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# Origin and Removal of Adducts (Molecular Mass = 98 u) Attached to Peptide and Protein Ions in Electrospray Ionization Mass Spectra

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Electrospray ionization of peptides and proteins often produces intense adduct ions resulting from the attachment of a moiety with mass 98 u. The formation of these adduct ions results in a substantial reduction in the mass spectrometric sensitivity and an undesirable increase in the complexity of the mass spectra. In the present study it was shown that the removal of the attached adducts from peptide and protein ions can be affected by collisional activation and that the adducts arise from the attachment of sulfuric acid or phosphoric acid to peptide and protein ions. When sulfate and phosphate ions are removed from the samples by chemical means, adduct free ions are obtained from proteins yielding spectra with improved quality and sensitivity. (*J Am Soc Mass Spectrom* 1990, 1, 382-388)

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Electrospray ionization is a soft ionization technique in which intact, multiply charged gas phase ions of large, involatile and thermally labile molecules are produced by electro spraying a dilute solution of the analyte of interest [1]. Although the technique was introduced some twenty years ago [2, 3], it did not receive wide attention until recently. The renewal of interest in electrospray ionization resulted from important developments made by Fenn and co-workers [4-6]. These investigators successfully interfaced an atmospheric pressure electrospray ion source to a quadrupole analyzer and demonstrated the ability of the technique to analyze large, involatile molecules such as organic polymers [5] and proteins [6]. Because protein ions produced by electrospray are frequently highly charged, the observed mass-to-charge ratios ( $m/z$ ) of the protein ions can be relatively small. Therefore, conventional quadrupole mass analyzers with limited  $m/z$  range can be used to determine the molecular masses of large proteins. Other workers [7, 8] have also used electrospray ionization, and the related technique called ion spray, to determine accurately the molecular masses of a large number of proteins.

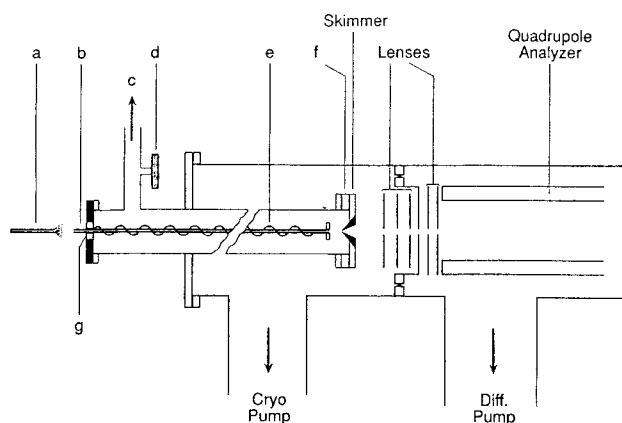
Recently, we constructed an electrospray ionization source that we coupled directly to a commercial quadrupole analyzer and measured the molecular masses of a large number of peptides and proteins [9, 10]. In this instrument ions generated by electro spray-

ing the analyte solution at atmospheric pressure enter the vacuum of the mass spectrometer through a 20-cm long metal capillary tube. Desolvation of the ions is carried out by heat transfer to the charged droplets during their transport through the metal capillary tube, as well as by collisional activation in a region of reduced pressure after the ions exit from the capillary tube. By controlling the heat transfer and collisional activation, ions can be generated that are either solvated to a desired degree or completely desolvated. When desired, additional energy can be deposited in the ions to obtain fragmentation spectra [11, 12]. Desolvation in the present system is convenient and effective and does not require the use of the counter current flow of heated gas employed by other researchers [4-8]. The results obtained with the present system [9, 10] are comparable to those obtained with previous systems [4-8].

During the course of the analysis of a large number of peptides and proteins we noticed that the mass spectra often contained ion species corresponding to the attachment to the analyte ions, of a moiety of unknown origin and identity, with molecular mass 98 u [9]. For certain peptides and proteins intense ions resulting from the attachment of as many as five such moieties to the protonated molecule ions were observed. Loo et al. [13] and Henry et al. [14] also reported the observation of similar intense adduct ions in the mass spectra of several proteins. For example, the spectra of ribonuclease A and ribonuclease S reported by Loo et al. were almost completely dominated by the adduct ions. Covey et al. [7] also observed similar adduct ions (see, for example, Figure 6

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**Figure 1.** A schematic diagram of the electrospray ionization mass spectrometer (not drawn to scale). a = 152- $\mu\text{m}$  i.d. syringe needle maintained at a potential of  $\sim 5.0$  kV; b = 0.5-mm i.d., 20-cm long stainless steel capillary tube maintained at a potential of 100–300 V; c = to rotary pump; d = pirani gauge; e = heating tape; f = teflon insulating plate; g = Swagelock teflon fitting. The skimmer (0.5-mm i.d. orifice), lens elements and quadrupole analyzer are from a Vestec 201 thermospray mass spectrometer (Houston, TX) (Reprinted with permission.)

of ref 7). Formation of these adduct ions reduces the intensities of the protonated molecule ions (and hence the mass spectrometric sensitivity) and complicates the mass spectra. We undertook the present investigation to gain an understanding of the nature and origin of the adducts and to develop a method for their removal.

