R' = $-\overset{O}{\overset{\|}{C}}-OCH_3$, R'' =



1b Chlorophyll *b*, 1a, R = CHO

2a Pheophytin *a*, 1a - Mg + 2H

2b Pheophytin *b*, 1b - Mg + 2H

3a Chlorophyllide *a*, 1a, R'' = H

3b Chlorophyllide *b*, 1b, R'' = H

4a Pheophorbide *a*, 3a - Mg + 2H

4b Pheophorbide *b*, 3b - Mg + 2H

5a Pyropheophytin *a*, 2a, R' = H

5b Pyropheophytin *b*, 2b, R' = H

Fig. 1. Structures of chlorophylls and chlorophyll derivatives.

spectrum recorded on a diode-array detector [26] and confirmed using FAB-MS–MS [18]. Fresh spinach leaves containing chlorophylls *a* and *b* (**1a** and **1b** in Fig. 1) were incubated at 65°C for 30 min to activate the enzyme chlorophyllase. During this incubation period, the chlorophylls were partially converted into chlorophyllides *a* and *b* (**3a** and **3b**). Next, the mixture of chlorophylls and chlorophyllides was extracted from the spinach leaves using acetone as previously described [26] and then analyzed by HPLC–MS as described below. In another procedure, pheophytins *a* and *b* (**2a** and **2b**) were extracted using diethyl ether from an acidified chlorophyll extract [18]. The pheophytin extract was then analyzed by HPLC–MS. Pyropheophytins *a* and *b* (**5a** and **5b**) were prepared from heat-processed (145°C for 7 min) spinach puree as previously described [27] and then analyzed by HPLC–MS. Although pheophorbide *a* (**4a**) was detected in several samples, pheophorbides *a* and *b* (**4a** and **4b**) were obtained by acidification (1.0 *M* HCl) of a chlorophyllide extract. Prior to analysis by HPLC–MS, the chlorophyll derivatives contained in each sample extract were identified by RP-HPLC based on their visible light absorbance spectra recorded on a diode-array detector [26]. All samples containing chlorophyll derivatives were handled under subdued light.

HPLC separations were carried out using an Applied Biosystems (Foster City, CA, U.S.A.) Model 140A dual syringe solvent delivery system, that had been mod-

ified so that the dynamic mixer was replaced with a "T" union to minimize dead volume. The HPLC system was equipped with a Rheodyne (Cotati, CA, U.S.A.) Model 8125 injector and Vydac (Hesperia, CA, U.S.A.,) C_{18} narrow-bore column (15 cm × 2.1 mm) packed with 300 Å pore size, 5-μm diameter silica particles. The solvent flow-rate was 70 μl/min for all separations. Each extract was dissolved in diethyl ether or acetone (approximately 1 μg/μl), and 15 μl (15 μg) were injected onto the reversed-phase column per analysis.

The mixture of chlorophylls and chlorophyllides, extracted from spinach leaves that had been incubated at 65°C, was separated by RP-HPLC using a procedure modified from that of Canjura and Schwartz [26]. The solvent system consisted of a 20-min linear gradient from 100% solvent A to 100% solvent B. Solvent A consisted of ethyl acetate–methanol–water–glycerol (15:65:20:0.5, v/v/v/w), and solvent B contained the same solvents in a ratio of 60:30:10:0.5 (v/v/v/w). In order to shorten the retention times of the more hydrophobic pheophytins in the acid-treated spinach extract, a 10-min linear gradient was used from 50 to 100% solvent B. For extracted mixtures of pheophytins and pyropheophytins from heat-treated tissue, the original 20-min gradient from 100% solvent A to 100% solvent B was used, but mass spectra were recorded beginning 15 min after sample injection. Otherwise, recording of frit-FAB mass spectra was begun 5 min after sample injection, and the solvent front eluted approximately 7 min after injection or at approximately 2 min on the total-ion chromatograms. The delay before recording mass spectra minimized the use of computer disk memory to store background scans during HPLC–MS.

