Journal of Porphyrins and Phthalocyanines
J. Porphyrins Phthalocyanines **3**, 283–291 (1999)

Investigation of MALDI-TOF Mass Spectrometry of Diverse Synthetic Metalloporphyrins, Phthalocyanines and Multiporphyrin Arrays

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Received 10 July 1998
Accepted 24 August 1998

ABSTRACT: We investigated the utility of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) for analyzing porphyrinic compounds using a variety of different synthetic porphyrins, azaporphyrins, phthalocyanines and multiporphyrin arrays. Comparisons of spectra obtained from these analytes deposited either as neat samples or codeposited with neutral or acidic matrices have been made with the goal of identifying conditions that yield minimal demetalation, transmetalation, adduct formation and fragmentation. It was found that the molecular masses of many porphyrins can be successfully measured from neat sample preparations and do not require a matrix to facilitate desorption and ionization, although the measurement of large multiporphyrin arrays was facilitated by the use of matrices. Demetalation of magnesium porphyrins occurred in the presence of acidic matrices, but not with neutral matrices such as 1,4-benzoquinone. Positive ion spectra were obtained for each compound and negative ion spectra were also collected for the azaporphyrins and phthalocyanines. Examination of selected samples (prepared neat, with 1,4-benzoquinone, 2,3,5,6-tetrachloro-1,4-benzoquinone or α-cyano-4-hydroxycinnamic acid) showed that the dominant process of ionization involved oxidation yielding the radical cation $M^{+\cdot}$ rather than the protonated molecule $[M+H]^+$. MALDI-TOF-MS is shown to be a powerful analytical tool for the characterization of diverse synthetic porphyrinic compounds. Copyright © 1999 John Wiley & Sons, Ltd.

KEYWORDS: mass spectrometry; LDI; MALDI; radical cations; metalloporphyrins; porphyrin arrays; phthalocyanines

INTRODUCTION

Naturally occurring porphyrinic molecules constitute the most important class of biological cofactors, including hemes, the chlorophylls and vitamin B_{12}. Synthetic porphyrins have been examined as biomimetic models, catalysts, sensors and therapeutic agents. Phthalocyanines, which are closely related to porphyrins, also have a rich synthetic chemistry and have been widely used as dyes. More recently, covalent multiporphyrin arrays have been constructed that function as light-harvesting energy funnels [1], molecular wires [2] and optoelectronic gates [3].

A major challenge in the mass spectrometric analysis of porphyrinic compounds is to avoid metalation of free base porphyrins and demetalation or transmetalation of metalloporphyrins. Many metals in the periodic chart have been incorporated into porphyrins, and the stability of the metalloporphyrins varies dramatically. Magnesium porphyrins are among the most labile metalloporphyrins and are demetalated by weak acids such as acetic acid or by silica gel. Preservation of the porphyrin metalation state during mass spectrometric investigation is essential. Of equal concern is the detection of impurities. For example, the reliable spectroscopic analysis of synthetic multiporphyrin arrays requires samples of ≥99% purity. An ideal mass spectrum would give no demetalation, fragmentation or adduct formation, as

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CCC 1088–4246/99/040283–09 $17.50

