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Recent advances in liquid chromatography–mass spectrometry and capillary zone electrophoresis–mass spectrometry for protein analysis

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ABSTRACT

The utility of the combination of separations techniques, such as liquid chromatography and capillary zone electrophoresis, with mass spectrometry in applications involving protein analysis is discussed. The use of continuous-flow fast atom bombardment and electrospray ionization mass spectrometry is compared for the analysis of tryptic digests. For liquid chromatography, both microbore and slurry-packed capillary bore columns were used to separate peptides from proteolytic digests.

INTRODUCTION

Mass spectrometry is playing an ever widening role in the investigation of analytical and structural problems encountered in the biological sciences. To date, a large number of separations techniques have been combined with mass spectrometry (MS) to provide the biotechnologist with very sensitive and specific tools with which to unravel complex biological processes. These include gas chromatography, high-performance liquid chromatography (HPLC), electrophoresis, thin layer chromatography, supercritical fluid chromatography, counter current distribution, gel permeation chromatography, and microdialysis. Over the past six years, instrumental combinations such as liquid chromatography–mass spectrometry (LC–MS) and capillary zone electrophoresis–mass spectrometry (CZE–MS) have made substantial gains both in their capabilities and mechanical utility and are becoming important tools for the separation and detection of molecules in complex mixtures.

The combination of liquid chromatography with mass spectrometry requires the use of an interface to handle the high gas loads which are produced by vaporization of the LC solvent. For example, 1 ml of liquid water gives approximately 1200 ml of gas [standard temperature and pressure (STP)]. However, most commercial MS systems are only able to handle a small fraction of this with standard pumps. A number of potential solutions to this high gas load problem have been devised; (i) evaporation of the solvent outside of the mass spectrometer, *i.e.*, use of a moving belt

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or similar transport system, (ii) direct introduction of a fraction of the eluent such as that used in the direct liquid introduction (DLI) technique, (iii) use of micro columns to provide lower flow-rates as utilized in continuous-flow fast atom bombardment (CF-FAB), (iv) use of additional pumps and heat, as in thermospray, and (v) use of an atmospheric pressure ionization technique in which a portion of a spray of ions is sampled by the spectrometer, as in electrospray. Generally, all of these techniques have been utilized by at least one MS interface, albeit with variable success.

One of the most useful applications for LC–MS analysis in biotechnology is that of peptide mapping. In this procedure, a protein or polypeptide is digested with a proteolytic enzyme such as trypsin, chymotrypsin, subtilisin, or another similar protease. An aliquot of the digest is then injected directly into the liquid chromatograph, usually without further sample concentration or clean-up. Separations are generally accomplished using gradient elution systems and reversed-phase columns, often C_8 and C_{18} columns, eluted with water and an organic phase such as acetonitrile. The mass spectrometer is scanned continuously, usually between m/z 200 and 2000. Data analysis consists of the identification of all of the specific masses, usually protonated molecular species. The focus of this paper will be on the use of LC–MS, and to some extent CZE–MS, for the analysis of peptides and peptide digests. Data obtained from analyses by LC–MS will be compared to that obtained for the direct analysis of the hydrolysis mixture, *i.e.*, direct injection of the sample without the use of an LC column. A comparison will also be made of LC–MS data obtained from electrospray and CF-FAB.

EXPERIMENTAL

Liquid chromatography was performed on an Applied Biosystems Model 130A microbore liquid chromatograph, modified for LC–MS and operated as previously described [1]. Microbore separations were done using a Brownlee Aquapore RP-300 (C_8) column, 50 × 1 mm I.D.), at gradient flow-rates of approximately 25 μl/min. Typically, gradient elutions were performed from 0–20% solvent B in 10 min, 20–45% B in 35 min, and 45–100% B in 10 min, holding 100% B for 2 min. For CF-FAB solvent A was water–glycerol–trifluoroacetic acid (TFA) (95:5:0.1); solvent B was acetonitrile–water–glycerol–TFA (60:35:5:0.1). For electrospray the glycerol in the eluents was replaced by water. The UV detector was set to monitor at 215 nm. The eluent from the column was split 4:1, allowing a flow of 5 μl/min to enter the source of the mass spectrometer. Sample injections containing 300 pmol were applied to the column, and 60 pmol were actually transferred into the ion source.

Microcapillary LC separations were obtained using a 320 × 0.25 mm I.D. fused-silica capillary slurry packed with Spherisorb ODS2 (3 μm). The outlet of the microcapillary column was fitted with a 0.025 mm I.D. capillary about 25 cm long. This outlet capillary was in turn connected via a short sleeve of PTFE tubing to a 1 m length of 0.048 mm I.D. fused-silica capillary (Polymicro Technologies, Tucson, AZ, U.S.A., not deactivated) that served as the transfer line to the mass spectrometers. About 5 cm from the beginning of this transfer line a small area from which the polyimide coating had been removed served as the window for on-line UV detection at 210 nm (Applied Biosystems). A Waters gradient HPLC system consisting of Models 590 and 510 pumps and a Model 680 solvent programmer was used to pro-

duce gradients at a flow-rate of 1.2 ml/min. The solvent was then split prior to the injector (Rheodyne 8125, variable volume). Two Waters Nova-pak C_{18} (15 × 0.39 cm I.D.) columns in series were attached to the high flow side of the split tee, thus providing the back pressure needed to achieve a flow-rate of 2–2.5 μl/min through the microcapillary column. The following gradient program was used: 0–50% B over 7.5 min, 50–88% B over 22.5 min, then stepped and held at 100% B. For LC–MS with CF-FAB, solvent A was water–glycerol–TFA (95:5:0.1) and solvent B was water–glycerol–acetonitrile–2-propanol–TFA (39:5:45:11:0.1). The glycerol was replaced by water for LC–MS with electrospray. A 0.1-μl injection volume equivalent to 50 pmol of digest was used to load the microcapillary column. No post-column split was necessary.