Positive-ion FAB mass spectra were obtained using a JEOL (Tokyo, Japan) JMS-HX110HF double-focusing mass spectrometer equipped with a JMA-DA5000 data system and HPLC–frit-FAB-MS interface. Xenon fast atoms at 6 kV were used for FAB ionization. The accelerating voltage was 10 keV, and the resolving power was 1000 for all measurements. The range m/z 300–1000 was scanned over approximately 7 s except during the analysis of pyropheophytins, in which this mass range was scanned in approximately 15 s. Although standard FAB mass spectra were recorded in profile mode (Fig. 2 A and B), centroided data were recorded during HPLC–frit-FAB-MS (Fig. 2C) because of limited computer disk space.

For compatibility with the vacuum system of the mass spectrometer, the HPLC column eluate was split so that approximately 5 μl/min entered the HPLC–frit-FAB-MS interface. At a column flow-rate of 70 μl/min, this resulted in a split ratio of 1:14. Because approximately 15 μg of each sample was injected, approximately 1 μg reached the mass spectrometer per analysis. Operation of the column at higher flow-rates would have resulted in a smaller split ratio and loss of sensitivity. The ion source temperature was maintained at 40°C, which was sufficient to prevent solvent from freezing in the frit. Glycerol contained in the mobile phase functioned as the FAB matrix.

RESULTS AND DISCUSSION

In recent studies of chlorophylls by positive-ion FAB-MS using a standard FAB probe [17,18], abundant molecular ions, $M^{+\cdot}$, were detected using 3-nitrobenzylalcohol as the FAB matrix. Protonated and deprotonated molecules, $[M+H]^+$ and $[M-H]^+$, were also observed although at lower relative abundance. The use of more

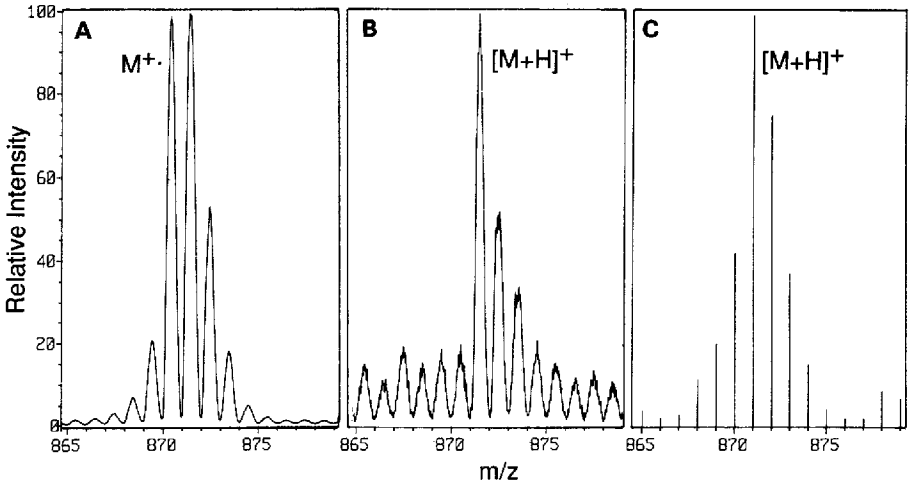


Fig. 2. Comparison of molecular-ion species of pheophytin *a* obtained using (A) standard probe FAB-MS with neat 3-nitrobenzylalcohol as the matrix, (B) standard probe FAB using neat glycerol as the matrix, or (C) HPLC–frit-FAB-MS with a mobile phase containing ethyl acetate–methanol–water–glycerol. In each positive-ion FAB mass spectrum, note the relative abundances of the radical cation, $M^{+\cdot}$, and the protonated molecule, $[M+H]^+$.

acidic matrices such as dithiothreitol–dithioerythritol or thioglycerol resulted in an increase in the relative abundance of protonated molecules of chlorophyll *a* [17].