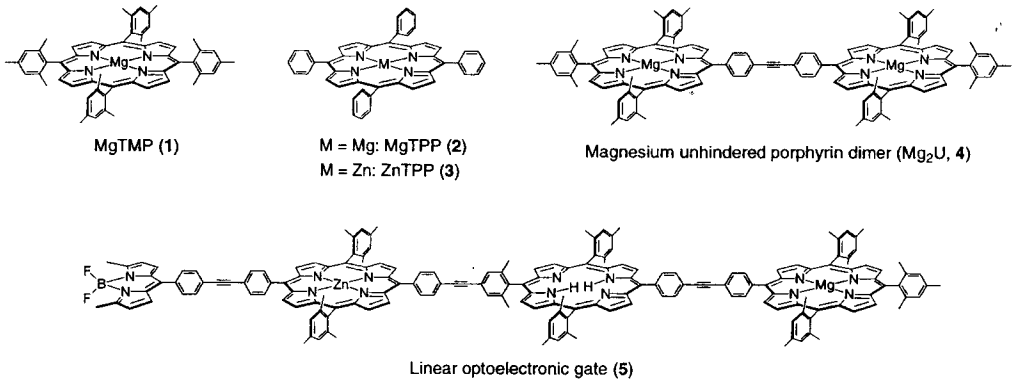

Fig. 1. Synthetic magnesium-containing porphyrins and arrays.

peaks due to these mass spectral processes are not easily distinguished from impurities in the sample [4]. The ability to collect mass spectra reliably is of utmost importance for the characterization of synthetic monomeric porphyrins as well as of multiporphyrin arrays.

Mass spectral methods for examining these synthetic products are essential for carrying out research in this area. Porphyrins have been examined by diverse mass spectral methods, including EI [5–11], CI [12–16], DCI [8, 17–19], ^{252}Cf PD [20–23], ESI [24–29], FAB [6, 8, 30–33] and MALDI [4, 34–38]. Previously we found that MALDI-TOF-MS could be used to obtain useful mass spectra of arrays containing up to nine porphyrins from neat samples as well as from samples codeposited with matrices [4].

In this paper we perform a more expansive study of MALDI-TOF-MS with an emphasis on magnesium-

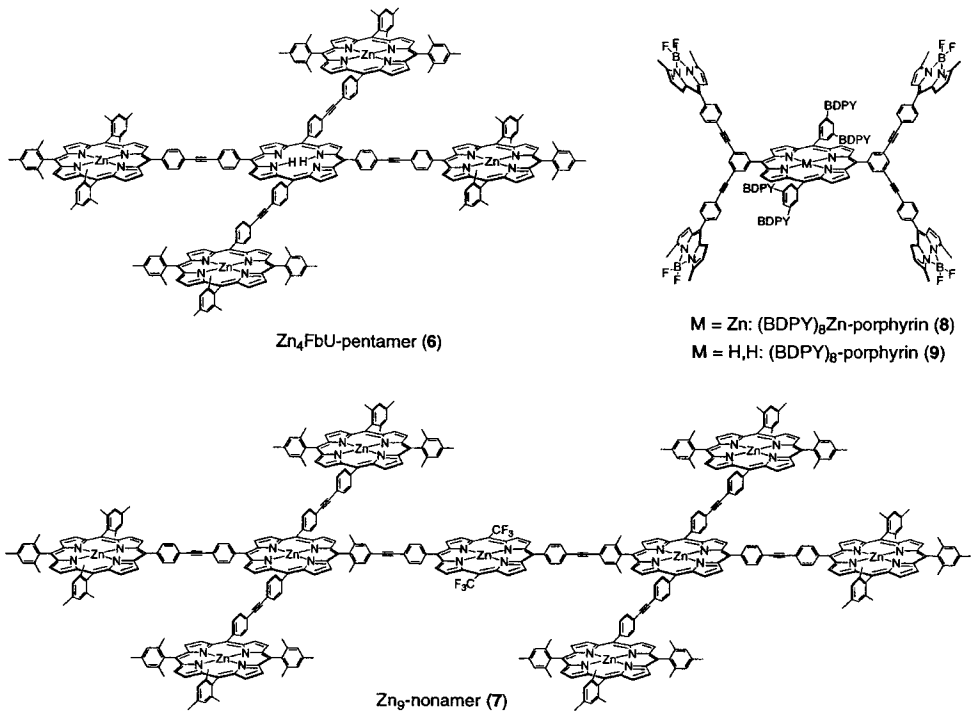

Fig. 2. Large multiporphyrin arrays. BDPY denotes the boron-dipyrrin dye with attached phenylethyne unit. Only four of the eight BDPY structures are displayed for clarity.

