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A STUDY OF THE FRAGMENTATION OF SINGLY AND MULTIPLY CHARGED IONS PRODUCED BY ^{252}Cf FISSION FRAGMENT BOMBARDMENT OF POLYPEPTIDES BOUND TO NITROCELLULOSE

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ABSTRACT

A time-of-flight mass spectrometric study of the prompt and metastable decomposition of ions produced by ^{252}Cf fission fragment bombardment is presented. Results for a series of polypeptides with molecular weights between 1000 and 14000 u are given. Comparisons are made between the amount of fragmentation observed in ions desorbed from bulk (electrosprayed) polypeptide samples and the amount of fragmentation observed in the corresponding ions desorbed from polypeptides bound to nitrocellulose. Ions desorbed from nitrocellulose were found to undergo much less decomposition than those desorbed from bulk polypeptide. Details of the slow unimolecular decay of high molecular weight multiply protonated polypeptides were investigated for the first time. The metastable decomposition of multiply protonated polypeptides was found to occur, in substantial part, with the formation of two charged fragmentation products.

INTRODUCTION

The analysis of involatile, high molecular weight biomolecules such as polypeptides by mass spectrometry requires that condensed-phase sample material be converted into intact gas-phase ions. The required conversion can be effectively accomplished by bombardment of a thin solid film of the material of interest with high energy ($\sim$ 100 MeV) ^{252}Cf fission fragments [1]. This high-energy desorption and ionization technique has facilitated the successful molecular weight determination of polypeptides containing as many as 223 residues [2,3]. Until recently, electrospray deposition [4] has been the method of choice for producing the thin solid sample films used in fission fragment ionization mass spectrometry. Films produced by electrospray deposition consist of a great many contiguous macroscopic clusters of sample deposited on a metallic substrate. Since the typical dimensions of the

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clusters are 0.1–1.0 μm, desorption of sample ions occurs primarily from bulk material. We have shown previously [5–8] that a very high proportion of the ions produced from such bulk electrosprayed samples undergo both prompt ($< 10^{-9}$ s after impact) and metastable decomposition ($> 10^{-9}$ s after impact), which indicates that considerable energy enters the molecules during the desorption and ionization processes. The large amount of fragmentation observed effectively decreases the yield of ionized intact sample molecules and also causes considerable broadening of the time-of-flight mass spectral peaks [5].

Recently, a new class of method for polypeptide sample preparation has been devised [9–12] which yields fission fragment mass spectra of higher quality than that previously obtained from samples produced by electrospray. The method involves the non-covalent attachment of monolayer amounts of polypeptide to a supporting matrix which has an affinity for the polypeptide. Jordan et al. [9] first noted that bovine insulin adsorbed on the perfluorinated cation-exchange polymer Nafion yielded a fission fragment spectrum with an enhanced intensity protonated molecule peak when compared with the spectrum from a bulk insulin sample, a lower metastable ion population, and the appearance of an intense doubly protonated molecule peak. Sundqvist and co-workers [11,12], using nitrocellulose as a support matrix, have demonstrated even more dramatic improvements in the quality of the fission fragment mass spectra obtained from peptides and small proteins. Their results indicate that protonated polypeptides desorbed from nitrocellulose undergo substantially less fragmentation than polypeptides desorbed from bulk material. A strong enhancement of the desorption yield of multiply charged (protonated) intact molecule species was also observed from nitrocellulose. Similar changes and improvements in fission fragment ionization mass spectra have also been obtained by Alai et al. [13] using samples prepared by electrospraying polypeptides dissolved in solutions containing reduced glutathione.

In the present study, we compare the amount of fragmentation occurring in ions desorbed by fission fragments from bulk polypeptide samples with the amount of fragmentation of the corresponding ions desorbed from polypeptides bound to nitrocellulose. Results are presented for a series of polypeptides with molecular weights between 1000 and 14000 u. Comparisons are made for fragmentation reactions which occur relatively rapidly after the ion formation process ($\leqslant 10^{-8}$ s) as well as for those which occur more slowly ($\geqslant 10^{-6}$ s). We also describe for the first time an investigation of some characteristics of the unimolecular decay modes of multiply protonated polypeptide ions. These multiply protonated ions are frequently the dominant species observed in ^{252}Cf fission fragment ionization time-of-flight spectra for proteins with molecular weights > 6000 u.