LC–CF-FAB-MS conditions were used as previously reported [1] and were performed on a Finnigan MAT 90 high-performance magnetic mass spectrometer. For LC–MS analyses, the spectrometer was scanned in one of three mass ranges for each sample injection; *e.g.* m/z 350–1100, 1100–1700, and 1700–2650 at a scan speed of 10 s/decade and at a resolution of approximately 1200.

Electrospray ionization was performed on a Finnigan MAT TSQ 70 quadrupole mass spectrometer. For LC–MS analysis, the general operating conditions previously reported [2] were employed except that a sheath liquid, isopropyl alcohol, was used at a flow-rate of 2 μl/min. The instrument was scanned from 300 to 1500 a.m.u. in 5 s. The flow-rates for the columns were 5 μl/min for the microbore column and 2.1 μl/min for the packed capillary column. For direct injection of a proteolytic digest, approximately 50 pmol of digest in 1 μl of 0.1% TFA was injected into a solvent carrier consisting of water–acetonitrile–TFA (80:20:0.1). The flow-rate was 5 μl/min, with an additional sheath liquid (isopropanol) flow of 4 μl/min.

The CZE–CF-FAB-MS apparatus was essentially the same as that reported previously [3]. The CZE capillary (Polymicro Technologies) was 90 cm × 50 μm I.D. For the analysis of tryptic digests, approximately 25 pmol of the digested protein mixture was pneumatically loaded into the capillary. Electrophoresis was performed at 15 kV (8 μA) with 40 m*M* sodium citrate and 40 m*M* sodium chloride, pH 2.5, as the buffer. The CF-FAB carrier solution, present in the cathodic reservoir interface was composed of water–glycerol–acetonitrile (92:5:3), containing 2.3 m*M* acetic acid and 1 m*M* ammonium hydroxide. This solution was allowed to flow into the ion source at 5 μl/min.

RESULTS AND DISCUSSION

LC–MS

For LC–MS, generally three column options are available; full bore, microbore, and capillary bore. One of the major differences among these columns is the solvent flow-rates that are used. As a result of the high gas load that the evaporated solvent places on the vacuum system, microbore and capillary bore columns are generally favored for on-line mass spectrometry applications. While full bore columns can be used, ionization techniques such as FAB and electrospray would require solvent flows to be split 1/50 to 1/100, thereby significantly affecting sensitivity. On the other hand, only split ratios of 1:3 to 1:4 need be used with microbore columns and no splitter at all for capillary bore columns.

We have found that use of a microbore column at flow-rates of approximately 25 μl/min provides a satisfactory balance between chromatographic performance and a low sample-split ratio. The steepness or rapidity of the gradient program is an extremely important parameter which the investigator can utilize to achieve specific

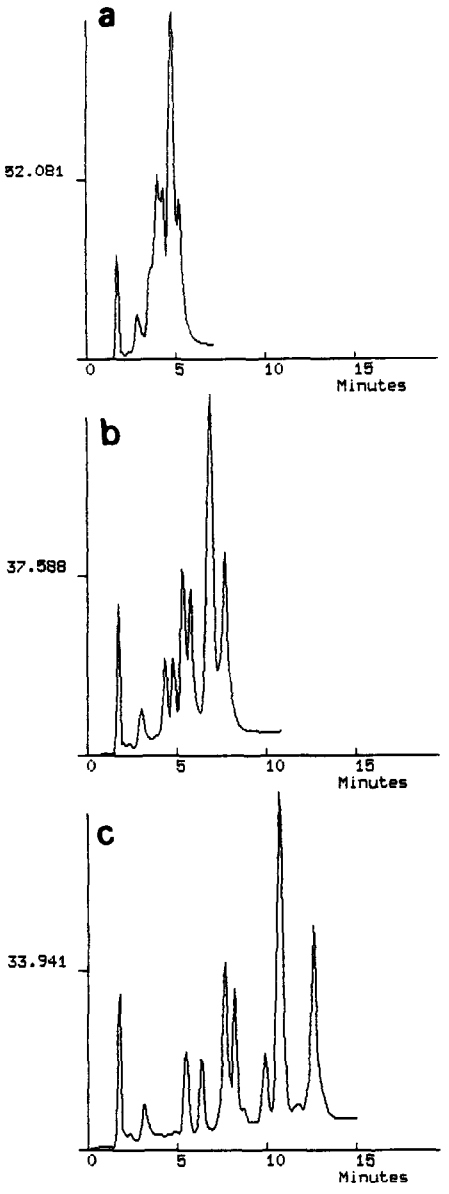

Fig. 1. The gradient elution of a sample of the tryptic digest of horse heart cytochrome *c* using UV (210 nm) detection on a 1 × 50 mm Aquapore RP-300 (C_8) column. The following gradient programs were used: (a) 5–40% B, 2.5 min; 40–60% B, 1.0 min; 60% B, hold; (b) 5–40% B, 5.0 min; 40–60% B, 1.0 min; 60% B, hold; (c) 5–40% B, 10.0 min; 40–60% B, 1.0 min; 60% B, hold. Approximately 100 pmol of peptides were injected into the column. Chromatographic conditions are given in the experimental section.