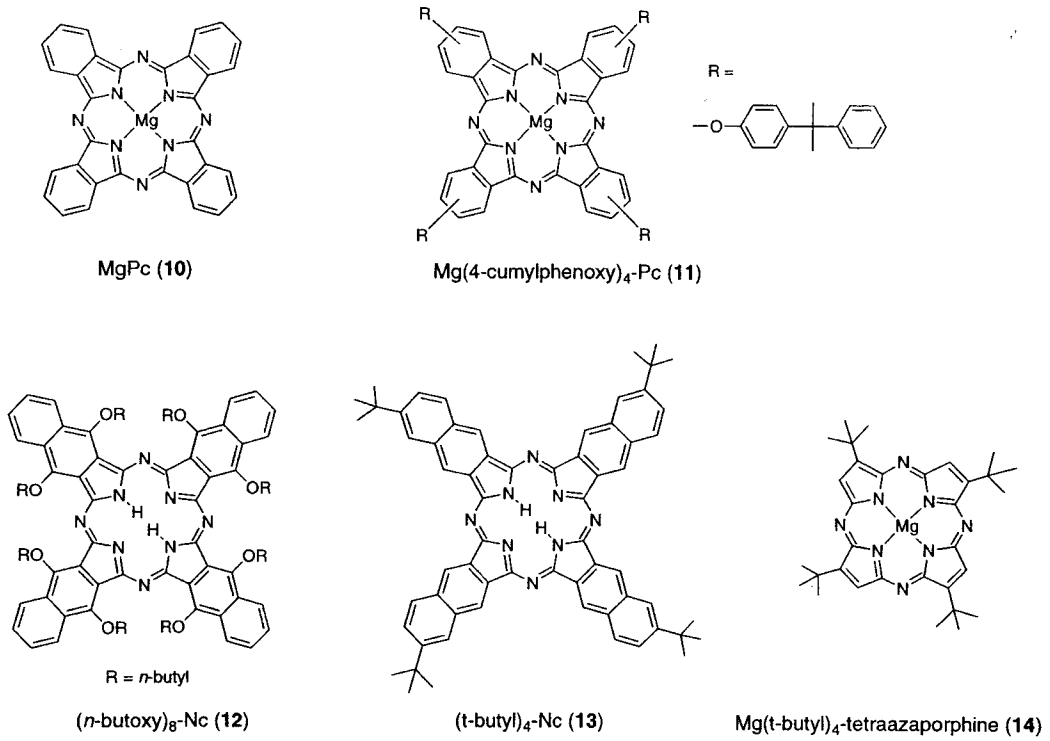

Fig. 3. Naphthalocyanines, phthalocyanines and a tetraazaporphyrin.

containing porphyrins, phthalocyanines and multiporphyrin arrays. The study includes 14 synthetic porphyrinic compounds (Figs 1–3). We extend our studies using quinones as matrices [39]. These studies also explore the ease of formation as well as the nature of the molecular (pseudomolecular) ion observed. Collectively, these studies aid in identifying appropriate conditions for mass spectral analysis of diverse porphyrins with limited demetalation, fragmentation and adduct formation, as well as enhanced signal-to-noise ratio.