EXPERIMENTAL

The mass spectral measurements were made with the time-of-flight fission fragment ionization mass spectrometer built in this laboratory and described previously [14]. A schematic representation of the instrumental configuration used for the present measurements is given in Fig. 1. In addition to the acceleration grid electrode A, the instrument contains three grid electrodes positioned close to the secondary ion detector, the primary purpose of which is to provide a retarding potential for the study of fragmentation reactions occurring in the 3.0 m long flight tube [5]. The intergrid distances are given in ref. 8. The acceleration potential was normally set to + 10 kV except in the studies of the decomposition of multiply charged ion species where it was set to + 5 kV. The instrument also incorporates an electrostatic particle guide (EPG) [15] to enhance the transport of ions through the 3.0 m long flight tube. The EPG does not enhance the transport of neutral fragments, which are thus strongly discriminated against in the present instrument. The detection efficiency for high molecular weight ion species is increased through the use of a post-accelerating potential difference of 8.5 kV, which is applied just prior to ion detection between grid D and the face of the microchannel plate detector E.

Samples of porcine insulin, bovine ribonuclease A, and horse heart cytochrome C were obtained from the Sigma Chemical Co. [16]. The sample of porcine proinsulin was provided by Dr. Ronald E. Chance [17]. The sample of the protected heptapeptide [Boc-Leu-Gln-Ser(Bzl)-Ala-Tyr (2,6Cl_2Bzl)-Gln-Leu] was provided by T. Sasaki [18] and was prepared by the Merrifield solid phase synthesis.

The electrosprayed sample films [4] were prepared as described previously [8] by dissolving the sample in glacial acetic acid and spraying 5–15 nmol of polypeptide on to an aluminized polyester foil with an area of 1 cm^2. The method used for preparing the solid polypeptide film bound to nitrocellulose (NC) is based on a previously described technique [12]. Details of the

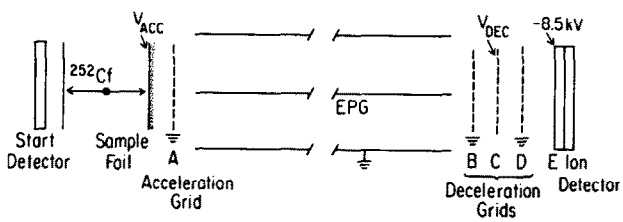

Fig. 1. Schematic representation of fission fragment ionization time-of-flight mass spectrometer. EPG is the electrostatic particle guide.

present method are given in ref. 19. The NC support matrix was prepared by electrospraying 50 μg NC (1 μg μl^{-1} in acetone) on to an aluminized polyester support with a surface area of 1 cm^2. 1–2 nmol of polypeptide dissolved in 2–4 μl of appropriate solvent was spread on the NC layer. The solvents used were 10% acetic acid in trifluoroethanol (v/v) for the protected heptapeptide sample, 0.10% aqueous trifluoroacetic acid for the proinsulin and ribonuclease A samples, and 0.25% aqueous heptafluorobutyric acid for the insulin and cytochrome C samples. Following adsorption of the insulin and cytochrome C samples to the NC surface, the surface films were thoroughly washed by insertion into a large volume (16 ml) of 0.25% heptafluorobutyric acid for a period of 2 min. After removal from the washing solution, the sample foils were inserted into the vacuum lock of the mass spectrometer where the film was dried by evacuation, leaving a bare layer of sample molecules bound to the surface of the NC. In the case of the protected heptapeptide, the sample film was not washed following adsorption of the material to the NC surface. The fission fragment flux through the sample foil was 2600 fission fragments s^{-1}.

RESULTS AND DISCUSSION

The partial ^{252}Cf fission fragment ionization mass spectra between m/z 410 and m/z 2560 of the protected peptide Boc-Leu-Gln-Ser(Bzl)-Ala-Tyr(2,6-Cl$_2$Bzl)Gln-Leu are compared for sample films produced by electrospray [Fig. 2(a)] and by adsorption to NC [Fig. 2(b)]. Both these spectra were obtained with the deceleration grid (C in Fig. 1) potential set to the flight-tube potential (0 V). Under these conditions, no ions arising from unimolecular decompositions in the flight tube are rejected. Rather, these decomposition product ions are detected with the same mean times-of-flight as the precurser ion species from which they originate. Thus, these spectra give "snapshots" of the ion population at times between 1×10^{-7} s and 3×10^{-7} s after the desorption and ionization event [5–7]. This interval represents the time required to accelerate the various ions shown in the spectra to full energy. Comparison of Fig. 2(a) and (b) reveals striking differences between the spectra obtained using the two different sample preparation techniques. The spectrum obtained from the NC-bound peptide [Fig. 2(b)] exhibits intense peaks corresponding to the $(M + Na)^+$, $(M + 2Na - H)^+$ and $(2M + 3Na - 2H)^+$ ion species with measured and calculated (in parentheses) isotopically averaged m/z values of 1194.7 (1194.4), 1216.7 (1216.6), and 2410.3 (2410.8), respectively. Peaks corresponding to these same ion species are also observed in the spectrum obtained from the electrosprayed sample [Fig. 2(a)] but are much weaker. Thus, the ratio of the yield per unit time from the electrosprayed sample to the yield from the