types of analyses. Fig. 1 shows the separation of a cytochrome *c* tryptic digest on a 50 × 1 mm I.D. C_8 column using three different gradient programs. The highest resolution separation is, of course, achieved with the shallower gradient, and would be preferred if component separation and collection are required. On the other hand, the most rapid gradient program is effective if the analyst wishes to simply separate the compounds in the digest from salt or large molecules, as might be required in on-line process monitoring where many aliquots need to be analyzed in a narrow time domain. In fact, most of the mass spectral data obtained from the slow, higher resolution LC analysis is obtainable from the rapid gradient analysis as well. However, ion suppression problems, coelution of compounds of the same mass, and lack of the ability to collect fractions of separated compounds remain drawbacks of the rapid elution technique.

CF-FAB–MS

CF-FAB is a technique that is based on the flow of a carrier solvent into the source of a mass spectrometer with subsequent atom bombardment of the liquid as it flows over the surface of a target [4,5]. Liquid samples may be superimposed on this flow by flow-injection techniques, or this carrier flow can be the effluent of an LC. A typical microbore LC–CF-FAB set-up is shown in Fig. 2. A splitter is used prior to the sample injection valve so that gradients can be formed rapidly and mixed efficiently before being put through the column. Typically, gradients are formed at 200–500 μl/min and then split so that approximately 25 μl/min flow through the injector to the column. After the column, a 1:4 split of the eluent is used to give 5 μl/min flow into the MS ion source. The remaining 20 μl/min is allowed to flow through a UV detector and on into a fraction collector, if desired. For the separation of peptides, a TFA–acetonitrile gradient system is often used with a C_8 column. Both eluting solvents contain 5% (v/v) glycerol to help stabilize the flow of liquid over the FAB target surface.

For the purpose of comparing the performances of microbore and capillary columns in LC–MS applications, and CF-FAB with electrospray, we have chosen to use the tryptic digest of human growth hormone (hGH) as a typical example. A detailed analysis of the tryptic digest of this protein has been published by other

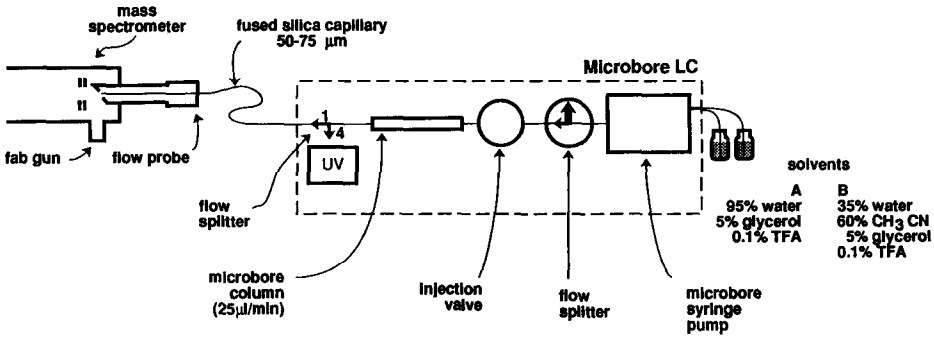

Fig. 2. LC–CF-FAB-MS instrumental arrangement for microbore analyses. (Reprinted with permission from ref. 1.)

investigators [6]. Twenty-one tryptic peptides are normally present in the limit digest, although several others can be seen at low levels presumably due to the action of contaminating proteases. In some of the figures that include chromatograms, we have selected only six of these fragments in order to simplify the comparisons. The $(M+H)^+$ values of these are m/z 383, 693, 1254, 1362, 1400, and 2055. These particular peptides not only represent a distribution of different molecular weights, but also have retention times which fall throughout the entire time period of peptide elution.

Chromatograms for the microbore LC–CF-FAB-MS analysis of the tryptic digest mixture of hGH are shown in Fig. 3. The mobile phase and gradient conditions are described in the experimental section. The amount of sample injected onto the microbore column was 300 pmol, with 60 pmol flowing into the mass spectrometer. The top panel of the figure shows the UV absorbance trace at 215 nm, and the bottom panel the selected ion chromatograms for the six peptides listed above. The ion chromatogram plots are generated by superimposing the six selected ion chromatograms on a single axis. Thus, each selected ion chromatogram is independently normalized and in such a summary plot, absolute intensities are not given. We prefer such a plot