EXPERIMENTAL

Materials and Methods

The synthetic porphyrins and phthalocyanines were prepared as described: magnesium tetramesitylporphyrin (MgTMP, **1**) [40], magnesium tetraphenylporphyrin (MgTPP, **2**) [40], magnesium unhindered porphyrin dimer (Mg_2U, **4**) [1], linear optoelectronic gate (**5**) [3], Zn_4FbU-pentamer (**6**) [41], Zn_9-nonamer (**7**) [4], $(BDPY)_8$Zn-porphyrin (**8**) [42], $(BDPY)_8$-porphyrin (**9**) [42] and magnesium tetrakis(4-cumylphenoxy)phthalocyanine (Mg(4-cumylphenoxy)$_4$-Pc, **11**) [43]. Zinc tetraphenylporphyrin (ZnTPP, **3**) magnesium phthalocyanine (MgPc, **10**), 5,9,14,18,23,27,32,36-octabutoxy-2,3-naphthalocyanine ((*n*-butoxy)$_8$-Nc, **12**), 2,11,20,29-tetra-*tert*-butyl-2,3-naphthalocyanine ((*t*-butyl)$_4$-Nc, **13**) and 2,7,12,17-tetra-*tert*-butyl-5,10,15,20-tetraaza-21*H*,23*H*-porphine ((*t*-butyl)$_4$-tetraazaporphine, **14**) were obtained from Aldrich Chemical Co. The matrices were obtained from Aldrich (Milwaukee, WI). 1,4-Benzoquinone was recrystallized from ethanol before use. CH_2Cl_2 was the solvent of choice and was distilled from CaH_2 to remove any residual acid. Analytes insoluble in CH_2Cl_2 were examined in solvents such as methanol, water or acetone.

Sample Preparation

Stock solutions of the samples having concentrations 10^{-3}–10^{-4} M were prepared in acid-free CH_2Cl_2. The mixture to be analyzed was prepared by combining 10 μL of the sample stock solution with 10 μL of a saturated solution of the matrix/oxidant/reductant in acid-free CH_2Cl_2. The mixture was vortexed and 1 μL of this mixture was spotted on a stainless steel target

Table 1. Compounds examined

Compound	Molecular formula	Calculated mass
Porphyrin monomers		
MgTMP (**1**)	$C_{56}H_{52}N_4Mg$	804.4*
MgTPP (**2**)	$C_{44}H_{28}N_4Mg$	636.2*
ZnTPP (**3**)	$C_{44}H_{28}N_4Zn$	676.2*
Multiporphyrin arrays		
Mg_2U (**4**)	$C_{108}H_{90}Mg_2N_8$	1546.7*
Linear optoelectronic gate (**5**)	$C_{178}H_{141}BF_2N_{14}MgZn$	2614.7†
Zn_4FbU-pentamer (**6**)	$C_{264}H_{206}N_{20}Zn_4$	3920.2†
Zn_9-nonamer (**7**)	$C_{460}H_{342}F_6N_{36}Zn_9$	7076.4†
$(BDPY)_8$Zn-porphyrin (**8**)‡	$C_{196}H_{132}N_{20}B_8F_{16}Zn$	3223.2†
$(BDPY)_8$-porphyrin (**9**)‡	$C_{196}H_{134}N_{20}B_8F_{16}$	3159.9†
Phthalocyanines		
MgPc (**10**)	$C_{32}H_{16}N_8Mg$	536.1*
Mg(4-cumylphenoxy)$_4$-Pc (**11**)	$C_{92}H_{72}N_8O_4Mg$	1376.6*
(*n*-Butoxy)$_8$-Nc (**12**)	$C_{80}H_{90}N_8O_8$	1290.7*
(*t*-Butyl)$_4$-Nc (**13**)	$C_{64}H_{58}N_8$	938.5*
(*t*-Butyl)$_4$-tetraazaporphine (**14**)	$C_{32}H_{42}N_8$	538.4*

* Monoisotopic mass.
† Average mass.
‡ BDPY denotes the boron-dipyrrin dye with attached phenylethyne unit.

wheel. Co-crystallization was achieved by allowing the mixture to air dry.

Instrumentation

A Bruker Proflex TOF mass spectrometer equipped with gridless delayed extraction capabilities, 1.2 m flight tube, nitrogen laser (337 nm), microchannel plate detector and 1 GHz transient recorder was used to obtain the linear mode data. The instrument provided a mass accuracy of 0.1% with external standards and 0.01% with internal standards. An instrumental mass resolution $m/\Delta m$ [where Δm is the full width at half-maximum (FWHM) of the peak] of several thousand was obtained for species of 2500 Da or less. The ion acceleration voltage was 20 kV.