## Experimental

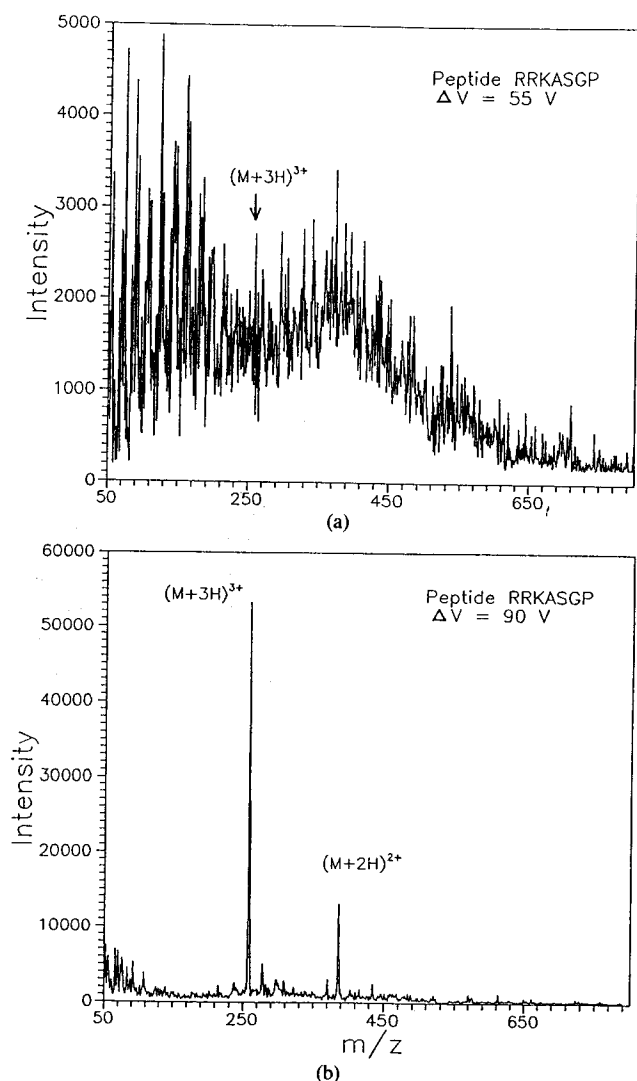
The electrospray ionization mass spectrometer used in the present investigation is shown in Figure 1. The mass spectrometer and the procedure adopted to acquire mass spectra were described previously [9]. Briefly, the sample solution is electrosprayed from a hypodermic needle, a, in ambient laboratory air at a rate of 0.5 to 0.75  $\mu\text{L}/\text{min}$ . The resulting charged droplets were transported into the vacuum of the mass spectrometer through a 20-cm long, 0.5-mm i.d. metal capillary tube, b, into a region of reduced pressure (1–10 torr). Because electrospray is carried out directly from solution at atmospheric pressure, the ions formed in this process are strongly solvated. Desolvation of analyte ions is carried out efficiently by two sequential processes. The first step involves controlled heat transfer to the charged droplets during their transport through the long capillary tube, b. The capillary tube is heated to approximately 85  $^{\circ}\text{C}$ . Ions that exit from the capillary tube are observed to be solvated to various extents. The remaining solvent molecules bound to the analyte ions are then removed completely in the second step by collisional activation induced by applying an electric field in the region of reduced pressure between the exit of the capillary tube and the skimmer (Figure 1). The procedure for optimization of the electric field by varying the volt-

age applied to the capillary tube is described elsewhere [9]. The desolvated ions finally pass through a set of lenses and enter the analyzer chamber, where their  $m/z$  values are determined in a quadrupole analyzer.