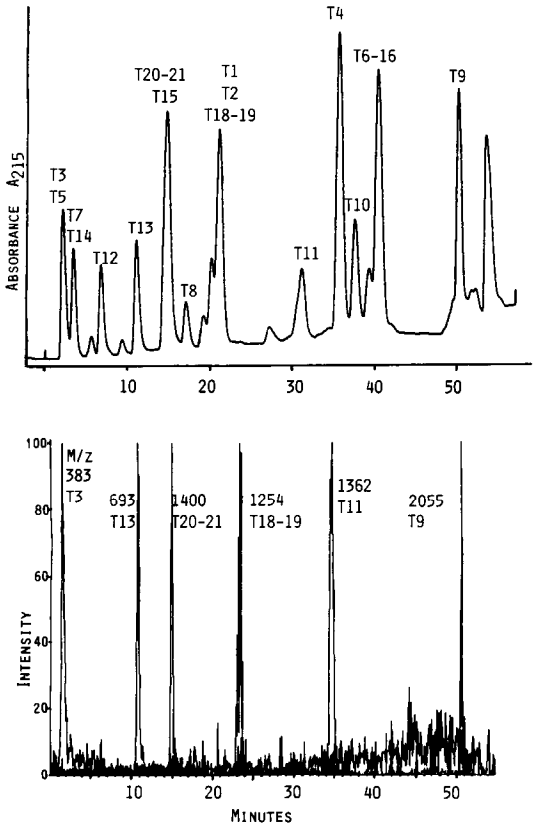

Fig. 3. The microbore LC–CF-FAB-MS analysis of 60 pmol of hGH tryptic digest; (top) the UV absorbance profile, and (bottom) the selected ion chromatograms, independently normalized, for six of the peptides in the mixture. Chromatographic conditions are given in the experimental section.

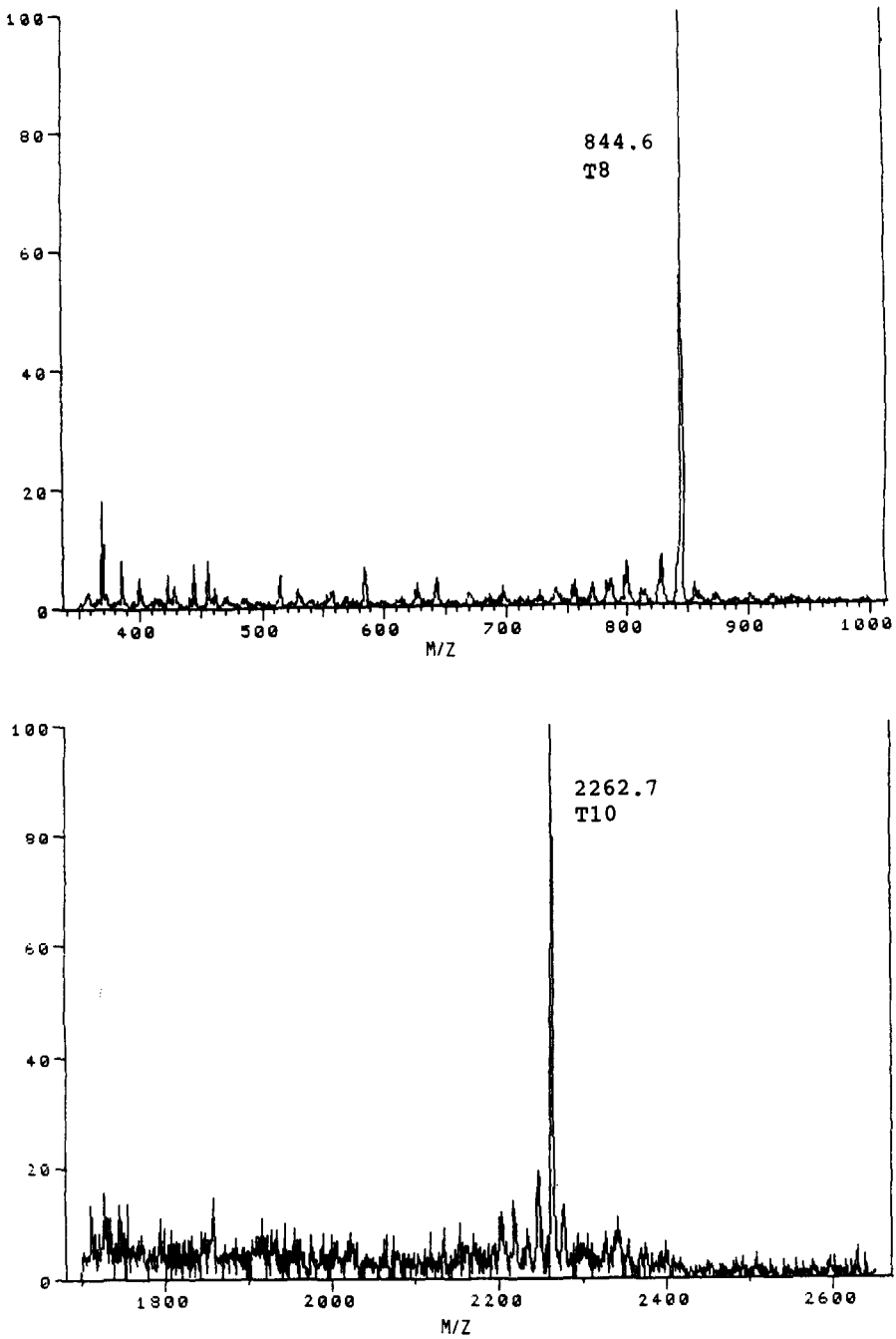

Fig. 4. The CF-FAB mass spectra of two peptides from the digest shown in Fig. 3; *m/z* 844, fragment T8, and *m/z* 2262, T10. Approximately 60 pmol of peptides were transmitted into the mass spectrometer.

because it quickly and easily indicates the position of a given mass, the essential parameter in the data workup. Of course, absolute ion intensities can be obtained from the individual selected ion chromatograms used to make this summary plot.