Because of its greater volatility and proven compatibility with continuous-flow FAB-MS [28], glycerol instead of 3-nitrobenzylalcohol was added to the mobile phase for HPLC–MS analysis of chlorophyll derivatives. However, neat glycerol is a poor solvent for hydrophobic compounds and is not an ideal matrix for standard probe FAB-MS of chlorophylls. For example, the molecular-ion species for 1-μg aliquots of pheophytin *a* are compared in Fig. 2 using a matrix of either 3-nitrobenzylalcohol or glycerol on a standard FAB probe. In these mass spectra, the use of 3-nitrobenzylalcohol (Fig. 2A) produced $M^{+\cdot}$ ions with a signal-to-noise ratio that was approximately 10-fold higher than that of the $[M+H]^+$ ions obtained using glycerol (Fig. 2B). However, HPLC–frit-FAB-MS of approximately 1 μg pheophytin *a* (after split, Fig. 2C) produced protonated molecules with a signal-to-noise ratio comparable to that of the molecular-ion radicals obtained using standard probe FAB with 3-nitrobenzylalcohol.

Abundant protonated molecules, $[M+H]^+$, were detected for all chlorophyll derivatives investigated using RP-HPLC separation in combination with positive-ion frit-FAB-MS. The compounds investigated by HPLC–frit-FAB-MS included chlorophylls *a* and *b*, chlorophyllides *a* and *b*, pheophytins *a* and *b*, pheophorbide *a*, and pyropheophytins *a* and *b* (see chlorophyll structures in Fig. 1). During HPLC–frit-FAB-MS, the combination of glycerol with large proportions of ethyl acetate and methanol in the HPLC–MS mobile phase provided both a good solvent for the chlorophylls and a protic environment for the formation of $[M+H]^+$ ions.

The total-ion chromatogram and mass chromatograms for the HPLC–MS

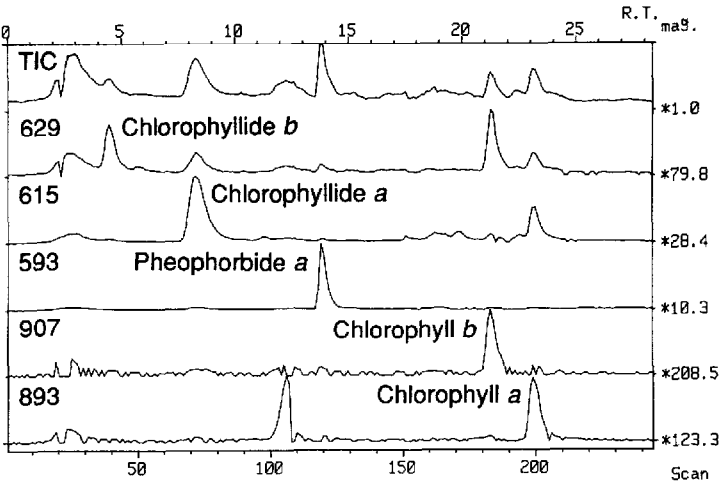


Fig. 3. Total-ion chromatogram and mass chromatograms obtained by positive-ion HPLC–frit-FAB-MS showing the detection of chlorophyllides *a* and *b*, pheophorbide *a*, and chlorophylls *a* and *b*, extracted from spinach that had been incubated to activate chlorophyllase. R.T. = Retention time in min. Right-hand scale: mag = magnification factor.

analysis of a mixture of chlorophylls *a* and *b*, chlorophyllides *a* and *b*, and pheophorbide *a* are shown in Fig. 3. Chlorophyllides *a* and *b* (retention times 8.2 and 4.3 min, respectively) were formed by chlorophyllase action on chlorophyll prior to extraction from the spinach. Intact chlorophylls *a* and *b* were detected at 23.2 and 21.2 min, respectively. Pheophorbide *a* and chlorophyllides *a* and *b* eluted before their more hydrophobic precursors, chlorophylls *a* and *b* (Fig. 3). Pheophorbide *a*, detected at a