A PerSeptive Biosystems Voyager-DE STR TOF mass spectrometer equipped with delayed extraction capabilities, a nitrogen laser (337 nm), dual-microchannel plate detector and 2 GHz oscilloscope was used to obtain the reflectron data. The instrument provided a mass accuracy of 0.05% with external standards and 0.005% with internal standards. An instrumental mass resolution of 7000 FWHM was obtained for species of 800 Da. The ion acceleration voltage was 25 kV.

In all the spectra, mass assignments were made via external calibration using peptide and matrix ions as calibrants. The mass spectra reported here were obtained by summing the signals from 100–600 laser shots in order to obtain sufficient counting statistics to observe trace impurities and trace adduct ions.

RESULTS AND DISCUSSION

We examined positive ion MALDI mass spectra of a series of synthetic magnesium and zinc porphyrins either in the presence or absence of a matrix (Table 1). Although each porphyrin studied yielded a peak corresponding to the ionized intact porphyrin, detailed differences were observed in the spectra obtained from

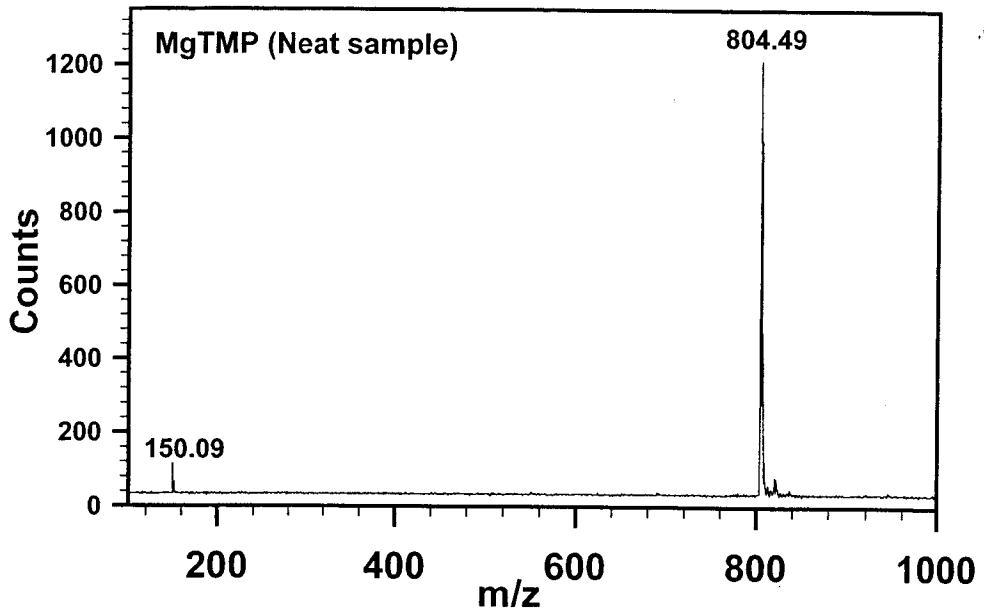

Fig. 4. MALDI-TOF delayed extraction reflectron mass spectrum of MgTMP (**1**) obtained from a neat sample preparation. The peak at *m/z* 150 is an uncharacterized impurity.

the neat porphyrin samples versus those examined in the presence of a matrix (*vide infra*).

Figure 4 shows a delayed extraction reflectron time-of-flight mass spectrum of MgTMP (**1**) obtained from a neat sample preparation. The mass resolution and mass accuracy were sufficiently high to allow unambiguous definition of the ion species. The spectrum is dominated by a single ion species corresponding to the porphyrin radical cation. Evidence for the radical cation $M^{+\cdot}$ is provided in Figure