The analyte solution for electrospray is prepared by dissolving the sample in a mixture of solvents comprised of 40–50% water, 45–50% methanol, and 3–5% acetic acid. Bovine ribonuclease A (catalog # R-5125), chicken egg lysozyme (L-6876), bovine trypsinogen (T-1143), bovine ribonuclease S (R-6000), equine myoglobin (M-0630), bovine trypsin (T-8642), bradykinin (B-3259), and the peptide, RRKASGP (A-3651) were obtained from the Sigma Chemical Company, St. Louis, MO. Human  $\beta$ -endorphin was obtained from Boehringer Mannheim Biochemicals, Indianapolis, IN. The peptides porcine dynorphin A(1-17), dynorphin model 1 (dyn m1), and dynorphin model 2 (dyn m2) were kindly provided by Dr. J. Taylor of the Rockefeller University. Dyn m2 is a synthetic peptide analogue of dynorphin A(1-17) (sequence: YGGFLRRIRPKLKWDNQ) in which alternate lysine and valine residues were incorporated in positions 7–15 and several other amino acid residues were replaced. The amino acid sequence of dyn m2 is YGGFLKVKVKVKVSS. The peptide dyn m1 has the same sequence as dyn m2, except that valine-10 is replaced by a proline. High purity water and methanol were obtained from Burdick and Jackson, Muskegon, MI, and acetic acid (ultra) from J.T. Baker Chemical Co., Phillipsburg, NJ.

## Results and Discussion

The procedure for removing solvent molecules bound to analyte ions of interest exiting from the capillary tube is demonstrated in Figure 2. Shown in Figure 2a is the electrospray ionization mass spectrum of the peptide RRKASGP. The peptide was dissolved in a solvent mixture of water, methanol, and acetic acid in the proportion 47.5:47.5:5.0% v/v at a concentration of 20 pmol/ $\mu\text{L}$  and electrosprayed at a rate of 0.5  $\mu\text{L}/\text{min}$ . The spectrum shown between  $m/z$  50 and  $m/z$  800 is a single scan acquired in 62 s. Thus, 10-pmol sample was consumed in acquiring the spectrum. The skimmer was operated at 20 V and the capillary tube at 75 V. The potential difference,  $\Delta V$ , applied between the exit of the capillary tube and the skimmer is therefore 55 V. The mass spectrum shows the presence of a large number of ion peaks of composition  $\text{H}^+(\text{H}_2\text{O})_i(\text{CH}_3\text{OH})_j$ , with  $i$  ranging from 0 to 13 and  $j$  from 0 to 3 in the  $m/z$  span 50–250 (Figure 2a). A weak  $[\text{M} + 3\text{H}]^{3+}$  ion (observed  $m/z = 258.3$ ; calculated  $m/z = 258.0$ ) is observed together with a large number of ions whose composition is  $[\text{M} + k\text{H}_2\text{O} + l\text{CH}_3\text{OH} + 3\text{H}]^{3+}$ , where  $k$  and  $l$  have a wide range of values. Under these operating conditions the majority of ions are observed to be highly solvated with water and methanol. When the poten-



**Figure 2.** Electrospray ionization spectra of the peptide RRKASGP, measured at two different values of the potential difference ( $\Delta V$ ) applied between the capillary tube and the skimmer. The temperature of the tube is  $\sim 85^\circ\text{C}$  and the potential applied to the skimmer,  $V(\text{skimmer}) = 20\text{ V}$ . (a)  $\Delta V = 55\text{ V}$ ; (b)  $\Delta V = 90\text{ V}$ . The spectra are single scans, each acquired in 62 s. Each data point in the scan is averaged three times by the data system.

