Narrowing the Gap between keV and Fission Fragment Secondary Ion Yields with Nitrocellulose

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The choice of a suitable matrix represents a critical step in obtaining mass spectra of high molecular weight biomolecules such as polypeptides by particle induced emission (fission fragment or keV ion bombardment). Desired features of matrices include reduction of intermolecular or sample-surface binding forces and reduction of secondary ion internal energies, which can lead to mass spectra of better quality. These are characterized by increased yields of singly or multiply charged molecular ions and reduced fragmentation. Glycerol and analogs have been widely used as liquid matrices, although they usually produce significant chemical noise and normally require relatively large amounts of sample. For solid samples, useful matrices include Nafion,[1] cationic[2] and anionic[3] surfactants, glutathione[4] and nitrocellulose.[5–8] In particular, Jonsson *et al.*[5] have found remarkable enhancements in quasimolecular ion yields for small proteins, e.g. insulin, desorbed from nitrocellulose under fission fragment bombardment. These results encouraged us to determine whether the use of nitrocellulose would lead to similar improvements when the sample was bombarded by keV ions. In a previous comparative analysis of a series of peptides,[9] molecular ion yields were found to decrease as the sample molecular weight increased. However, the yields dropped off more steeply for keV Cs^+ ions than for ^{252}Cf fission fragments, corroborating earlier findings by Kaminsky *et al.*[10] For example, bovine insulin (MW 5733 u) which is easily observable with plasma desorption mass spectrometry (PDMS), could barely be detected in keV ion spectra, when deposited on Ag or aluminized polyester substrates by electrospray. We now report that differences in relative yields between the two bombardment techniques have been reduced significantly by the use of a nitrocellulose (NC) matrix.

Experimental

The peptides analyzed included substance P (MW 1348 u), renin substrate (MW 1760 u), bovine insulin-chain B (MW 3496 u), bovine insulin (MW 5733 u), and aprotinin (MW 6512 u). Targets were prepared by first electrospraying an acetone solution (0.3 μg/μl) of NC onto an aluminized mylar film to yield a coverage of about 20 μg/cm^2 of NC. About 500 pmol of peptide dissolved in trifluoroacetic acid (TFA) 0.1% was then mixed with an equal amount (~500 pmol) of leucine-enkephalin-arginine (LEA), used for normalization, in 0.1% TFA. A few μl of the mixture were deposited on a microscope coverglass, over which the target foil with an NC outerlayer was placed. After ~2 min. the coverglass was removed from the foil; following solvent evaporation the target was introduced into the mass spectrometer. Targets were not washed with TFA prior to analysis; although this procedure slightly improves peak shape and abundance of quasimolecular ions,[5] it causes disappearance of the LEA used for normalization.

In Manitoba, targets were bombarded by a pulsed beam of 12 keV Cs^+ ions focused to ~1 mm diameter at the surface, whereas a ~100 MeV ^{252}Cf fission fragment source collimated to ~3 mm diameter was used at Rockefeller. In both cases, secondary ions were accelerated to 10 keV, and detected by microchannel plates without post-acceleration. Samples were first analyzed at Manitoba, and then sent by courier to Rockefeller. The time between analyses was about 72 h.

The Rockefeller spectrometer is a linear time-of-flight instrument with an electrostatic particle guide and a 3 m flight tube.[11] The Manitoba spectrometer is a reflecting instrument,[12,13] but the measurements reported here were made with the mirror voltage turned off, so as to make its configuration more similar to the Rockefeller spectrometer. In this mode of operation (as a linear spectrometer), its path length is ~1.1 m.

Results and Discussion

Figures 1 and 2 show spectra in the molecular ion region for substance P, renin substrate, bovine insulin-chain B, and bovine insulin, obtained by keV ion or fission fragment bombardment, as indicated. Although such spectra have been observed routinely with fission fragments, this appears to be the first time that intact molecular ions of a molecule as large as insulin have been detected using low energy primary particles on solid targets. Figure 3 illustrates the dramatic improvements achieved in the keV mass spectra of bovine insulin when deposited on NC instead of being electro-

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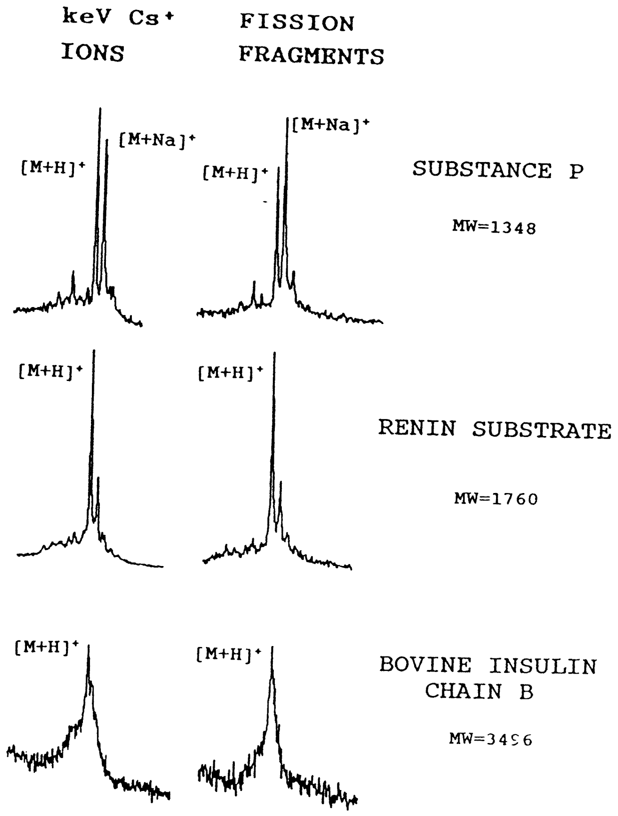