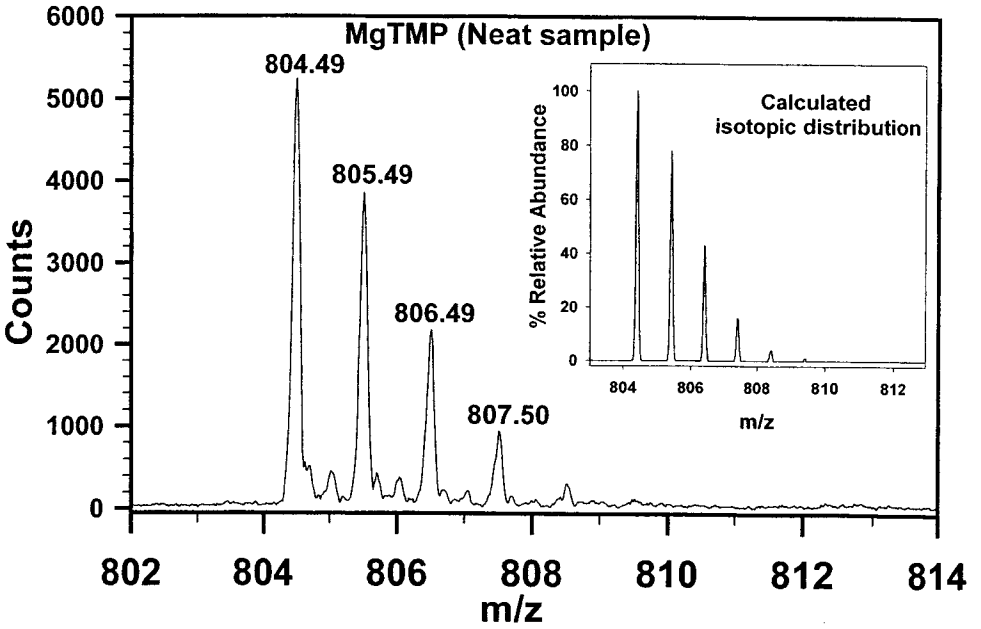

Fig. 5. Molecular ion region of mass spectrum of MgTMP (**1**) and calculated isotopic distribution (inset).

Table 2. Comparison of molecular masses obtained in presence and absence of matrix

Compound	Molecular formula	Calculated mass	Data obtained in reflector mode (delayed extraction)		
			BQ	Neat	4-HCCA
Porphyrin monomers					
MgTMP (**1**)	$C_{56}H_{52}N_4Mg$	804.4*	804.5*	804.5*	804.5*
MgTPP (**2**)	$C_{44}H_{28}N_4Mg$	636.2*	636.2*	636.2*	636.2*
ZnTPP (**3**)	$C_{44}H_{28}N_4Zn$	676.2*	676.4*	676.3*	676.3*
Multiporphyrin arrays					
Linear optoelectronic gate (**5**)	$C_{178}H_{141}BF_2N_{14}MgZn$	2614.7†	2614.2†	2614.6†	2614.1†
Zn_4FbU-pentamer (**6**)	$C_{264}H_{206}N_{20}Zn_4$	3920.2†	3920.1†	3919.9†	3919.6†
Zn_9-nonamer (**7**)	$C_{460}H_{342}F_6N_{36}Zn_9$	7076.4†	7077.1†	No Signal	7073.4†

* Monoisotopic mass.
† Average mass.

5, where the observed isotope distribution corresponds closely to the calculated isotopic distribution. It is of interest to note that contributions from protonation of the porphyrin, $[M+H]^+$, appear relatively minor. Identical results were obtained for MgTPP (**2**) and ZnTPP (**3**) (Table 2). The data obtained with neat sample and the two matrices give comparable masses for molecules ranging from monomers to multiporphyrin arrays.

We also examined the porphyrin monomers in a series of matrices that included conventional carboxylic acid matrices (e.g. α-cyano-4-hydroxycinnamic acid, ferulic acid) as well as neutral matrices (e.g. 1,4-bis(5-phenyloxazol-2-yl)benzene (POPOP), 1,4-benzoquinone (BQ), 2,3,5,6-tetrachloro-1,4-benzoquinone (TCQ)) [39]. Although the acidic matrices yielded intense intact porphyrin radical cations, exposure of the porphyrin to acid resulted in some demetalation of magnesium (but not zinc) as well as adducts of undetermined origin. We therefore feel it inadvisable to use acidic matrices with magnesium porphyrins. Although the neutral matrices yielded results comparable with those obtained in the neat preparations, we observed no advantage in their use for measuring porphyrin monomers.