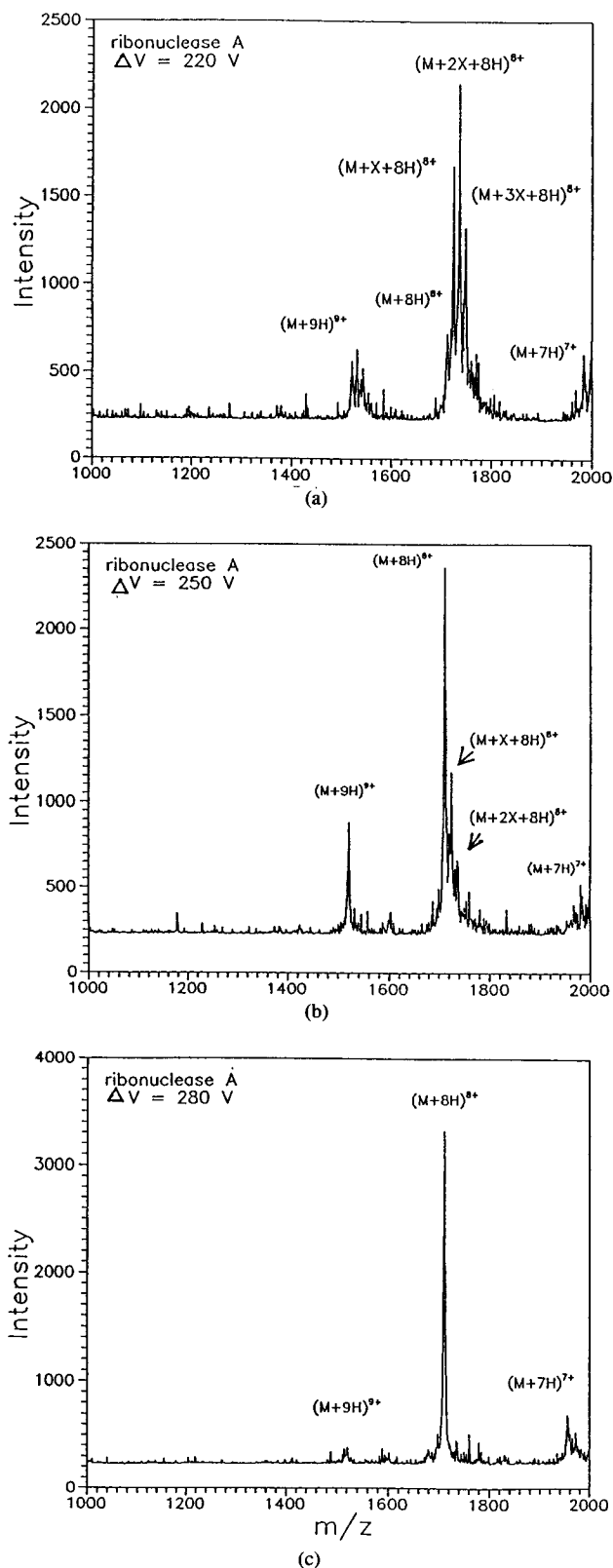
tial difference,  $\Delta V$ , between the skimmer and the capillary tube is increased from 55 V to 90 V, all other conditions being the same, intense  $[\text{M} + 3\text{H}]^{3+}$  and  $[\text{M} + 2\text{H}]^{2+}$  ions are observed (Figure 2b) with no attached water or methanol. The intensity of the bare  $[\text{M} + 3\text{H}]^{3+}$  ion has increased dramatically from  $\sim 3,000$  to  $\sim 55,000$  (arbitrary units). In general, the maximum intensity of peptide and protein ions are observed at a value of  $\Delta V$ , for which all solvent molecules (water, methanol, etc.) bound to the analyte ions are removed completely. Increasing  $\Delta V$  beyond this optimum value results in the fragmentation of the molecule ions [11, 12] and a decrease in molecule ion intensities.

In addition to the observation of adduct ions resulting from the attachment of solvent molecules, which

can be removed efficiently, a different type of adduct ion is often observed in the electrospray ionization spectra of certain peptides and proteins. An example of this latter type of adduct formation is illustrated in the electrospray ionization spectra of bovine ribonuclease A, shown in Figure 3a. The potential difference,  $\Delta V$ , applied between the skimmer and the capillary tube was 220 V. The spectrum consists of three clusters of ions centered at  $m/z$  1530,  $m/z$  1730, and  $m/z$  2000, respectively. The observed  $m/z$  values of all the ions are provided in Table 1, together with the deduced [1] charge ( $z$ ) of each of these ions. Also given are the molecular masses derived from the observed  $m/z$  and  $z$  values. The three ions observed between  $m/z$  1500 and  $m/z$  1600 are identified as  $[\text{M} + n\text{X} + 9\text{H}]^{9+}$ , where M is ribonuclease A, X is an unknown species ( $\text{MM} = 98\text{ u}$ , see Table 2), and  $n = 0-2$ . Similarly, the ions observed between  $m/z$  1700 and  $m/z$  1800,  $[\text{M} + n\text{X} + 8\text{H}]^{8+}$ , also arise from ribonuclease A ( $n = 0$ ) or the attachment of 1-3 of the unknown moiety, X, to the ribonuclease A ion. The absence of solvent adducts demonstrates that the species X binds to the ribonuclease A ion more strongly than does water or methanol. The electrospray ionization spectrum of ribonuclease A, reported by Loo et al. [13], is also dominated by these adduct ions. These investigators did not discuss the origin of these adduct species.

The relative intensities of the components of the  $[\text{M} + n\text{X} + 9\text{H}]^{9+}$  cluster of ions are different than those in the  $[\text{M} + n\text{X} + 8\text{H}]^{8+}$  cluster (Figure 3a). Although the intensities of the bare protein ions  $[\text{M} + 9\text{H}]^{9+}$  and  $[\text{M} + 8\text{H}]^{8+}$  are similar, the relative intensities of the adduct ions are much higher in the 8+ ion cluster. The observed differences in the relative ion intensities of the  $[\text{M} + n\text{X} + m\text{H}]^{m+}$  ions of different charge states ( $m$ ) can be attributed to different collisional activation energies experienced by the ions of different charge state. For a given potential difference,  $\Delta V$ , applied between the skimmer and the capillary tube, ions with nine charges will have 12.5% more collisional activation energy than those with eight charges. Thus the additional collisional activation energy results in more efficient removal of the adduct moieties, X, from the more highly charged adduct ions.