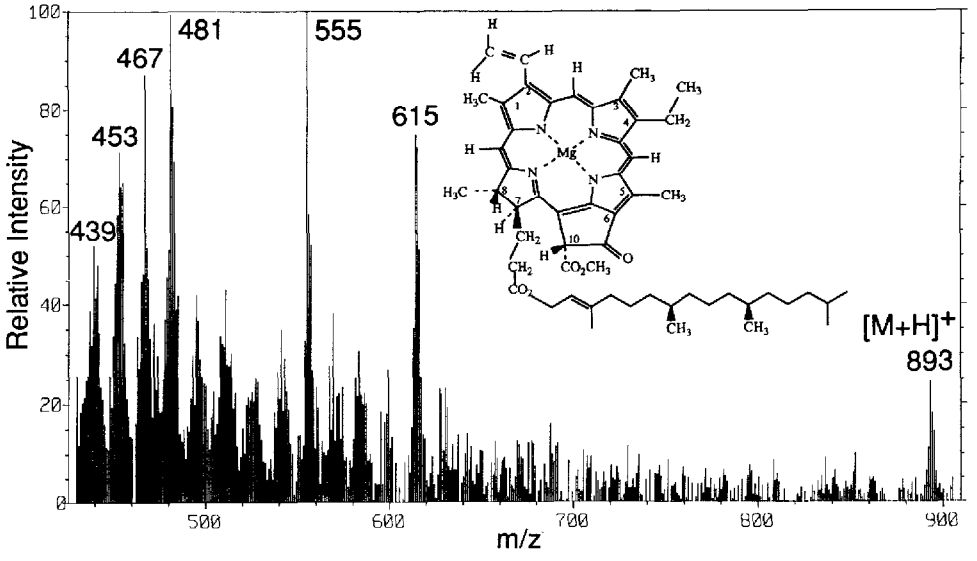


Fig. 4. Positive-ion frit-FAB mass spectrum of chlorophyll *a* (retention time 23.2 min) from the HPLC–MS analysis shown in Fig. 3.

retention time of 13.8 min, was formed by decomposition of chlorophyllide *a* during handling and extraction. No pheophorbide *b* was detected in this sample because of the greater stability of its precursor, chlorophyll *b*.

In addition to protonated molecules, fragment ions were detected in all mass spectra. The fragmentation patterns corresponded to those reported by Grese *et al.* [17] and van Breemen *et al.* [18]. For example, the positive-ion frit-FAB mass spectrum of chlorophyll *a*, recorded during the HPLC–MS analysis discussed above, is presented in Fig. 4. In this mass spectrum, fragment ions of chlorophyll *a* were detected at *m/z* 439, 453, 467, 481, 555 and 615. The most abundant fragment ion in the mass spectrum of chlorophyll *a* was detected at *m/z* 555, which was formed either by loss of $HCOOCH_3$ from *m/z* 615, $[MH-C_{20}H_{38}-HCOOCH_3]^+$, or by loss of $CH_3COOC_{20}H_{39}$ from the $[M+H]^+$ ion [17]. The ion detected at *m/z* 615, indistinguishable from the protonated molecule of chlorophyllide *a*, was formed by loss of the phytyl chain ($C_{20}H_{38}$) from the $[M+H]^+$ ion of chlorophyll *a* with transfer of a hydrogen from the leaving group back to the ester oxygen [17,18]. Therefore, the mass chromatogram of *m/z* 615 in Fig. 3 shows two bands, one corresponding to the protonated molecule of chlorophyllide *a* (retention time 8.2 min) and the other formed by fragmentation of chlorophyll *a* (retention time 23.2 min). Similarly, the mass chromatogram of *m/z* 629 (Fig. 3) shows two major bands corresponding to the $[M+H]^+$ ion of chlorophyllide *b* (retention time 4.3 min) and a fragment ion of chlorophyll *b* (retention time 21.2 min). The origin of the extra band in the mass chromatogram of chlorophyll *a* at a retention time of 12 min was not determined. However, the mass spectrum corresponding to this unknown band contained no fragment ions indicative of known chlorophylls or their derivatives. Instead, ions were detected at *m/z* 520, 571, 737, 765, 781, 839, 855, 877 and 893 (data not shown).

The total-ion chromatogram generated from the HPLC–frit-FAB-MS analysis of the pheophytin extract is shown in Fig. 5. Mass chromatograms corresponding to