Figure 6(a) shows the delayed extraction reflectron time-of-flight mass spectrum of Zn_4FbU (**6**) obtained from a neat sample preparation. In addition to the intact radical cation, an intense fragment ion is observed at *m/z* 729.3 which arises from the loss of a single porphyrin unit from $Zn_4FbU^{\cdot+}$ through cleavage at the *meso*-phenyl bond. The delayed extraction time-of-flight mass spectrum collected in the linear mode exhibits an apparent lower degree of fragmentation than that observed in the reflectron spectrum, because ions that undergo metastable decay in the field-free flight tube are collected as parent ions in the former but not in the latter. The other multiporphyrin arrays investigated (Table 1) gave comparable results. However, as the size of the array increased, the degree of fragmentation increased. Thus, for example, the Zn_9-nonamer (**7**) yields a considerably weaker intact ion peak in the reflectron versus linear mass spectrum. We previously observed that the large Zn-porphyrin-containing arrays yield a lower degree of fragmentation when the spectra are obtained in the presence of the matrix 4-HCCA compared with the neat preparations [4]. We have observed similar results with the quinone matrices (BQ, TCQ), which appear to facilitate survival of the intact porphyrin ion species. Given these observations, we speculate that large Mg-porphyrin-containing arrays would be best analyzed using quinone matrices.

We also examined a broader range of porphyrinic pigments (Table 1). In each compound studied, an intense ion corresponding to the intact radical cation was observed. Phthalocyanines and azaporphyrins are strongly electron–deficient and would be predicted to yield radical anions more easily than radical cations. Given the electron-withdrawing effects of the four additional nitrogens in phthalocyanines and azaporphyrins compared with porphyrins, we sought to