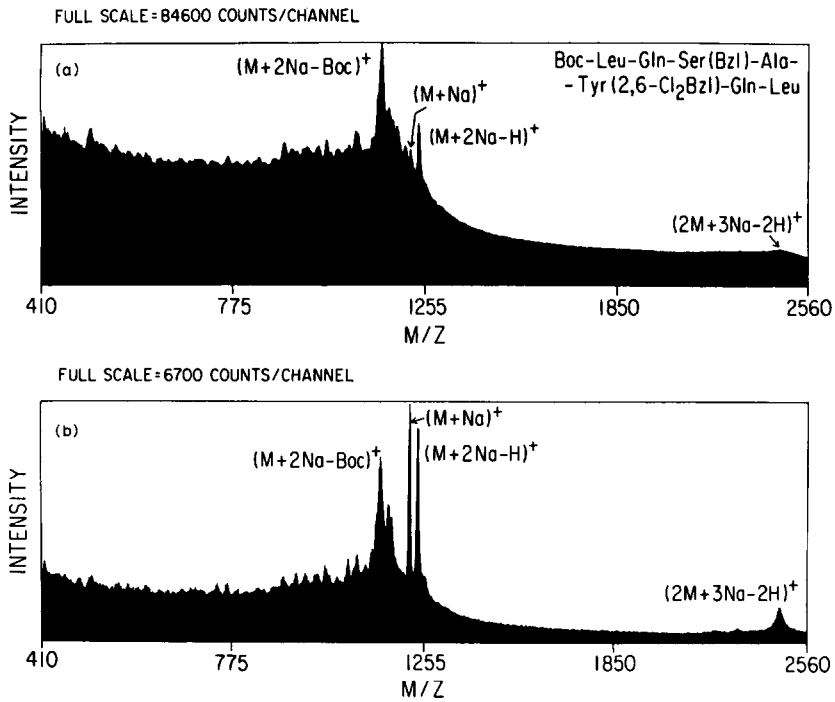

Fig. 2. Partial time-of-flight mass spectra of the protected heptapeptide Boc-Leu-Gln-Ser(Bzl)-Ala-Tyr(2,6-Cl_2Bzl)-Gln-Leu. (a) Mass spectrum obtained from an electrosprayed sample. Run time = 960 min. (b) Mass spectrum obtained from peptide deposited on nitrocellulose. Run time = 30 min.

NC-adsorbed sample are respectively 1 : 47, 1 : 7, and 1 : 15 for the $(M + Na)^+$, $(M + 2Na - H)^+$, and $(2M + 3Na - 2H)^+$ ions. A second and probably related difference seen from the data presented in Fig. 2 is the much higher ratio of fragment ions to sodium cationized intact molecule ions obtained from the electrosprayed sample. In particular, the broad fragment ion peak at m/z 1117 which we attribute to the loss of the Boc group from the $(M + 2Na - H)^+$ ion is much more dominant in the electrosprayed sample spectrum, as are the ions comprising the broad continuum [6–8]. The lower fragment ion yield and increased sodium cationized intact molecule yield from the NC-bound sample is consistent with a considerably lower energy injection into ion species desorbed from NC compared with those desorbed from bulk electrosprayed layers.