When the potential difference,  $\Delta V$ , is increased from 220 V to 250 V, all other conditions being the same, the moieties, X, attached to the  $[\text{M} + 9\text{H}]^{9+}$  ions are removed completely and the intensity of the  $[\text{M} + 9\text{H}]^{9+}$  ion is increased by a factor of two (Figure 3b). The intensities of the adducts related to the  $[\text{M} + 8\text{H}]^{8+}$  ion have also decreased substantially, but the removal is not complete. When  $\Delta V$  is increased further to 280 V (Figure 3c), the adducted molecules, X, are removed completely from the  $[\text{M} + 8\text{H}]^{8+}$  ion and the intensity of this ion is increased. At the same time, however, the  $[\text{M} + 9\text{H}]^{9+}$  ion has disappeared almost completely. The disappearance of the  $[\text{M} + 9\text{H}]^{9+}$  ion can be attributed to proton stripping and/or



**Figure 3.** Electro spray ionization spectra of bovine pancreatic ribonuclease A obtained from a solution of water, methanol, and acetic acid (48.5:48.5:3.0 v/v). Concentration = 10 pmol/ $\mu$ L, flow rate = 0.5  $\mu$ L/min. Each spectrum is an average of five scans, requiring 145 s/scan. Thus, 60 pmol of ribonuclease A was consumed in acquiring each of the spectra.  $V(\text{skimmer}) = 20$  V. (a)  $\Delta V = 220$  V; (b)  $\Delta V = 250$  V; (c)  $\Delta V = 280$  V. Data was obtained to  $m/z$  2000, the upper limit of the mass spectrometer.

**Table 1.** Observed  $m/z$  values, charges ( $z$ ), and experimentally observed molecular masses corresponding to each ion observed in the electro spray ionization spectra of ribonuclease A\*

$z$	$m/z$	Mass(u)	Identified species
9	1521.15	13681.4	$M^a$
	1532.10	13779.9	$M + X^b$
	1543.0	13878.0	$M + 2X$
8	1711.25	13682.0	$M$
	1723.60	13780.8	$M + X$
	1735.70	13877.6	$M + 2X$
	1747.95	13975.6	$M + 3X$

\*See Figure 3.

<sup>a</sup> $M$  represents the protein, ribonuclease A.

<sup>b</sup> $X$  denotes a species of mass 98 u (see Table 2).

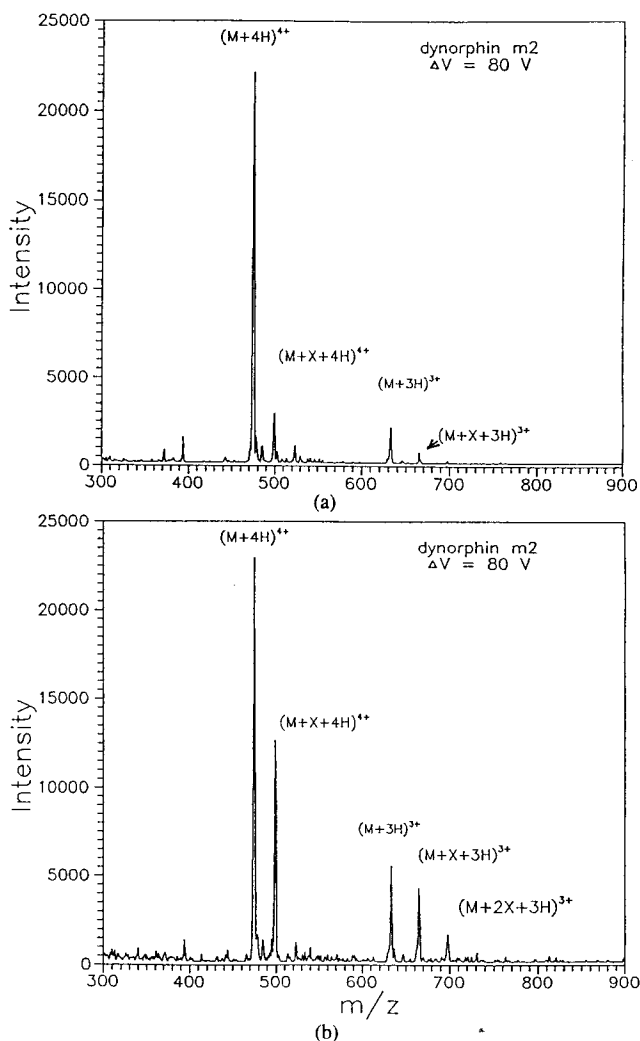
fragmentation of the ion [11, 12]. The data shown in Figure 3 demonstrate that although the species  $X$  binds quite strongly to protein ions in the gas phase, it can be removed completely by collisional activation, and appears to be bound *noncovalently* to the protein ions. The attachment of  $X$  was also observed in the electro spray ionization spectra of many other proteins, including bovine ribonuclease S, chicken egg lysozyme, bovine trypsinogen, bovine trypsin, and equine myoglobin. The extent of adduct formation was found to vary from protein to protein and could be removed by collisional activation.