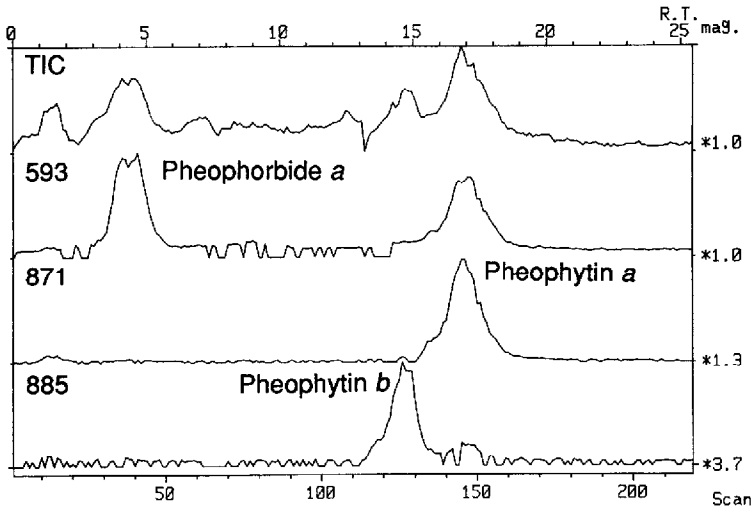


Fig. 5. Total-ion chromatogram and selected-ion chromatograms using positive-ion HPLC–frit-FAB-MS showing the detection of pheophytins *a* and *b*, and pheophorbide *a* from an acid-treated spinach extract. R.T. = Retention time in min. Right-hand scale: mag = magnification factor.

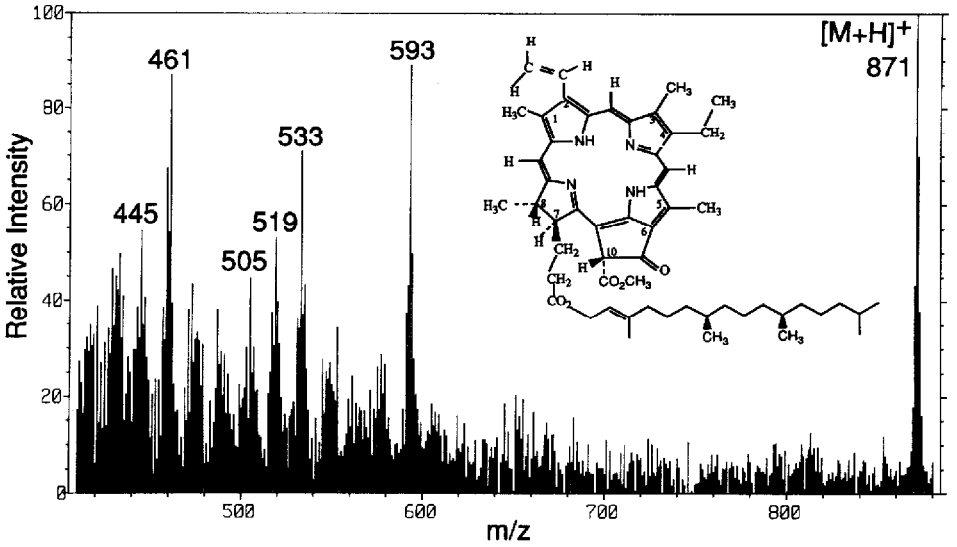


Fig. 6. Positive-ion frit-FAB mass spectrum of pheophytin *a* corresponding to a retention time of 19.1 min in the HPLC–MS chromatogram shown in Fig. 5.

the protonated molecules of pheophytins *a* and *b* and pheophorbide *a* are also plotted. These bands appeared wider than those of the chlorophylls and chlorophyllides shown in Fig. 3 or the pheophytins and pyropheophytins shown in Fig. 7. The primary explanation for the broader bands was the different gradient used for this elution. The mass spectrum of pheophytin *a*, showing an abundant protonated molecule at *m/z* 871 and several fragment ions, is shown in Fig. 6.

Pyropheophytins *a* and *b*, formed by heat treatment of spinach leaves, were extracted and then analyzed by HPLC–MS. The total-ion chromatogram and mass chromatograms for this analysis are shown in Fig. 7. A mixture of pheophytins and pyropheophytins was detected, since the conversion of pheophytin to pyropheophytin is incomplete during thermal treatments [29]. The mass spectrum of pyropheophytin *b* obtained during HPLC–MS is shown in Fig. 8. An abundant protonated molecule was detected at *m/z* 827 as well as fragment ions at *m/z* 461, 475, 489, 503 and 549. These fragment ions were similar to those observed using standard probe FAB-MS [18]. For example, the ion at *m/z* 549 was formed by loss of the phytyl chain with hydrogen transfer and is analogous to the ion at *m/z* 615 in the mass spectrum of chlorophyll *a* discussed above.

