BIOLOGICAL MASS SPECTROMETRY, VOL. 20, 783–788 (1991)

Coaxial Continuous Flow Fast Atom Bombardment for Higher-Molecular-Weight Peptides: Comparison with Static Fast Atom Bombardment and Electrospray Ionization

Kenneth B. Tomer,† John R. Perkins, Carol E. Parker and Leesa J. Deterding
Laboratory of Molecular Biophysics, National Institute for Environmental Health Sciences, Research Triangle Park, North Carolina 27709, USA

A comparison of coaxial continuous flow fast atom bombardment (FAB) with static FAB and with electrospray ionization (ESI) for the analysis of 'high'-mass peptides (M_r = 3000–4000) is presented. Sensitivities of the peptides by coaxial continuous flow FAB is nearly an order of magnitude better than by static FAB. Single-scan spectra with good signal-to-noise can be obtained from as little as 200 fmol (by flow injection analysis). Detection limits by ESI mass spectrometry were found to be equivalent to 20 times higher than by coaxial continuous flow FAB on a per mole basis, but 4–20 times lower on a concentration basis, owing to the greater flow per unit time employed in the ESI mass spectrometric experiments.

INTRODUCTION

Fast atom bombardment (FAB) has proven itself to be a powerful technique for the determination of polar molecules such as peptides. The utility of the technique for biological samples, especially tryptic digests, was significantly enhanced by the advent of continuous flow FAB[1] and the related frit FAB.[2]

These flow FAB techniques have met with wide acceptance and success. A number of workers have used the technique for tryptic digests[3–11] and Caprioli reported an increased signal-to-noise ratio by continuous flow FAB versus static FAB (signal averaged over an equal number of scans rather than over an equal amount of analyte consumed) for protonated bovine insulin.[1] There has been, however, a widespread concern (mentioned at meetings) that the sensitivity of continuous flow FAB for heavier peptides (M_r > 3000) is significantly lower than that obtained by introduction of the sample on a static FAB probe. A recent publication by Hemling *et al.* provided experimental confirmation of this observation.[10] The authors examined several 'high'-mass peptides including glucagon, C-peptide, Tyr-C-peptide and oxidized insulin B-chain at the 150–800 pmol level and found that they responded better to static FAB. These results were confirmed in a further series of experiments.[12]

Electrospray ionization (ESI)[13,14] has become an extremely popular technique for the determination of peptides and proteins. The formation of multiply charged molecular ions permits analysis of proteins with masses in excess of 100 000 Da on quadrupole mass spectrometers.[15] These measurements typically can consume only low picomole amounts of analytes.[15] In the report by Hemling *et al.*, a comparison was also made of ESI with conventional continuous flow FAB for the determination of tryptic fragments.[10] Although detection limits were not reported under ESI mass spectrometric conditions, the authors noted that ESI mass spectrometry yielded better sensitivity for glycopeptides.

Our laboratory has developed a variation on the theme of continuous flow FAB in which the analyte and matrix flows are delivered independently to the probe tip through a coaxial arrangement.[16,17] Using this approach and scanning over a narrow range, we have obtained detection limits in the attomole range for the small peptide MLF[17] and in the femtomole range when scanning over a wider range.[18] We therefore decided to investigate the sensitivity of coaxial continuous flow FAB relative to static FAB and ESI for 'high'-mass peptides (M_r > 3000) and now report our results.

EXPERIMENTAL

Materials

C-peptide, Tyr-C-peptide, glucagon and oxidized insulin B-chain were purchased from Sigma and used as delivered.

Mass spectrometry

All FAB mass spectra were acquired on a VG ZAB 4F tandem double-focusing mass spectrometer. Only MS-I was utilized in these experiments. Owing to the mass range of this instrument at full accelerating voltage, the instrument was operated at a reduced accelerating voltage, 6 kV. To maximize sensitivity, all experiments were performed at a resolution of ~900. Ions were produced by bombardment with 10 keV xenon atoms. The

† Author to whom correspondence should be addressed.