The analysis shown in Fig. 3 was obtained from a high-performance magnetic double-focusing mass spectrometer. In order to achieve the optimum signal-to-noise ratio in recording these data, the instrument was scanned in three different scan ranges; 350–1000, 1100–1500, 1700–2650. Each of these mass ranges was recorded using a separate LC–MS analysis and, therefore, the ion chromatogram shown in Fig. 3 represents the sum of these three sample analyses. The UV trace in Fig. 3 is from one of these analyses. Four of the individual tryptic fragments are found in disulfide linkage; T6–T16 and T20–T21. The mass spectra of two peptides identified in the analysis are presented in Fig. 4 to illustrate the signal-to-noise ratio obtained in the mass spectrometric analysis of 60 pmol of the digest. The ion at *m/z* 844 is the peptide T8 (tryptic fragment 8) and *m/z* 2262, the peptide T10. The separation and analysis of 50 pmol of the hGH tryptic digest injected onto a packed capillary column (C_{18}, 32 cm × 0.25 mm I.D.) gave the same results as that obtained from the microbore LC–MS analysis, *i.e.*, all of the expected peptides were identified and were eluted over a time of about 50 min (data not shown).

The advantages of CF-FAB are several; it produces relatively intense $(M + H)^+$ ion species for the various peptides, providing suitable ion intensities for MS–MS analyses at the 5–50 pmol level, it is compatible with many types of buffers normally used in biological reactions, and it can be used with either aqueous or organic solvents. The CF-FAB set-up is generally available as a relatively inexpensive option and can be retrofitted to most modern MS systems.

Electrospray MS

Electrospray ionization is an MS technique which produces ions in a spray of microfine solvent droplets at atmospheric pressure and in a high electric field [7,8]. In the source used in this study, ions created in the spray are transported through a glass capillary into the ion source of a mass spectrometer, as shown in Fig. 5. This partic-

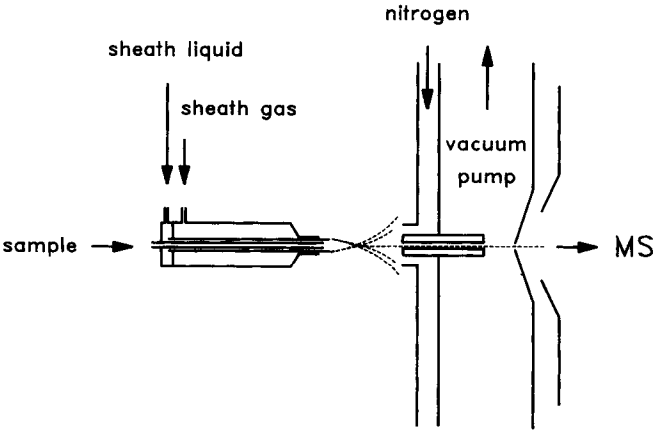

Fig. 5. The schematic diagram of an electrospray ion source. The microfine droplet spray containing ions is shown as dotted lines.