An analogous comparison between the mass spectra obtained from an electrosprayed sample [Fig. 3(a)] and a NC-bound sample [Fig. 3(c)] was carried out with porcine insulin (MW = 5777.7 u). Both these spectra were obtained with the deceleration grid (C in Fig. 1) set to the flight-tube

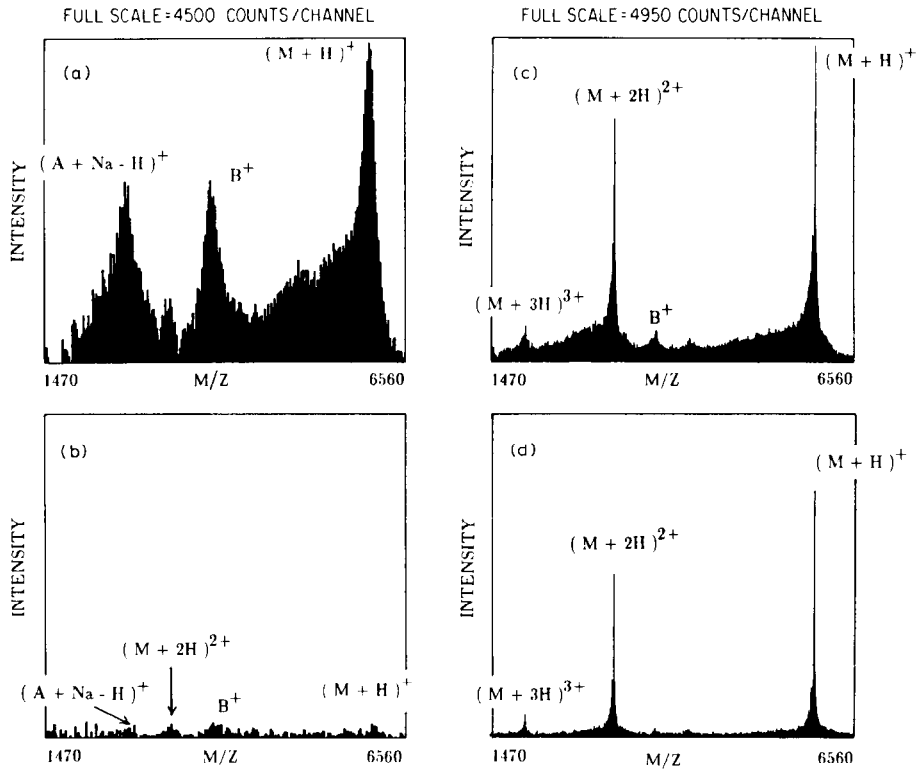

Fig. 3. Partial time-of-flight mass spectra of porcine insulin. (a) Electrosprayed sample. No metastable suppression applied, $V_{dec}/V_{acc} = 0.0$ kV/10.0 kV. Run time = 287 min. (b) Electrosprayed sample. Metastable suppression applied, $V_{dec}/V_{acc} = 8.4$ kV/10.0 kV. (c) Nitrocellulose-bound sample. No metastable suppression applied, $V_{dec}/V_{acc} = 0.0$ kV/10.0 kV. Run time = 61 min. (d) Nitrocellulose-bound sample. Metastable suppression applied, $V_{dec}/V_{acc} = 9.0$ kV/10.0 kV.

potential (0 V). Under these conditions, no ions which undergo metastable decomposition in the flight tube are rejected [5] so that these spectra give "snapshots" of the ion populations in the time range between 2×10^{-7} s and 4×10^{-7} s after the ion-forming event. To accentuate the ion peaks, the smooth, featureless continua [8] have been subtracted from these and all the remaining spectra shown in this study. The fission fragment mass spectrum obtained from the electrosprayed sample of porcine insulin [Fig. 3(a)] corresponds closely with that previously obtained by Sundqvist et al. [3] using the same sample preparation technique.

Comparison of Fig. 3(a) and (c) reveals several striking differences between the spectra obtained using the two different sample preparation techniques. The spectrum obtained from the NC-bound sample exhibits

much sharper peaks, a much smaller intensity of fragment ion species, a lower intensity of ions comprising the broad features observed between the various peaks, and a considerably higher intensity of doubly and triply charged (protonated) intact peptide ions. Comparison of the absolute collected ion yields for the $(M + H)^+$ ion is problematic because of the greatly different peak widths observed with the two different sample preparation techniques. One measure of the collected ion yield is the number of counts included under that part of the peak subtended by the full width at half maximum of the peak. Using this measure, the collected $(M + H)^+$ ion yield from the electrosprayed sample is 1.7 times higher than that from the NC-bound sample. On the other hand, using the peak height as a measure, we have found that the number of $(M + H)^+$ ions collected per unit time from the NC-bound sample is five times higher than that from the electrosprayed sample.