1052-9306/91/120783-06 $05.00

Received 29 July 1991
Revised 9 September 1991

mass spectrometer was scanned exponentially from mass 4050 down to mass 2950 at a scan rate of 50 s/decade (10 s/scan). Ions were detected in the third field-free region using a photomultiplier-based detection system. All data were acquired in the profile (continuum) mode with no electrical filtering using a VG 11-250 data system, with data processing being performed on a SUN 360 workstation using MACH 3 software (Kratos Analytical). In the coaxial continuous flow experiments, data from sufficient scans were added together so that the amount of analyte consumed during those scans added up to the quantity designated in the text below. In the static probe experiments, the instrument was scanned until analyte was no longer detected, and all of these scans were added together. The summed scans from both sets of experiments were submitted to a 21-point Savitsky-Golay smooth followed by a 21-point moving average smooth.

In the static probe experiments, 1.0 μl of FAB matrix (dithiothreitol–dithioerythritol) was placed on the FAB probe. Then, 1.0 μl of a solution of the peptide in MilliQ/MilliRho water was added to the FAB tip.

The coaxial continuous flow FAB system has been described in detail elsewhere.[16–20] Briefly, the analytical column was a 10 μm i.d. × 150 μm o.d. fused silica column 2.0 m in length. This column was threaded inside of the matrix delivery column (160 μm i.d., 365 μm o.d. fused silica column). The analytical column was withdrawn slightly (1 mm) into the matrix column at the probe tip. The matrix was 25 : 75 glycerol–0.5 mM aqueous heptafluorobutyric acid. The matrix was delivered by an ISCO μLC-500 syringe pump at a flow rate of 0.4 μl min^{-1}. Sample introduction was by means of a pressurized injection vessel that has previously been described.[19,21] In this technique a microvial of solution is placed in the vessel and the fused silica analytical column is inserted through the lid into the vial. The vessel is then pressurized with helium to inject the sample onto the column. This injection system requires approximately 5 μl of solution for manipulation. Thus, a detection limit of 1 μM translates into 5 pmol of analyte required. All of the coaxial continuous flow FAB experiments were performed by flow injection analysis. The flow rate of the column (1.4 nl s^{-1}) was determined by the amount of time needed for the sample to reach the mass spectrometer and by the column volume.

ESI mass spectra were acquired on a VG 12-250 quadrupole mass spectrometer that had been retrofitted with a Vestec electrospray source, model 611B (Vestec Corp., Houston, Texas). The needle voltage was held at ~3 kV with a spray current of 0.2–0.25 μA. The mass spectrometer was scanned from 100 Da to 1200 Da in 10 s at a mass resolution of 1.5 (50% valley). The instrument was fitted with a photomultiplier-based detection system identical to that used on the ZAB 4F. All data were acquired and processed in the same manner as the FAB data.

The probe utilized was of a coaxial flow design.[22] The analytical column was a 75 μm i.d., 1.2 m long fused silica column. The make-up fluid (sheath flow) flowed around the analytical column and mixed with the analyte at the probe tip (the electrospray needle). The sheath fluid in the ESI experiments was 50:50 Methanol–3% aqueous acetic acid. The sheath flow was 5 μl min^{-1}, while the analytical column flow was determined to be 28 nl s^{-1}.

RESULTS AND DISCUSSION

The quantities consumed in acquiring the spectra of the peptides are compared in Table 1. Spectra for summed scans equal to the amounts listed in the table for the coaxial continuous flow experiments (Figs 1(a)–4(a)) and the static experiments (Figs 1(b)–4(b)) and single scans from the coaxial continuous flow experiments (Figs 1(c)–4(c)) are shown in the figures. The spectra obtained are unremarkable except for C-peptide and Tyr-C-peptide. The spectra of these two peptides obtained by static probe show ions 100 and 200 Da above the mass of the protonated molecule, presumably due to adducts with the FAB matrix. These adducts were not observed using glycerol as the FAB matrix in the continuous flow FAB experiments. The intensities of these adduct ions are included in the intensities listed in Table 1.