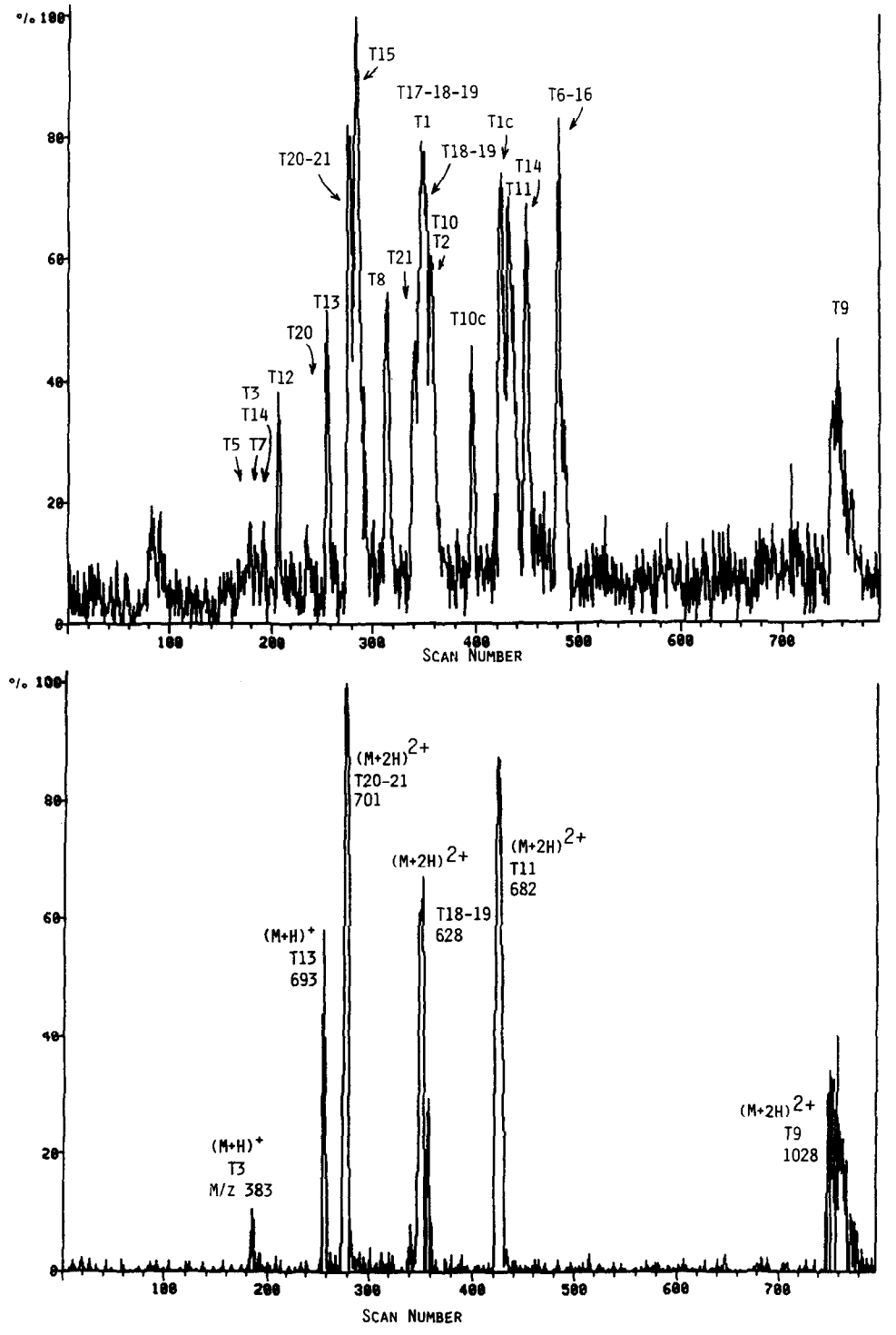

Fig. 6. The LC–electrospray analysis of 50 pmol of the tryptic digest of hGH using the capillary bore C_{18} column. (Top) the total ion chromatogram with background subtracted, and (bottom) the selected ion chromatograms of six peptides in the mixture showing their relative intensities. Chromatographic conditions given in the experimental section.

ular source contains three concentric capillary tubes; the inner capillary delivers the sample or LC eluent, the middle capillary delivers a sheath liquid, such as isopropyl alcohol, to stabilize the spray formation under LC conditions, and the outer capillary delivers nitrogen gas to help form and define the spray so it may be efficiently sampled by the mass spectrometer. Generally, quadrupole mass spectrometers are used for this ionization method because they have relatively low voltage ion sources. Like other MS ionization methods which directly take liquid from the LC, electrospray also works best at relatively low flow rates, *i.e.*, about 2–5 μl/min.

Ion chromatograms obtained from the separation and analysis of 50 pmol of the hGH tryptic digest injected onto a C_{18} slurry packed capillary column are shown in Fig. 6. The upper panel gives the total ion chromatogram for the peptides in the mixture, and the lower panel, the selected ion chromatograms for the six peptides showing their relative intensities. Four of the peptides in the selected ion chromatogram were detected as $(M+2H)^{2+}$ ions, *i.e.*, peptides of molecular weight 1253 (T18, 19), 1361 (T11), 1400 (T20–T21), and 2054 (T9) were recorded as *m/z* 628, 682, 701, and 1028, respectively. Fig. 7 shows the selected ion chromatograms for the singly and doubly charged molecular species of tryptic fragment T13. The peak at *m/z* 347 is the $(M+2H)^{2+}$ species and that at *m/z* 693, $(M+H)^{+}$. The mass spectrum taken from this chromatographic peak is shown in Fig. 8, upper panel. The lower panel of this figure shows a second mass spectrum for two tryptic fragments linked by

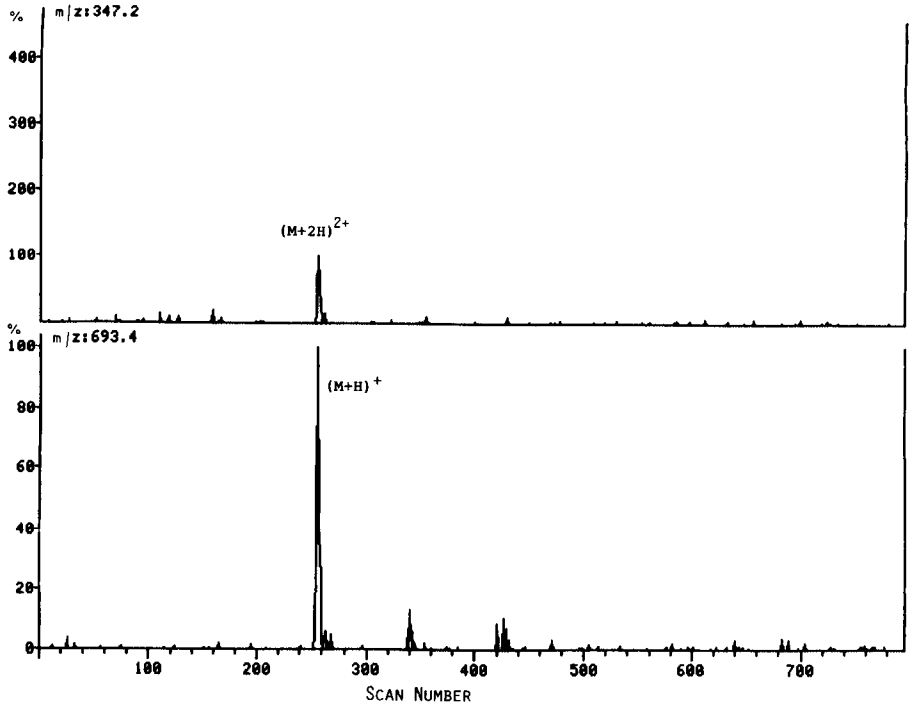

Fig. 7. The selected ion chromatograms for peptide fragment T13 from the analysis given in Fig. 6 showing both the singly (*m/z* 693.4) and doubly charged (*m/z* 347.2) ion chromatograms.