The data shown in Fig. 3(a) and (c) are consistent with a considerably lower energy injection into the protonated porcine insulin ions desorbed from NC compared with the corresponding ions desorbed from bulk electrosprayed layers. Further information concerning the relative degree of excitation of insulin ions desorbed from the two different sample preparations was obtained by a study of the metastable fragmentation of the ions during transit through the 3 m long flight tube. Such flight-tube fragmentation reactions occur for the $(M + H)^+$ ion at times greater than 3.8×10^{-7} s but less than 1.7×10^{-4} s after the ion-forming event. The spectrum shown in Fig. 3(b) was obtained from the same electrosprayed sample as that shown in Fig. 3(a) except that a potential $V_{dec} = 8.4$ kV was applied to the deceleration grid C (Fig 1). The effect of applying such a potential to the deceleration grid is to reflect and thus reject all ion fragments with mass

$$M_f < \frac{q_f V_{dec}}{q_p V_{acc}} \times M_p \tag{1}$$

where V_{acc} is the acceleration potential (10.0 kV in the case under consideration), M_p is the mass of the precursor ion, and q_f and q_p are, respectively, the charge on the fragment and precursor ions. Under these conditions, all fragment ions with $M_f < (q_f/q_p) \times (8.4/10.0) \times M_p$ are rejected from the spectrum. For the $(M + H)^+$ species, only 2.6% of the ions entering the flight-tube either survive the flight intact or decay to fragments with masses greater than 84% that of the precursor ion mass. Similar low survival rates are observed for the A- and B-chain fragment ions as well as ions constituting the broad features seen between the discrete peaks. Since the majority of the ions observed with $V_{dec} = 0$ are caused to be rejected by the application of the deceleration potential, we deduce that virtually all the ions comprising the spectrum in Fig. 3(a) are unstable to decomposition during flight. This

finding is in accordance with the results of our earlier investigation [8] and is in sharp contrast to the observed survival rate of porcine insulin ions desorbed from NC. Figure 3(c) shows the partial spectrum from NC-bound insulin with no metastable suppression, while Fig. 3(d) shows the corresponding spectrum from the same sample with $V_{dec} = 9.0$ kV so that all ion fragments with $M_f < (q_f/q_p) \times (9.0/10.0) \times M_p$ are rejected. Here, too, the ions constituting the broad features between the discrete peaks largely disappear. The $(M + H)^+$ ions, however, have a survival rate of 78% as measured from the relative peak heights. This data provide further evidence that protonated insulin molecules desorbed from NC contain considerably less internal energy of excitation than do the corresponding species desorbed from bulk insulin.

A dominant feature of the spectrum obtained from the NC-bound sample is the intense peak arising from doubly protonated insulin ions [12]. The peak height of the $(M + 2H)^{2+}$ ion after metastable suppression with $V_{dec}/V_{acc} = 9.0$ kV/10.0 kV [Fig. 3(d)] is 68% of the peak height without metastable suppression [Fig. 3(c)]. However, as previously noted [8], special consideration is needed for the interpretation of this doubly protonated insulin metastable fragmentation data. The doubly protonated peptide molecules are accelerated to twice the energy of the singly protonated species. Upon fragmentation, the doubly charged ion may decay into either a doubly charged fragment and a neutral fragment or into two singly charged fragments. Considering these two possible cases.

Case 1. $M_p^{2+} \rightarrow M_{f_1}^{2+} + M_{f_2}^{0}$.

From Eq. (1), application of a potential V_{dec} to the deceleration grid will cause all fragment ions with mass $M_{f_1} < (V_{dec}/V_{acc}) \times M_p$ to be rejected. When $V_{dec} > V_{acc}$, all doubly charged fragment ions will be rejected.

Case 2. $M_p^{2+} \rightarrow M_{f_1}^{1+} + M_{f_2}^{1+}$.

From Eq. (1), application of a potential V_{dec} to the deceleration grid will cause all fragment ions with mass $M_{f_i} < 1/2 \times (V_{dec}/V_{acc}) \times M_p$ to be rejected. When $V_{dec} = V_{acc}$, at least one of the two ion fragments will be detected as long as $M_{f_1} \neq M_{f_2}$.