These data show that the analyte intensity by coaxial continuous flow FAB is approximately tenfold greater than that obtained by the static probe, except for Tyr-C-peptide where the coaxial continuous flow FAB spectra are only twice as intense as the static spectra. These results are in marked contrast to the results reported by Hemling and co-workers.[10,12] Three possible reasons for these results can be postulated. One is that the coaxial approach constrains the analyte to the center of the probe tip, presenting a higher flux of analyte at the center of the FAB beam than is found in the standard continuous flow FAB approach. The second is that the source pressures in the coaxial continuous flow FAB experiments (2×10^{-5} Torr) are significantly lower than those encountered in standard

Table 1. Amounts analyzed: coaxial continuous flow *vs.* static FAB

	Coaxial continuous flow					
	Summed scans		Single scan		Static	
Compound	Amount	Intensity	Amount	Intensity	Amount	Intensity
Oxidized insulin B-chain	5 ng	2.2 V	0.7 ng	0.33 V	5 ng	0.27 V
Glucagon	5 ng	234 mV	0.7 ng	81 mV	5 ng	0[a]
C-peptide	29 ng	13 V	1.4 ng	0.7 V	25 ng	1.5 V[b]
Tyr-C-peptide	100 ng	11 V	14 ng	1.7 V	100 ng	5.7 V[b]

[a] Below detection limit.
[b] Total of protonated molecule and adducts.

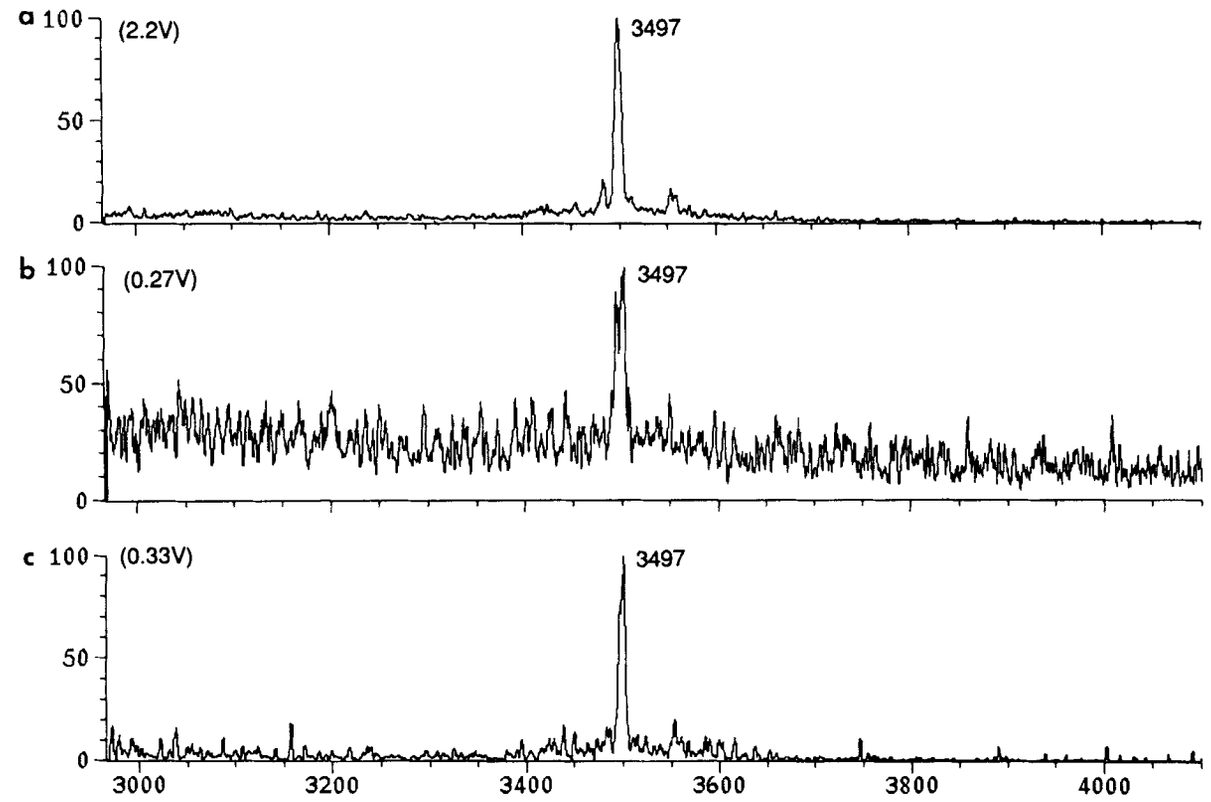

Figure .1 (a) Mass spectrum from 5 ng of oxidized insulin B-chain acquired by coaxial continuous flow FAB. (b) Mass spectrum from 5 ng of oxidized insulin B-chain acquired by static FAB. (c) Single-scan mass spectrum of 0.7 ng of oxidized insulin B-chain acquired by coaxial continuous flow FAB.