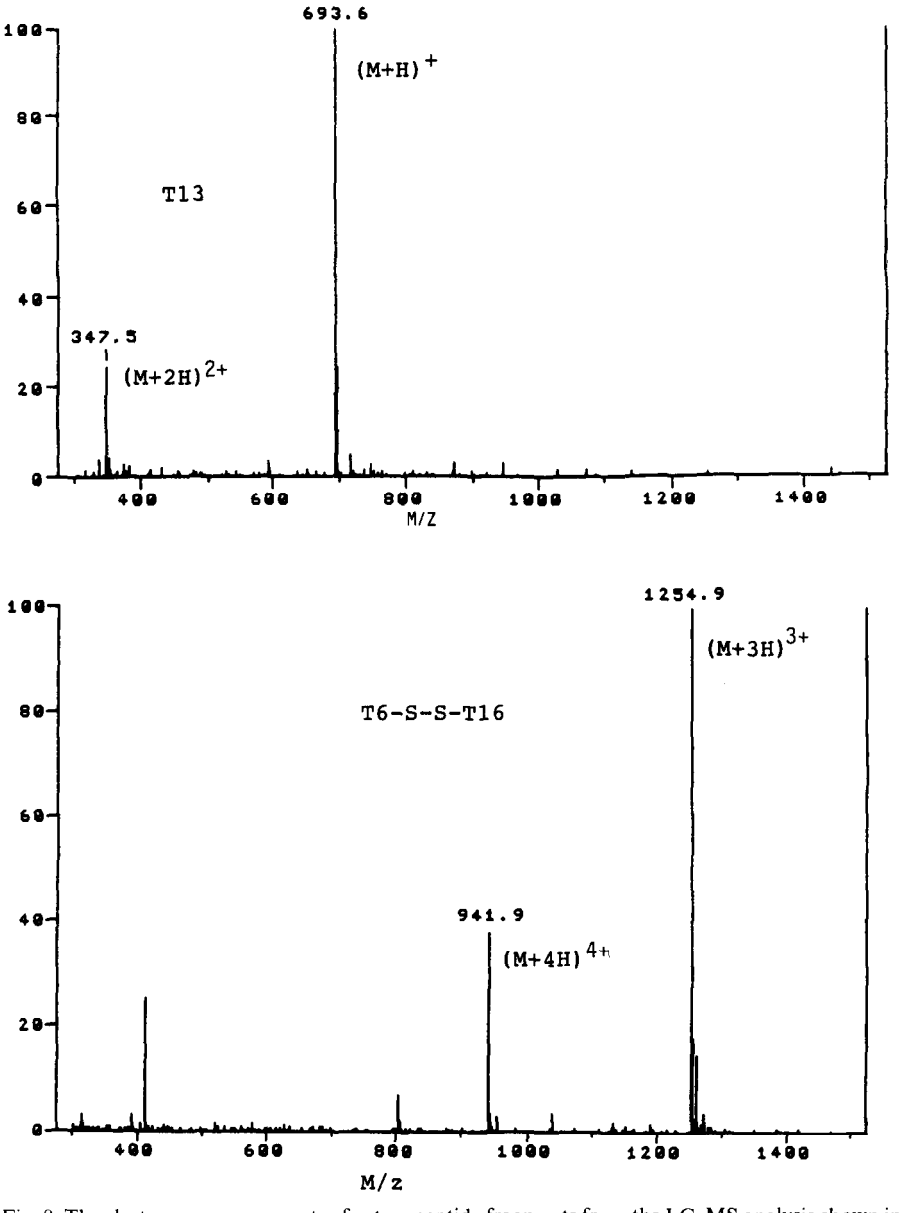

Fig. 8. The electrospray mass spectra for two peptide fragments from the LC–MS analysis shown in Fig. 6.

a disulfide bridge (T6–S–S–T16) at m/z 1255 for $(M+3H)^{3+}$ and m/z 941 for $(M+4H)^{4+}$.

One of the common analysis procedures for a tryptic digest is the direct analysis of an aliquot either by FAB or, more recently, electrospray without the use of an LC column. This procedure is generally termed FAB-mapping when the FAB ionization method is used. Although it takes considerably less time and effort to perform such an

analysis, the results are inferior in comparison to the information obtained from the LC–MS system. This is shown in Table I for the comparison of the capillary LC–electrospray MS analysis of 50 pmol of the hGH digest compared to the direct injection of 50 pmol/μl of the digest into the electrospray source. It is seen that the LC–MS analysis is superior in permitting the identification of 20 of the 21 peptides in the scan range and at higher comparative intensities than that obtained by direct injection, where only 11 of the 21 peptides were identified. The LC system thus performs an extremely important function for the mass spectrometric ionization process in that it separates compounds entering the ion source in time, minimizing ion suppression effects that often occur when many compounds arrive in the source at the same time.