The relative contributions of the two possible decay modes discussed above have not previously been investigated for high molecular weight multiply charged polypeptides. We thus undertook to determine their relative importance by measuring the yields of multiply charged species and their decay products as a function of V_{dec}/V_{acc}. The spectra from NC-bound porcine insulin at four different values of V_{dec}/V_{acc} are shown in Fig. 4. Figure 4(a) shows the partial mass spectrum between m/z 1460 and m/z 7940 obtained with $V_{dec}/V_{acc} = 0.0$ kV/5.0 kV = 0.0. Under these conditions,

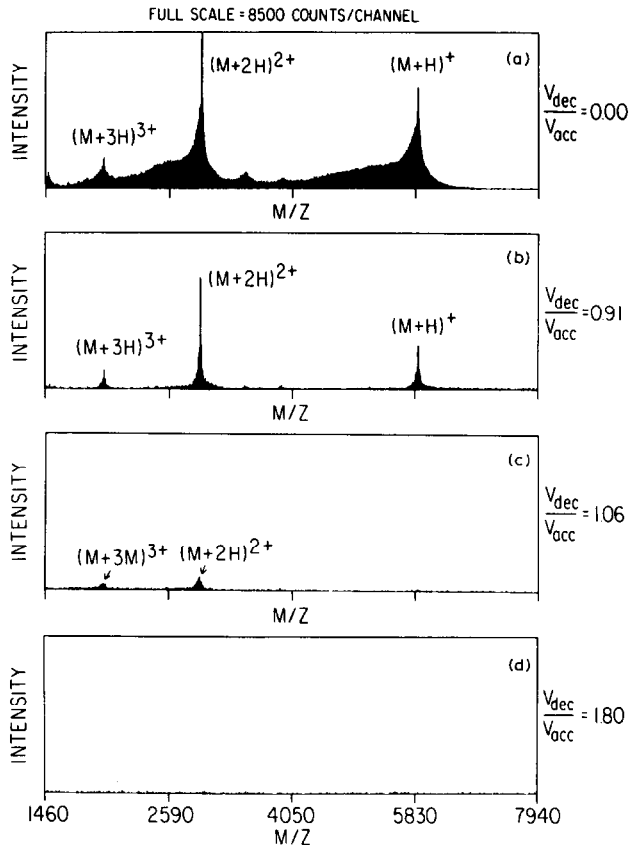

Fig. 4. Partial time-of-flight mass spectra of porcine insulin deposited on NC taken with four different magnitudes of the ratio of the deceleration potential to the acceleration potential, V_{dec}/V_{acc}.

no ions undergoing unimolecular decomposition in the flight tube are rejected. The spectrum is comparable with that shown in Fig. 3(c) except that the lower value used for V_{acc} decreases the detection efficiency of the various ion species. The loss of detection efficiency is highest for intact $(M + H)^+$ ions which have a total energy of only 13.5 keV at the detector (5.0 keV from the initial acceleration and 8.5 keV from the postacceleration). The doubly and triply charged ions are detected with relatively higher efficiency because they acquire higher energies (27.0 keV and 40.5 keV, respectively) than do the singly charged ions.

Figure 4(b) shows the partial mass spectrum with $V_{dec}/V_{acc} = 4.55$ kV/5.00 kV = 0.91. The data are comparable with that shown in Fig. 3(d). Figure 4(c) shows the partial mass spectrum with $V_{dec}/V_{acc} = 5.3$ kV/5.0 kV = 1.06. Because the deceleration potential is set higher than the acceleration poten-

tial, no intact $(M + H)^+$, $(M + 2H)^{2+}$, or $(M + 3H)^{3+}$ ions can be detected. Nor can singly charged fragments of $(M + H)^+$, doubly charged fragments of $(M + 2H)^{2+}$, or triply charged fragments of $(M + 3H)^{3+}$. Indeed, the only species which can be detected under these circumstances are singly charged decay products arising from the doubly charged precursors, singly and doubly charged products arising from the triply charged precursors, and neutral fragments. Thus the appearance of a peak at a position in the spectrum corresponding to the $(M + 2H)^{2+}$ ion constitutes direct evidence for the unimolecular fragmentation of some fraction of the doubly protonated molecule into *two singly charged* fragments (case 2, above). A similar conclusion can be made concerning the triply charged insulin ion. The possibility that the peaks shown in Fig. 4(c) arise from neutral fragments was eliminated by collecting a spectrum with $V_{dec}/V_{acc} = 9.0$ kV/5.0 kV = 1.80 [see Fig. 4(d)]. The detection of neutral species cannot be affected by the magnitude of the deceleration potential. Thus, the observed disappearance of the peaks demonstrates that the species giving rise to the peaks in Fig. 4(c) are charged. From the data shown in Fig. 4, we have deduced an approximate lower limit of 50% for the fraction of $(M + 2H)^{2+}$ primary flight tube fragmentation reactions which occur by the case 2 decay mode. The value we obtain is a lower limit because the doubly charged reaction products may themselves undergo in-flight decay prior to detection [6,8]. To deduce this lower limit, we made the following assumptions. (1) The height of the peak designated $(M + 2H)^{2+}$ in Fig. 4(c) reflects the number of heavy charged fragments which arise from case 2 decays. (2) The light charged fragments arising from case 2 decays are collected and detected in the spectrum shown in Fig. 4(a) with the same efficiency as are the heavy charged fragments. (3) The difference in the heights of the peaks designated $(M + 2H)^{2+}$ in Fig. 4(b) and (a) gives a measure of the total number of $(M + 2H)^{2+}$ disintegration reactions.