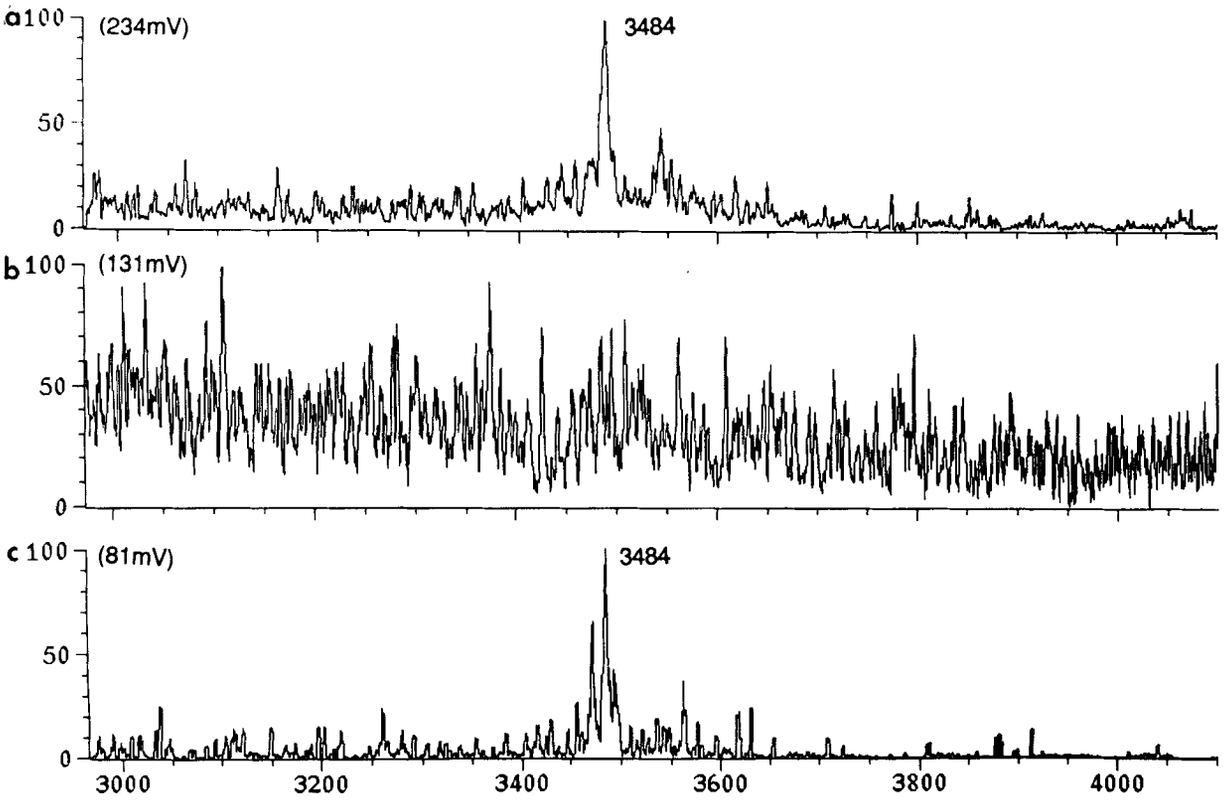

Figure 2. (a) Mass spectrum from 5 ng of glucagon acquired by coaxial continuous flow FAB. (b) Mass spectrum from 5 ng of glucagon acquired by static FAB. (c) Single-scan mass spectrum of 0.7 ng of glucagon acquired by coaxial continuous flow FAB.