A contributing factor to the broadening of pheophytin and pheophorbide bands in the chromatograms shown in Figs. 5 and 7 was epimerization at C-10 caused by acid treatment of chlorophylls *a* and *b* to generate the pheophytins and pheophorbides. The pairs of epimers have been completely resolved using a different chromatographic column, mobile phase, and gradient [26]. There was no difference between the mass spectra of each pair of the epimers, however.

Several bands were detected by HPLC–MS that did not correspond to chlorophyll derivatives. The major contaminants in the chlorophyll and chlorophyllide ex-

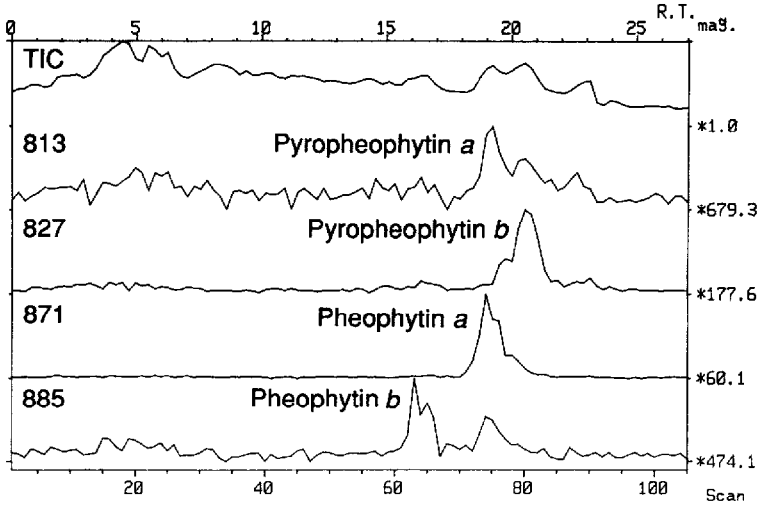


Fig. 7. Total-ion chromatogram and mass chromatograms for a mixture of pyropheophytins *a* and *b* and pheophytins *a* and *b* obtained using positive-ion HPLC–frit-FAB-MS. Mass spectra were recorded beginning 15 min after injection instead of 5 min as in Figs. 3 and 5. R.T. = Retention time in min. Right-hand scale: mag = magnification factor.

tract (Fig. 3) eluted at the solvent front (approximately 2 min), and at 3, 12 and 22.3 min. The most abundant contaminant band, eluting at 3 min, produced a base peak that corresponded to a protonated molecule at *m/z* 521. In the pheophytin extract (Fig. 5), the major bands that did not correspond to chlorophyll derivatives were