The results of metastable fragmentation experiments performed on still higher molecular weight polypeptides like proinsulin, ribonuclease A, and cytochrome C deposited on NC are qualitatively similar to those described above for insulin except that a lower proportion of protonated polypeptide ions survive intact the flight to the detector. Thus, for example, the measured survival rate of the $(M + H)^+$ ion from horse heart cytochrome C (MW = 12 361 u) was observed to be more than four times lower than the survival rate of the $(M + H)^+$ ion from porcine insulin (see Table 1). Table 1 gives the survival rates of mono-, di-, and tri-protonated insulin and cytochrome C determined by measuring the ratio of the peak intensities with $V_{dec}/V_{acc} = 9.0$ kV/10.0 kV to the peak intensities with $V_{dec}/V_{acc} = 0.0$ kV/10.0 kV. These results have not been corrected for detection efficiency differences between fragment ions and the precursor ions from which they

TABLE 1

The percentage survival for protonated insulin and cytochrome C which enter the flight tube intact when V_{dec}/V_{acc} is set equal to 9.0 kV/10.0 kV

	Percentage survival		
	$(M+H)^+$	$(M+2H)^{2+}$	$(M+3H)^{3+}$
Porcine insulin	78	67	65
Horse heart cytochrome C	17	31	41

arise nor for differences in the detection efficiencies of the various intact ion species. Furthermore, it is important to notice that the acceleration time and the time spent in free flight are shorter for the more highly charged species. The acceleration and free-flight times are also shorter for insulin ions than for the corresponding cytochrome C ions.

In order to obtain information about the decay of multiply charged polypeptides with molecular weights > 10000 u, we carried out an experiment on cytochrome C analogous to that described above for insulin. Figure 5(a) shows the partial mass spectrum of horse heart cytochrome C between m/z 780 and m/z 8400 obtained with $V_{dec}/V_{acc} = 0.0$ kV/5.0 kV. Under these conditions, no ions which undergo unimolecular decomposition in the flight tube are rejected. A series of peaks corresponding to the addition of 2, 3, 4, and 5 protons to cytochrome C is evident in the spectrum. In addition, the spectrum exhibits broad features which lie between and under the sharper peaks. The experimentally determined isotopically averaged molecular weights of 12361 u for the $(M + 2H)^{2+}$ ion and 12371 u for the $(M + 3H)^{3+}$ ion compares reasonably closely with the calculated value of 12361 u. Figure 5(b) shows the mass spectrum from the same sample with $V_{dec}/V_{acc} = 5.3$ kV/5.0 kV. The filled-in spectrum in Fig. 5(b) has been normalized in time with respect to the spectrum shown in Fig. 5(a). The spectrum in Fig. 5(b) is also shown magnified by a factor of 12.7 in intensity to facilitate inspection of spectral detail. Intact multiply protonated cytochrome C ions do not contribute to the spectrum shown in Fig. 5(b) because the deceleration potential is set higher than the acceleration potential [see Eq. (1)]. Furthermore, only those unimolecular reaction products which give rise to at least two charged fragments (case 2 reactions) can contribute to this spectrum. The observation of the spectrum with discrete peaks shown in Fig. 5(b) demonstrates that a significant proportion of the flight-tube fragmentation reactions of multiply protonated cytochrome C, occurs (as in the case of insulin) by the case 2 decay mode.