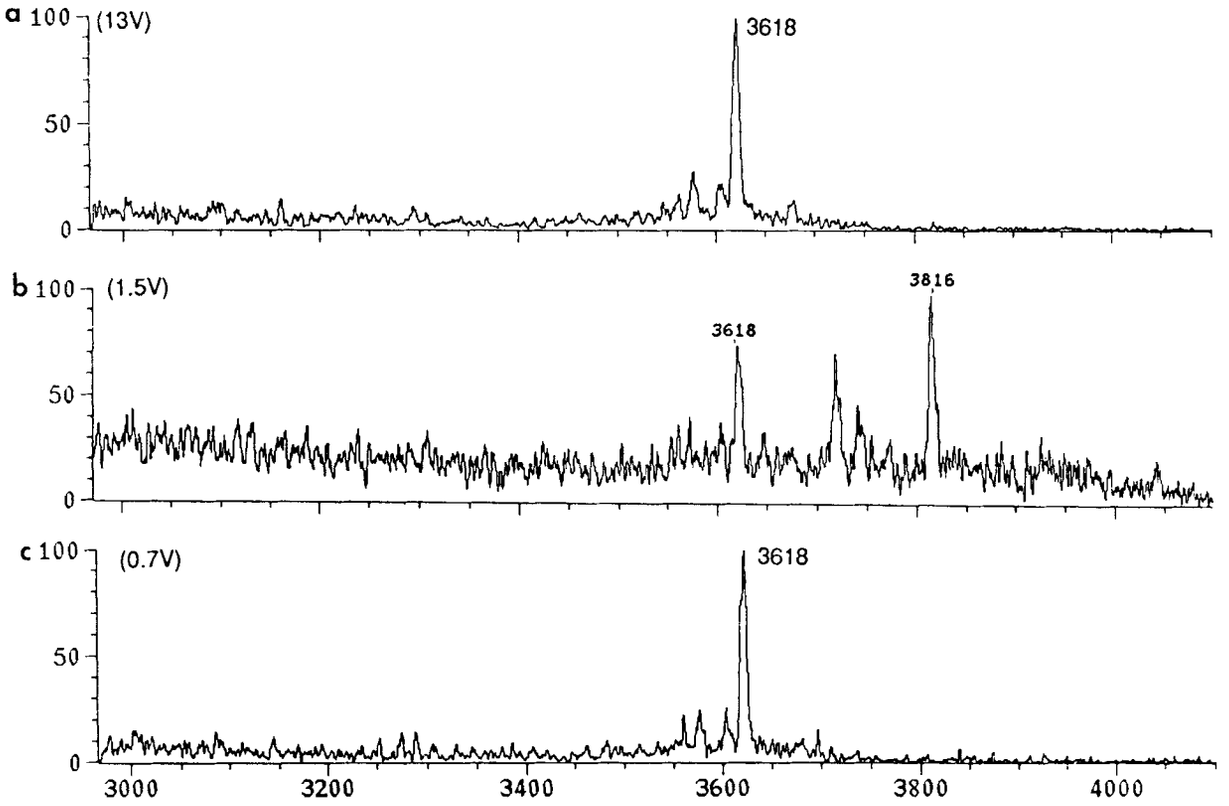

Figure 3. (a) Mass spectrum from 29 ng of C-peptide acquired by coaxial continuous flow FAB. (b) Mass spectrum from 25 ng of C-peptide acquired by static FAB. (c) Single-scan mass spectrum of 1.4 ng of C-peptide acquired by coaxial continuous flow FAB.