In an attempt to elucidate the origin and nature of the molecule  $X$ , we investigated the effect of the electro spray ionization spectra of adding various reagents into the peptide and protein spray solutions. Because the molecular mass of both  $H_2SO_4$  and  $H_3PO_4$  is 98 u, we hypothesized that the adduct ions resulted from the attachment of sulfuric acid or phosphoric acid molecules to the peptide or protein ions. To test this hypothesis, small amounts of sulfuric acid and phosphoric acid were added to the solutions of ribonuclease A. Unfortunately, however, the addition of these acids, even in submicromolar amounts, makes it difficult to obtain a stable electro spray. An alternative approach was adopted that involved the addition of trace amounts of sulfate or phosphate salts to the analyte solution, which already contained acetic acid (3-5%). One such experiment is illustrated in Figure 4a where the spectrum obtained from a sample of Dyn m2 is shown ( $\Delta V = 80$  V). In addition to the molecule ions  $[M + 4H]^{4+}$  and  $[M + 3H]^{3+}$ , low intensities of ions with composition  $[M + X + 4H]^{4+}$ ,  $[M + X + 3H]^{3+}$ , and  $[M + 2X + 3H]^{3+}$  are also observed in the mass spectrum (Figure 4a). The mass of the species  $X$  is determined to be 98 u (Table 2). To the solution used to acquire the mass spectrum shown in Figure 4a, 40 pmol/ $\mu$ L of ammonium sulfate was added. The electro spray ionization spectrum of this solution is shown in Figure 4b. After the addition of ammonium sulfate, the adduct ion intensity relative to the protonated molecule ion is seen to increase substantially. This experiment suggests that the in-

**Table 2.** Observed average molecular masses of peptides, proteins, and related adducts, deduced from different ions\*

Analyte	Identified species	Measured molecular mass (u)	Source of data
Ribonuclease A	M(13682.3) <sup>a</sup>	13681.7 ± 0.4	Figure 3 and Table 1
	M + X	13780.4 ± 0.6	
	M + 2X	13877.8 ± 0.3	
	M + 3X	13975.6 <sup>b</sup>	
	X	98.0 ± 0.7	
Dynorphin m2	M(1895.4) <sup>a</sup>	1895.5 ± 0.7	Figure 4
	M + X	1993.1 ± 0.7	
	M + 2X	2091.3 ± 0.5	
	X	97.9 ± 0.4	
Dynorphin A(1-17)	M(2147.5) <sup>a</sup>	2147.0 ± 0.3	Not shown
	M + X	2245.1 ± 0.7	
	X	98.1 ± 0.8	
Peptide RRKASGP	M(770.9) <sup>a</sup>	771.5 ± 0.6	Figure 2

\*As observed in Figures 2-4.

<sup>a</sup>Calculated average molecular mass [19].<sup>b</sup>The value is from a single measurement.**Figure 4.** Electrospray ionization of dynorphin model 2 dissolved in a mixture of water, methanol, and acetic acid (47:50:3 v/v). Concentration = 2.0 pmol/ $\mu$ L; flow rate = 0.7  $\mu$ L/min. Each of the spectra is a single scan acquired in 57 s.  $V(\text{skimmer}) = 20$  V,  $\Delta V = 80$  V. (a) no salt added; (b) 40 pmol/ $\mu$ L ammonium sulfate added.