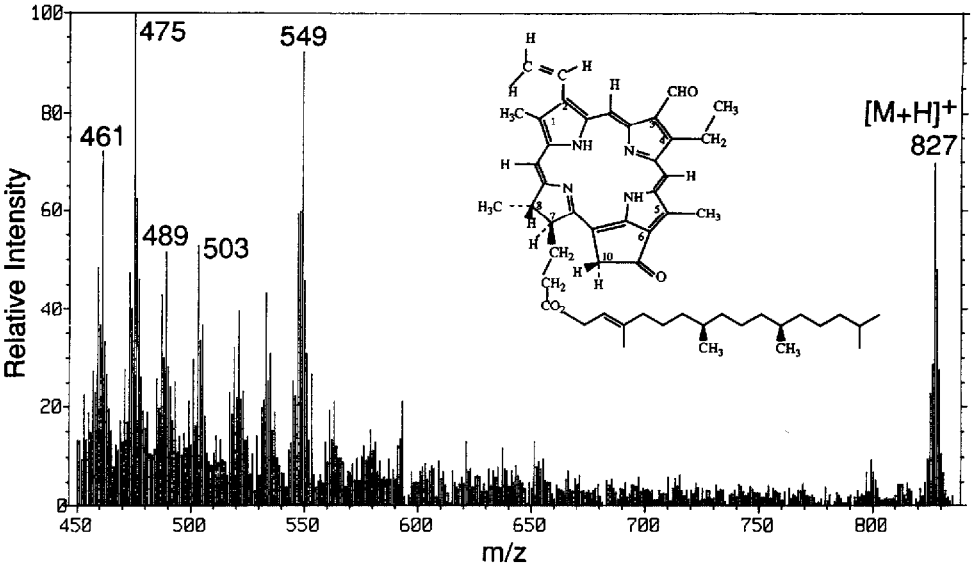


Fig. 8. Positive-ion frit-FAB mass spectrum of pyropheophytin *b* recorded at a retention time of 20.6 min in the HPLC–MS chromatogram shown in Fig. 7.

observed at 1.5, 6.8 and 12.3 min. Because these samples were prepared as crude extracts of spinach, the presence of hydrophobic compounds other than chlorophyll derivatives was expected. No further characterization of these contaminants was undertaken.

Grese *et al.* [17] have suggested and other investigators have shown [30,31] that formation of radical species in solution such as chlorophyll $M^{+\cdot}$ ions or perhaps radical matrix species can result in chlorophyl allomerization. These products typically form by oxidation of chlorophyll to a radical cation followed by nucleophilic attack at the C-10 position [31]. The longer chlorophylls are exposed to the FAB beam, the more oxidized by-products are produced [17]. During HPLC–frit-FAB-MS analysis of nine chlorophyll derivatives, no evidence of chlorophyll allomerization was observed. Because fresh sample and matrix are continuously flowing onto the frit-FAB probe, degradative processes such as reactions between chlorophyll and matrix are minimized compared to standard probe FAB-MS. Furthermore, the use of protic matrices such as glycerol minimizes the formation of radical ions and promotes the formation of protonated molecules instead.

CONCLUSIONS

The first HPLC–MS separation and identification of chlorophylls and their chlorophyllide, pheophytin, pheophorbide and pyropheophytin derivatives is reported here. The on-line combination of HPLC with FAB-MS provides a rapid and specific method to characterize chlorophyll derivatives. Although not used in this investigation, a UV–VIS absorbance detector could be added between the HPLC column and the mass spectrometer for further characterization of the pigments.

Compared to our previous HPLC analyses of chlorophyll derivatives using visible absorbance detection [26], HPLC–MS chromatograms showed the same order of elution for each chlorophyll derivative but with different retention times and lower chromatographic resolution. These differences were the result of the addition of glycerol to the mobile phase for HPLC–MS, the use of a shorter HPLC column, and a faster gradient program. Glycerol matrix was added to the mobile phase instead of post-column or coaxial addition for convenience and technical simplicity. The HPLC column and gradient were selected to increase the speed of each HPLC–MS analysis, which was important because of the high demand for MS analyses in our laboratory.

Compared to HPLC fraction collection, sample concentration *in vacuo*, and then analysis by MS or other techniques, HPLC–MS is much faster and reduces the time the sample might be exposed to light or atmospheric oxygen during handling. Furthermore, HPLC–frit-FAB-MS minimizes chlorophyl allomerization that occurs during standard probe FAB as a result of exposure to the FAB beam in the presence of nucleophilic matrix. Unlike moving-belt or thermospray HPLC–MS, the HPLC eluate is heated only slightly during HPLC–frit-FAB-MS so that pyrolysis of chlorophyll pigments does not occur. (The ion source was heated to 40°C to prevent freezing of solvent in the frit.) Given the ease of use and numerous advantages of the method, HPLC–frit-FAB-MS can be a powerful technique for the identification of new chlorophyll derivatives from natural sources and for biochemical studies of chlorophyll degradation products formed during senescence of leaves.