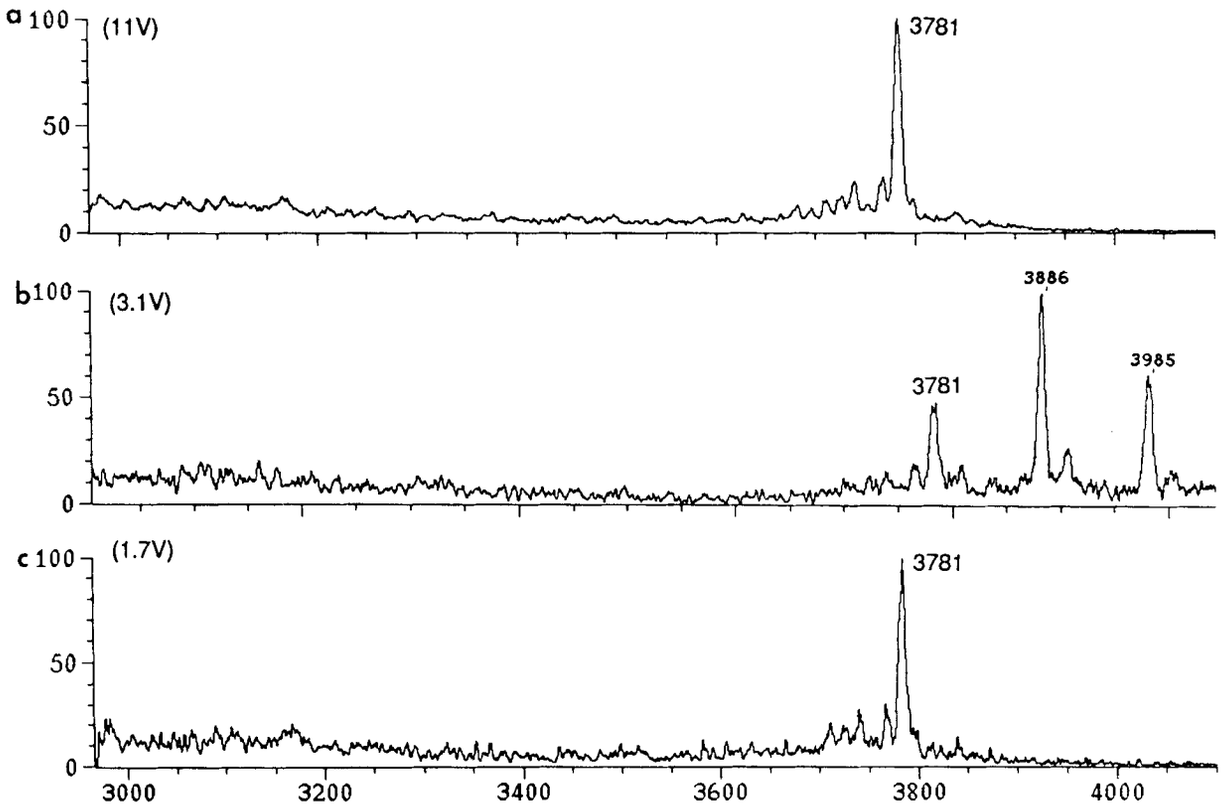

Figure 4. (a) Mass spectrum from 100 ng of Tyr-C-peptide acquired by coaxial continuous flow FAB. (b) Mass spectrum from 100 ng of Tyr-C-peptide acquired by static FAB. (c) Single-scan mass spectrum of 14.4 ng of Tyr-C-peptide acquired by coaxial continuous flow FAB.

Table 2. Limits of detection: coaxial continuous flow FAB *vs.* electrospray ionization

	Limits of detection			
	Coaxial continuous flow FAB		Electrospray ionization	
Compound	Concentration	Amount	Concentration	Amount
Oxidized insulin B-chain	1.6×10^{-6} M	20 fmol	0.4×10^{-6} M	100 fmol
Glucagon	28×10^{-6} M	20 fmol	1.4×10^{-6} M	400 fmol
C-peptide	1.4×10^{-6} M	10 fmol	0.6×10^{-6} M	150 fmol
Tyr-C-peptide	56×10^{-6} M	2 pmol	5.0×10^{-6} M	1.6 pmol

continuous flow FAB owing to the low total flows involved. The lower source pressure may yield better sensitivity through lowered collisional scattering of the analyte. The third may be due to the design of the continuous flow FAB probe used in the Hemling experiments—this was an early design which yielded poorer sensitivity than later probe tip designs.

The single scan data in Figs 1(c)–4(c) also show that reasonable full-scan data can be acquired from femtomole levels of analytes using the coaxial approach with detection limits determined to be in the tens of femtomoles for all peptides except Tyr-C-peptide.

Table 2 compares the detection limits realized using ESI and coaxial continuous flow FAB. ESI mass spectra of the peptides obtained at levels near the single-scan levels shown for coaxial continuous flow FAB (shown in Figs 1(c)–4(c) are shown in Fig. 5. The detection limits were determined by a serial dilution of the sample until no signal (S:N > 3:1) was observed. The relative sensitivities noted here include not only differences in sensitivities between coaxial continuous flow FAB and ESI, but also differences in sensitivities between the specific magnetic sector instrument and quadrupole instrument used in this study. Thus, these