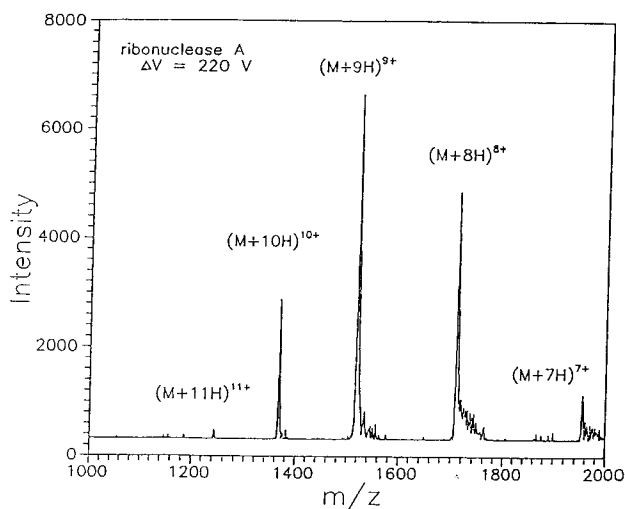
crease in the intensities of adduct ions is caused by the attachment of  $\text{H}_2\text{SO}_4$  to the protein ions. The experiment was repeated for several other concentrations of added ammonium sulfate to the ribonuclease A solutions. The ratio of the intensity of the adduct ions relative to the protonated molecule ions is observed to increase linearly with the increase in concentration of the added ammonium sulfate up to 40 pmol/ $\mu$ L. A further increase in salt concentration perturbs the stability of the electrospray and reduces the spectral quality. The above experiments were also repeated with several other peptides, including dynorphin A(1-17), dynorphin m1, human  $\beta$ -endorphin, and bradykinin. A similar increase in the intensity of adduct ions is observed in each case. However, the increase in the cases of bradykinin and human  $\beta$ -endorphin was relatively small.

The intensity of the adduct ions also increased upon addition of disodium hydrogen phosphate to a solution of dynorphin A(1-17), although the increase was lower than that observed for the addition of ammonium sulfate. Addition of ammonium sulfate (40 pmol/ $\mu$ L) increased the sum of the adduct ion intensities from 20% to 64% relative to the sum of the  $[\text{M} + 3\text{H}]^{3+}$  and  $[\text{M} + 4\text{H}]^{4+}$  ions, whereas addition of the same amount of disodium hydrogen phosphate only increased the adduct ion intensities from 20% to 33%. This observation indicates that the binding energy of sulfuric acid to protein and peptide ions in the gas phase is higher than that of phosphoric acid and/or that the sulfuric acid adduct concentration in solution is higher.

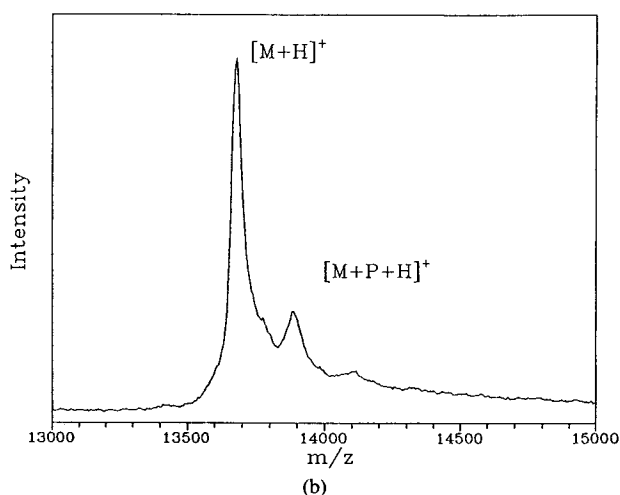
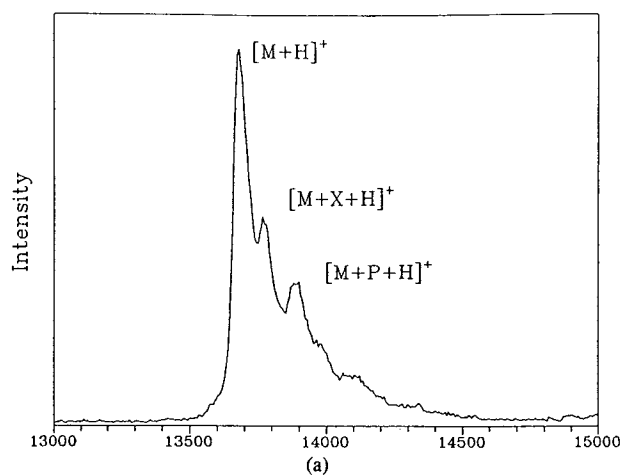
To further test our hypothesis, we carried out experiments designed to chemically remove sulfate and phosphate impurities from the protein solution prior to electrospray ionization mass spectrometric analysis. A barium acetate solution was added to a 250 pmol/ $\mu$ L aqueous solution of ribonuclease A to give a final salt concentration of 10,000 pmol/ $\mu$ L. A precipitate was