ACKNOWLEDGEMENTS

This material is based upon work supported by the North Carolina Biotechnology Center. Preliminary results were presented at the *38th ASMS Conference on Mass Spectrometry and Allied Topics, Tucson, AZ, June 3–8, 1990.*

REFERENCES

1 T. W. Goodwin, *Chemistry and Biochemistry of Plant Pigments*, Academic Press, New York, 2nd ed., 1976.
2 J. Gross, *Pigments in Fruits*, Alden Press, Oxford, 1987.
3 M. A. Posthumus, P. G. Kistemaker, H. L. C. Meuzelaar and M. C. Ten Noever de Brauw, *Anal. Chem.*, 50 (1978) 985.
4 J.-C. Tabet, M. Jablonski, R. J. Cotter and J. E. Hunt, *Int. J. Mass Spectrom. Ion Processes*, 65 (1985) 105.
5 R. S. Brown and C. L. Wilkins, *J. Am. Chem. Soc.*, 108 (1986) 2447.
6 J. Grotemeyer, U. Boesl, K. Walter and E. W. Schlag, *J. Am. Chem. Soc.*, 108 (1986) 4233.
7 J. Grotemeyer and E. W. Schlag, *Angew. Chem. Eng. Ed.*, 27 (1988) 447.
8 R. C. Dougherty, P. A. Driefuss, J. Sphon and J. J. Katz, *J. Am. Chem. Soc.*, 102 (1980) 416.
9 A. H. Jackson, *Phil. Trans R. Soc. Lond. A*, 293 (1979) 21.
10 J. E. Hunt, R. D. Macfarlane, J. J. Katz and R. C. Dougherty, *J. Am. Chem. Soc.*, 103 (1981) 6775.
11 B. T. Chait and F. H. Field, *J. Am. Chem. Soc.*, 104 (1982) 5519.
12 B. T. Chait and F. H. Field, *J. Am. Chem. Soc.*, 106 (1984) 1931.
13 M. Barber, R. S. Bordoli, G. J. Elliot, R. D. Sedgwick and A. N. Tyler, *Anal. Chem.*, 54 (1982) 645A.
14 R. G. Brereton, M. B. Bazzaz, S. Santikarn and D. H. Williams, *Tetrahedron Lett.*, 24 (1983) 5775.
15 D. L. Bricker and D. H. Russell, *J. Am. Chem. Soc.*, 108 (1986) 6174.
16 R. Guevremont and R. K. Boyd, *Int. J. Mass Spectrom. Ion Processes*, 84 (1988) 47.
17 R. P. Grese, R. L. Cerny, M. L. Gross and M. Senge, *J. Am. Soc. Mass Spectrom.*, 1 (1990) 72.
18 R. B. van Breemen, F. L. Canjura and S. J. Schwartz, *J. Agric. Food Chem.*, (1990) submitted for publication.
19 E. Constantin, Y. Nakatani, G. Teller, R. Hueber and G. Ourisson, *Bull. Soc. Chim. Fr.*, 7/8 (1981) II303.
20 C. Fenselau and R. J. Cotter, *Chem. Rev.*, 87 (1987) 501.
21 R. M. Caprioli, T. Fan and J. S. Cottrell, *Anal. Chem.*, 58 (1986) 2949.
22 Y. Ito, T. Takeuchi, D. Ishi and M. Goto, *J. Chromatogr.*, 346 (1985) 161.
23 R. M. Caprioli, W. T. Moore, B. Dague and M. Martin, *J. Chromatogr.*, 443 (1988) 355.
24 S. Pleasance, P. Thibault, M. A. Moseley, L. J. Deterding, K. B. Tomer and J. W. Jorgenson, *J. Am. Soc. Mass Spectrom.*, 1 (1990) 312.
25 D. E. Games, S. Pleasance, E. D. Ramsey and M. A. McDowall, *Biomed. Environ. Mass Spectrom.*, 15 (1988) 179.
26 F. J. Canjura and S. J. Schwartz, *J. Agric. Food Chem.*, (1991) in press.
27 F. J. Canjura, S. J. Schwartz and R. V. Nunes, *J. Food Sci.*, (1991) in press.
28 R. M. Caprioli, *Anal. Chem.*, 62 (1990) 477A.
29 S. J. Schwartz, S. L. Woo and J. H. von Elbe, *J. Agric. Food Chem.*, 29 (1981) 533.
30 J. E. Hunt, P. M. Schaber, T. J. Michalski, R. C. Dougherty and J. J. Katz, *Int. J. Mass Spectrom. Ion Phys.*, 53 (1983) 45.
31 P. M. Schaber, J. E. Hunt, R. Fries and J. J. Katz, *J. Chromatogr.*, 316 (1984) 25.