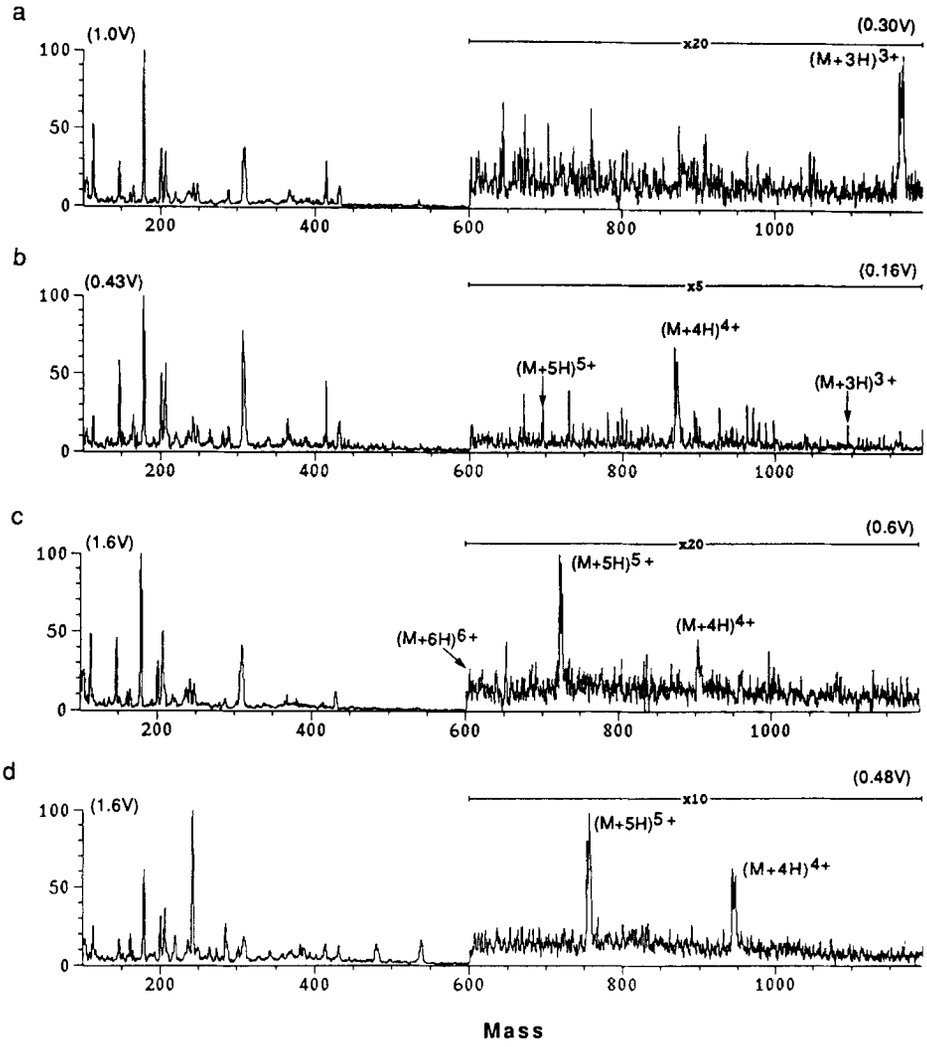

Figure 5. (a) ESI mass spectrum of oxidized insulin B-chain (0.7 ng consumed). (b) ESI mass spectrum of glucagon (1.4 ng consumed). (c) ESI mass spectrum of C-peptide (1.4 ng consumed). (d) ESI mass spectrum of Tyr-C-peptide (17 ng consumed).

results should not be interpreted as a rigorous comparison of coaxial continuous flow FAB and ESI. Table 2 shows that coaxial continuous flow FAB is as sensitive or more sensitive than ESI for the determination of these peptides on an absolute amount basis on the instruments used in this study. ESI, however, proves to be more sensitive when the concentration of the analyte is considered. Thus, ESI detection limits are in the micromolar to submicromolar level under these conditions, while the detection limits for coaxial continuous flow FAB are a factor of 4–20 higher. This difference in molar detection levels versus absolute quantity detection limits is due to the greater flow rate employed in the ESI experiments (28 nl s^{-1} for ESI versus 1.4 nl s^{-1} for continuous flow FAB).

Detection limits were not determined for static FAB. Based on the ratio of signal intensities for coaxial continuous flow FAB and static FAB analyses of identical amounts, however, the static FAB detection limits are at least an order of magnitude higher than the coaxial continuous flow FAB detection limits.