The spectrum of the case 2 decay products [Fig. 5(b)] exhibits sharper

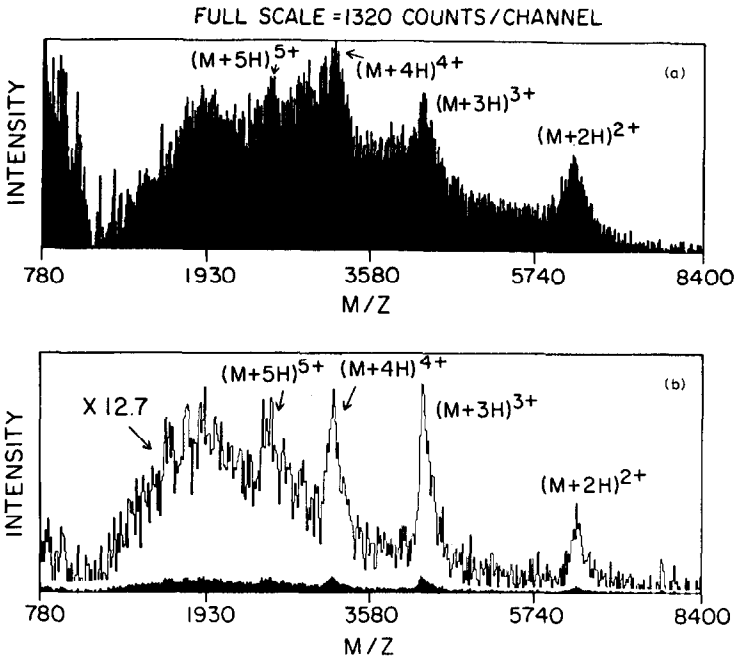

Fig. 5. Time-of-flight mass spectra of horse heart cytochrome C deposited on NC showing the region containing the multiply protonated ion peaks. (a) No metastable suppression applied, $V_{dec}/V_{acc} = 0.0$ kV/5.0 kV. (b) Metastable suppression applied, $V_{dec}/V_{acc} = 5.3$ kV/5.0 kV. Filled-in spectrum has been normalized in time with respect to the spectrum shown in (a). Unfilled-in spectrum is magnified in intensity by a factor of 12.7 compared with the filled-in spectrum.

peaks and less intense continua than does the corresponding spectrum obtained without any metastable ion rejection [Fig. 5(a)]. Thus, ions which contribute to the broad components of the discrete peaks and to the continua have been selectively rejected by the deceleration potential. These rejected ions have three likely sources. (1) The lighter of the two charged fragmentation products from case 2 reactions. Fragment ions acquire a velocity spread about the mean velocity of the precursor ion resulting from the conversion of internal energy of excitation of the precursor into kinetic energy of the fragments. Conservation of momentum requires that the ratio of the fragment velocities (in the centre of mass system of the precursor ion) is inversely proportional to the ratio of the fragment masses. Thus, the lighter fragment acquires a higher velocity than does the heavier fragment. (2) The heavier of the two charged fragmentation products of case 2 reactions which contain sufficient internal energy of excitation to undergo further decay in flight. (3) Fragmentation products of case 1 reactions.

CONCLUSION

We have demonstrated quantitatively that polypeptide ions desorbed from NC contain, on average, substantially less energy of excitation than do the corresponding ion species desorbed from bulk polypeptide samples. The present result confirms the earlier conclusions of Jordan et al. [9] using Nafion and Jonsson et al. [12] using NC and explains, to a large extent, improvements observed in the quality of spectra obtained from polypeptide samples adsorbed on NC. The detailed reasons for the reduced excitation of species desorbed from NC remain to be elucidated. However, as previously suggested [9–13], reduction of the binding energy of the molecule of interest to other molecules and to the surface is probably the dominant factor. Improvements in our understanding and control of the binding of biomolecules to surfaces holds the prospect of increasing our control over the amount and the type of fragmentation incurred during desorption.

We have also shown that the relative survival probability of polypeptide ions desorbed from NC by fission fragments decreases as a function of increasing polypeptide molecular weight. Thus, the injection of an excessive amount of energy during the ion-induced desorption and ionization process still severely limits the quality of mass spectra obtained from polypeptides with molecular weights > 10 000 u.

We have also demonstrated, for the first time, that a substantial fraction of the slow unimolecular fragmentation reactions of multiply protonated polypeptides give rise to two charged fragmentation products. The finding confirmed our expectation, based on general energetic and statistical considerations, that the multiple charges would frequently be shared between the fragmentation products. The finding also has interesting practical implications for fission fragment time-of-flight mass spectrometry since both charged fragments arising from a single disintegration reaction can, in principle, be efficiently detected as a correlated event.

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