observed and removed by centrifugation. The precipitate was insoluble in dilute HCl solution, confirming the precipitate to be  $\text{BaSO}_4$ . The supernatant, containing the protein, was analyzed by electrospray ionization. The results shown in Figure 5 can be directly compared with the spectrum obtained under the same conditions prior to the removal of the impurities (Figure 3a). Intense adducts are no longer present in the mass spectrum (although weak residual adduction is still observed), and the protein is more highly protonated. The increase in the intensity of ions with higher charge states likely results from the removal of sulfate impurities. After removal of the sulfate impurities the intensities of the  $[\text{M} + n\text{H}]^{n+}$  ion peaks, where  $n = 7-11$  (Figure 5), are considerably higher than those obtained prior to removal (Figure 3a). The presence of sulfate impurities in the ribonuclease A sample was confirmed by treating a 500 pmol/ $\mu\text{L}$  solution of ribonuclease A with lead acetate. A precipitate was obtained and was separated by centrifugation. The precipitate was soluble in ammonium acetate, a test for  $\text{PbSO}_4$ . The solution was also analyzed by negative ion  $^{252}\text{Cf}$ -plasma desorption mass spectrometry. The observation of an intense negative ion at  $m/z$  97 ( $\text{HSO}_4^-$ ) and a relatively weak ion at  $m/z$  79 ( $\text{PO}_3^-$ ) confirmed that the precipitate was mostly  $\text{PbSO}_4$  rather than a phosphate salt. By monitoring the removal of adducts from ribonuclease A as a function of the amount of barium acetate added, the sulfate impurity observed in the ribonuclease A sample was estimated at  $\sim 8\%$  by weight.

An independent confirmation of the above findings was obtained by matrix assisted laser desorption [15, 16] mass spectrometric analysis of the ribonuclease A sample before and after the treatment with barium acetate, performed in the authors' laboratory (Figure



**Figure 5.** Electrospray ionization spectra of bovine pancreatic ribonuclease A after treatment with barium acetate. All other conditions are the same as those given in the caption for Figure 3a.



**Figure 6.** The matrix assisted laser desorption mass spectrum of bovine ribonuclease A using sinapinic acid matrix. From (a) the untreated protein sample and (b) after treatment with barium acetate.

6). Shown in Figure 6a is the molecule ion region of the laser desorption spectra of ribonuclease A obtained with a sinapinic acid matrix [17]. The three partially resolved ion peaks observed are  $[\text{M} + \text{H}]^+$ ,  $[\text{M} + \text{X} + \text{H}]^+$ , and  $[\text{M} + \text{P} + \text{H}]^+$ , originating respectively from ribonuclease A ( $\text{M}$ ), the attachment of  $\text{X}$  (obs. mass = 95) to ribonuclease A ion, and the attachment to ribonuclease A ion of a photochemically generated species,  $\text{P}$  (mass = 206) from the matrix. The adduction of  $\text{P}$  is commonly observed in the matrix assisted laser desorption spectra of proteins [17]. The accuracy of the mass of the adduct,  $\text{X}$ , is not as high as that obtained in the electrospray ionization spectrum because the adduct ion is only partially resolved. The spectrum of the barium acetate treated ribonuclease A sample is shown in Figure 6b. The  $[\text{M} + \text{X} + \text{H}]^+$  ion has disappeared, confirming that sulfate ions from the ribonuclease A sample solution have been precipitated out by  $\text{Ba}^{2+}$  and, therefore, sulfuric acid adducts were not observed.

The peptide and protein ions that showed a strong propensity for the attachment of sulfuric acid and phosphoric acid contain basic sites that are not protonated. For example, bovine ribonuclease A has nineteen basic sites but was observed to acquire a maximum of only nine protons (Figure 3). Although details of the interaction between the acid molecules and the peptide and protein molecule ions have not yet been elucidated, we believe that attachment of these acids may occur at the strongly basic sites.

The source of sulfate and/or phosphate in the peptide and protein samples has not been determined. It is likely that the reagents or solvents used during purification and isolation have contributed small amounts of sulfate and/or phosphate salts. It is noteworthy that 0.25N sulfuric acid is commonly used in the initial extraction of ribonuclease A from bovine pancreas [18]. We have also learned from the Sigma Chemical Company that ammonium sulfate and phosphate buffers were used during isolation of the ribonuclease A and ribonuclease S. Ammonium sulfate is often used during the purification of proteins.

## Conclusion

The origin, identity, and removal of unwanted adducts (MM = 98 u) attached to peptide and protein ions observed in the electrospray ionization mass spectra were investigated. We demonstrated that these adducts can be removed from protein ions by collisional activation, suggesting that the adducts are non-covalently bound to the protonated protein molecule ions. Addition of ammonium sulfate to the peptide and protein solutions containing acetic acid increased the adduct ion intensities. Addition of disodium hydrogen phosphate also increased the adduct ion intensities, but to a lesser extent. We suggest that sulfate and/or phosphate impurities present in the peptide and protein samples produce small amounts of sulfuric acid and/or phosphoric acid that bind strongly to the accessible basic sites of the peptide and protein ions. When the sulfate and phosphate ions were removed by precipitation with barium ion, prior to electrospray ionization, adduct free protein ions were

observed in the electrospray ionization mass spectra with a concomitant increase in the sensitivity.

## Acknowledgments

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