CONCLUSIONS

These data show that the increased sensitivity shown for peptides by continuous flow FAB over static FAB for low-mass peptides can be realized for 'high'-mass peptides (M_r = 3000–4000) if the coaxial approach is used. In addition, detection levels by coaxial continuous flow FAB can be comparable to those obtained by ESI for these peptides (these results may be instrument dependent).

REFERENCES

1. R. M. Caprioli, T. Fan and J. D. Cottrell, *Anal. Chem.* **58**, 2949 (1986).
2. Y. Ito, T. Takeuchi, D. Ishi and M. Goto, *J. Chromatogr.* **346**, 161 (1985).
3. R. M. Caprioli and W. T. Moore, in *Methods in Enzymology*, ed. by J. D. McCloskey. Academic Press, New York, p. 214 (1990).
4. R. M. Caprioli, B. B. DaGue and K. Wilson, *J. Chromatogr. Sci.* **26**, 640 (1988).
5. J. E. Coutant, T.-M. Chen and B. L. Ackermann, *J. Chromatogr.* **529**, 265 (1990).
6. R. M. Caprioli, W. T. Moore, B. DaGue and M. Martin, *J. Chromatogr.* **443**, 355 (1988).
7. K. Mock, J. Firth and J. S. Cottrell, *Org. Mass Spectrom.* **24**, 591 (1989).
8. J. T. Stults, J. H. Bourell, E. Canova-Davis, V. T. Ling, G. R. Laramee, J. W. Winslow, P. R. Griffin, E. Rinderknecht and R. L. Vandlen, *Biomed. Environ. Mass Spectrom.* **19**, 655 (1990).
9. W. J. Henzel, J. H. Bourell and J. T. Stults, *Anal. Biochem.* **187**, 228 (1990).
10. M. E. Hemling, G. D. Roberts, W. Johnson, S. A. Carr and T. R. Covey, *Biomed. Environ. Mass Spectrom.* **19**, 677 (1990).
11. L. J. Deterding, C. E. Parker, J. R. Perkins, M. A. Moseley, J. W. Jorgenson and K. B. Tomer, *J. Chromatogr.*, **554**, 329 (1991).
12. G. D. Roberts, W. P. Johnson, M. E. Hemling and S. A. Carr, reported at the 39th ASMS Conference on Mass Spectrometry and Allied Topics, Nashville, Tennessee, 19–24 May (1991).
13. J. B. Fenn, M. Mann, C. K. Meng, S. F. Wong and C. M. Whitehouse, *Science* **246**, 64 (1989).
14. T. R. Covey, R. F. Bonner, B. I. Shushan and J. Henion, *Rapid Commun. Mass Spectrom.* **2**, 249 (1988).
15. R. D. Smith, J. A. Loo, C. G. Edmonds, C. J. Barinaga and H. R. Udseth, *Anal. Chem.* **62**, 882 (1990).
16. J. S. M. de Wit, L. J. Deterding, M. A. Moseley, K. B. Tomer and J. W. Jorgenson, *Rapid Commun. Mass Spectrom.* **2**, 100 (1988).
17. M. A. Moseley, L. J. Deterding, J. S. M. de Wit, K. B. Tomer, R. T. Kennedy, N. Bragg and J. W. Jorgenson, *Anal. Chem.* **61**, 1577 (1989).
18. M. A. Moseley, L. J. Deterding, K. B. Tomer and J. W. Jorgenson, *Anal. Chem.* **63**, 109 (1991).
19. R. M. Caprioli and K. B. Tomer, in *Continuous-flow Fast Atom Bombardment Mass Spectrometry*, ed. by R. M. Caprioli, p. 93. Wiley, New York (1990).
20. K. B. Tomer and M. A. Moseley, in *Continuous-flow Fast Atom Bombardment Mass Spectrometry*, ed. by R. M. Caprioli, p. 121. Wiley, New York (1990).
21. L. J. Deterding, M. A. Moseley, K. B. Tomer and J. W. Jorgenson, *Anal. Chem.* **61**, 2504 (1989).
22. J. R. Perkins, C. E. Parker and K. B. Tomer, *J. Am. Soc. Mass Spectrom.*, in press.