Figure 1. Molecular ion region of direct, positive ion mass spectra of substance P (MW 1348 u), renin substrate (MW 1760 u) and bovine insulin-chain B (MW 3496 u); left, 12 keV Cs^+ ion bombardment; right, ^{252}Cf fission fragment bombardment.

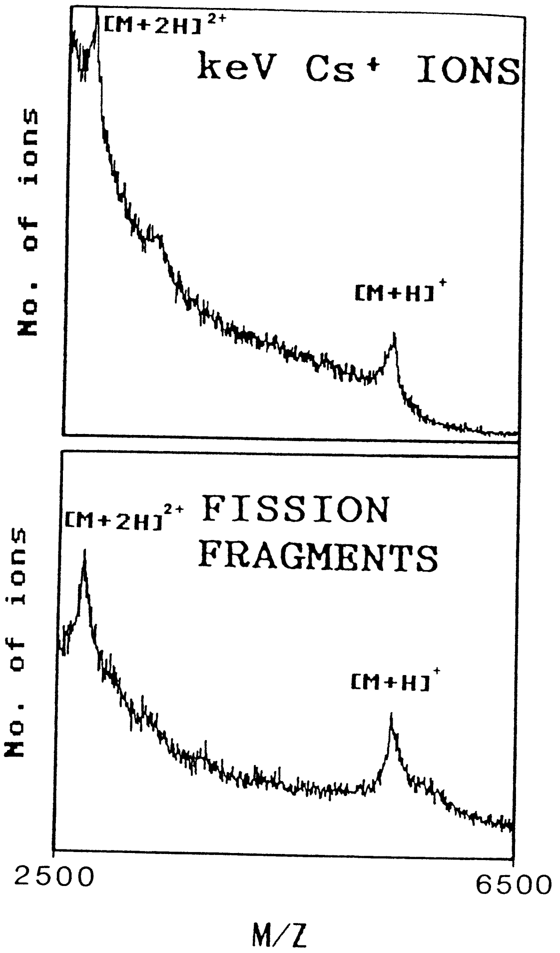

Figure 2. Bovine insulin (MW 5733 u) direct positive ion spectra (*m/z* 2500–6500); upper, 12 keV Cs^+ ion bombardment; lower, ^{252}Cf fission fragment bombardment.

sprayed on bare aluminized mylar. As was the case in our previous comparison,[9] we found remarkable similarity between the mass spectra obtained at the two different energies. Some differences remain, however; for example, note in Fig. 2 the background continuum which is larger for keV ion bombardment, probably indicating increased decomposition in the acceleration region.[14]

Molecular ion intensities were measured relative to the leucine-enkephalin-arginine (LEA) that had been mixed with each sample. Yields were calculated according to the same procedure used by Ens *et al.*[9] for the previous comparative studies. The ratio of these yields for keV and MeV bombardment is plotted as a function of molecular weight in Fig. 4. Also shown are the results of our previous measurements (which were normalized to leucine-enkephalin). Clearly the relative yields decrease much less rapidly with nitrocellulose; for example, the keV/MeV normalized yield ratio of insulin has increased by a factor of at least 5 in the present measurement.

Even though the irradiated target area was not exactly the same for fission fragments as for Cs^+ ions, target non-uniformity did not appear to be a problem when following the sample deposition procedure described in the experimental section. No significant variations in peptide/LEA ratios were obtained for a given target when the primary particle beam was collimated to various diameters in the fission fragment mass spectrometer.

The fission fragment measurements at Rockefeller were always made following the keV ion measurements at Manitoba, so changes in the targets with time could produce a systematic error in the results. To test this possibility, SIMS analyses of a bovine insulin target were performed at Manitoba over a period of 5 days, with removal of the target from the spectrometer to a desiccator between measurements. They showed measurable signal-to-background ratio reductions as a function of time for the quasimolecular ions as well as a decrease in the intensity of the sharper component of the $[M+H]^+$ peak with respect to its wider metastable component. However, no significant reductions in the ratio of the molecular ion peaks of insulin to LEA were observed. Normalization of our data to LEA is thus expected to yield results approximately independent of time over the <72 h intervals involved.

Conclusion

Differences in relative quasimolecular ion yields between ^{252}Cf fission fragment and keV Cs^+ ion bombardment have been reduced significantly by the use of a nitrocellulose sample matrix, instead of aluminized-polyester substrates. For the first time, intact molecular