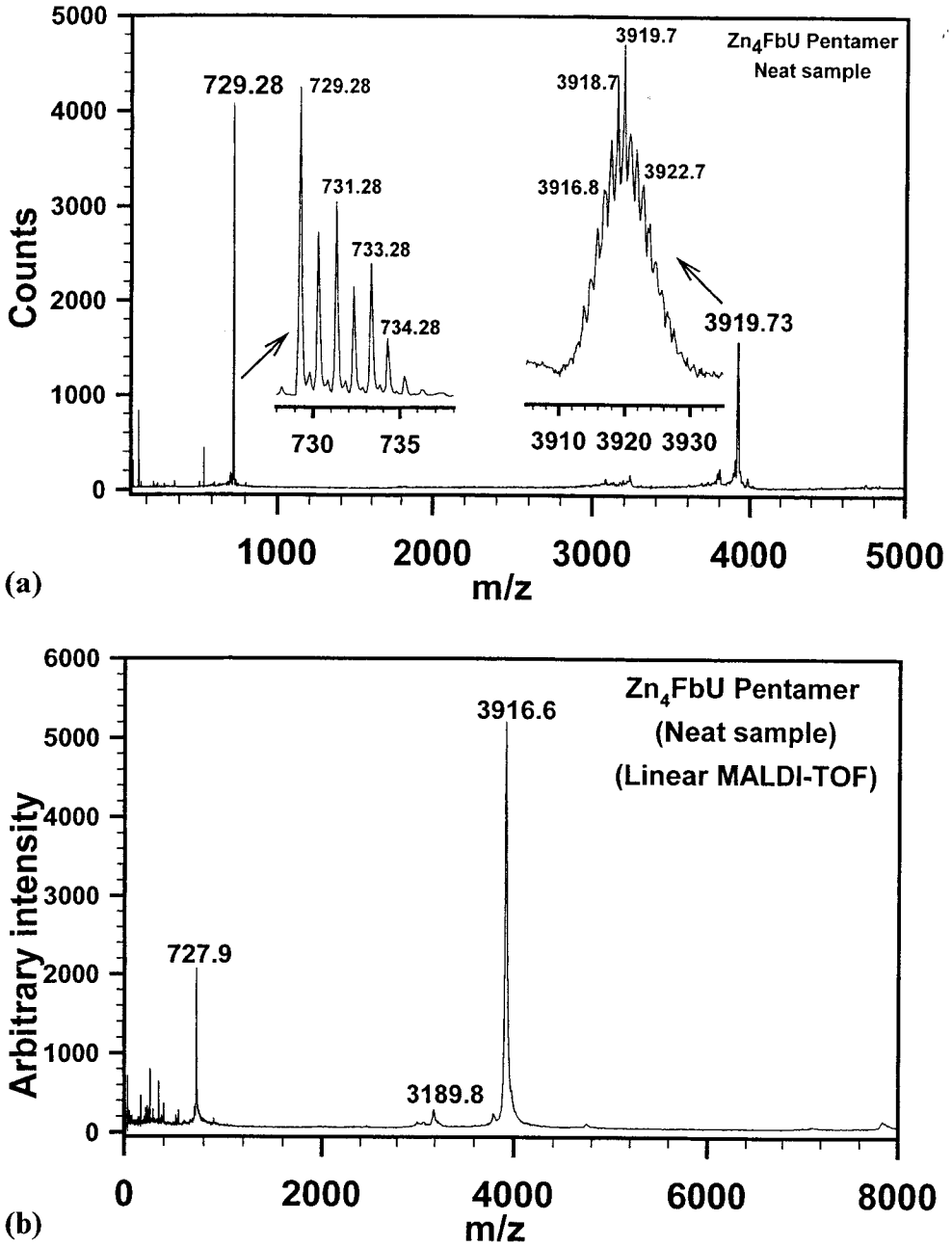

Fig. 6. MALDI-TOF delayed extraction mass spectra of Zn_4FbU-pentamer (**6**) obtained from a neat sample preparation. (a) Reflectron spectrum. Insets show a closer view of the molecular ion and the base peak regions. The peak at *m/z* 729.3 is due to the loss of a single porphyrin unit from Zn_4FbU and shows near-isotopic resolution. (b) Linear mode spectrum.

investigate whether reducing agents would afford enhanced ionization (via formation of the radical anions). *N,N,N′,N′*-Tetramethyl-1,4-phenylenediamine (TMPDA) was used as the reducing agent. Surprisingly, we observed that both species (the radical cation $M^{+\cdot}$ in the positive ion mode and the radical anion $M^{-\cdot}$ in the negative ion mode) were produced in good yield, but in general the alkyl- and alkoxy-substituted compounds (e.g. **11–14**) exhibited more extensive side-chain fragmentation from the

negative ion species. Therefore we believe that the positive ion mode is most useful for examining the intact porphyrinic pigments.

CONCLUSIONS

We have shown that positive ion MALDI-TOF mass spectrometry is a generally useful method for analyzing a diverse range of synthetic porphyrinic compounds. In all cases the dominant intact molecule ion species is the porphyrin radical cation $M^{+\cdot}$. Porphyrins are unusual in that they can be analyzed in neat form, i.e. they serve as their own matrices. However, large multiporphyrin arrays can be more readily analyzed using a MALDI matrix. Neutral quinones appear to be the matrices of choice for magnesium-containing porphyrins, because the more widely used carboxylic acid matrices induce demetalation.

Acknowledgements

Mass spectra were obtained at the NCSU Mass Spectrometry Facility or The Rockefeller University. Partial funding of the instrumentation was provided by North Carolina Biotechnology Center grant 9610-IDG-1001 (Bruker MALDI-TOF). This research was supported by NIH grants to J.S.L. (GM 36238) and B.T.C. (RR00862).

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