As indicated above, one of the great advantages of electrospray over other ionization techniques is its ability to form multiply charged ions. Since mass spectrometers measure mass-to-charge (m/z) ratios, large molecules whose singly charged molecular species normally fall outside the effective mass range of the spectrometer can be recorded as multiply charged ions. For example, a sample of soybean trypsin inhibitor (molecular weight 20 091) will form a distribution of multiply charged ions

TABLE I

ANALYSIS OF hGH TRYPTIC DIGEST BY ELECTROSPRAY MASS SPECTROMETRY, COMPARING LC–MS AND DIRECT INJECTION ANALYSIS

Tryptic fragment	Molecular ion observed	Relative ion intensity[a]	
		LC–MS[b] analysis of digest	Direct injection of digest[c]
4	2543^{2+}	+ + +	+
9	2056^{2+}	+ + +	+ +
11	1363^{2+}	+ + +	+ + +
14	627^{2+}	+ + +	+ + +
15	1491^{2+}	+ + +	+ + +
18(19)	1255^{2+}	+ + +	+ + +
21	786^{2+}	+ + +	–
1	931^{1+}	+ +	+
6	2618^{3+}	+ +	–
8	845^{1+}	+ +	+
10	2264^{3+}	+ +	–
20–S–S–21	1401^{2+}	+ +	+
2	980^{1+}	+	+
3	383^{1+}	+	–
6–S–S–16	3765^{4+}	+	–
12	773^{1+}	+	–
13	693^{1+}	+	+
20	618^{1+}	+	–
5	404^{1+}	+	–
7	762^{1+}	+	–
16	1150^{2+}	–	–

[a] Relative ion intensity shown as highest (+ + +) to lowest (+). Ions not observed noted by (–).
[b] Data from capillary LC–MS analysis shown in Fig. 6; 50 pmol of peptides transmitted into the MS. Sample solution contained 50 pmol in a 1 μl injection.

containing charged molecules from 16^+ to 22^+ [9]. These multiply charged species are, of course, related to a single molecule species and this multiply charged spectrum may be deconvoluted to give the molecular weight of the protein. Because these multiply charged species mainly fall between m/z 800 and 2000, relatively low cost quadrupole instruments can be used quite effectively for high-molecular-weight measurements of 10 000–50 000 daltons or more.

CZE–MS

CZE–MS is of great interest and potential in the field of analytical biochemistry because of its relatively high separation efficiency and ability to separate molecules based on their charge. It is generally a low cost and relatively easy analytical system to operate, although narrow bore capillaries present special handling problems. Nevertheless, CZE represents a breakthrough in high resolution, low flow-rate separations systems. Flow-rates essentially due to electroosmotic flow are typically in the range of 0.01–0.1 μl/min. With respect to combining CZE with mass spectrometry, these flow-rates are too low for stable operation of most ion sources and make-up liquid needs to be added to bring the flow-rate up to approximately 2–5 μl/min. A typical instrumental arrangement for CZE–CF-FAB is shown in Fig. 9. An interface is necessary because of the differences in the flow-rates of the CZE apparatus and the CF-FAB and electrospray systems. The CF-FAB system requires 2–5 μl/min of solvent flow for optimal performance and this is achieved using make-up solvent from a reservoir in the interface assembly. The interface can be of two types; the liquid-junction interface where the CZE capillary and the CF-FAB capillary are essentially butted up end-to-end in the interface [3], and the coaxial interface where the CZE capillary is threaded through the CF-FAB capillary almost to the target tip on the probe [10]. Each system has particular advantages and disadvantages. Basically, the liquid-junction interface is easier to set up and operate but gives significant band broadening. The coaxial interface provides higher resolution by giving minimal peak broadening but is mechanically more difficult to set up and run reproducibly.

The ion electropherograms for the analysis of 25 pmol of the tryptic digest of β-lactoglobulin A obtained by CZE–CF-FAB-MS (liquid-junction interface) is given in Fig. 10. The upper panel in the figure shows the total ion recording and the lower

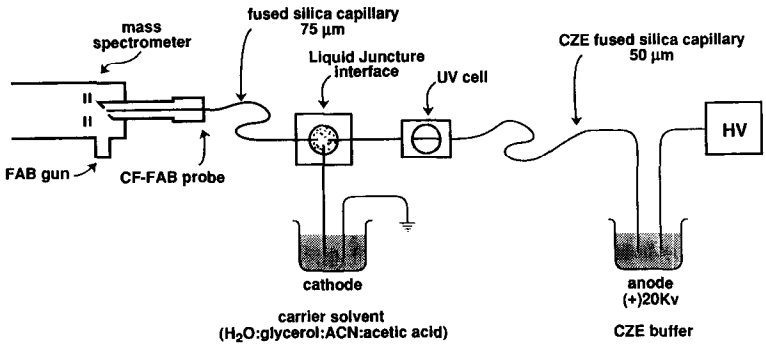

Fig. 9. The CZE–CF-FAB instrumental arrangement.

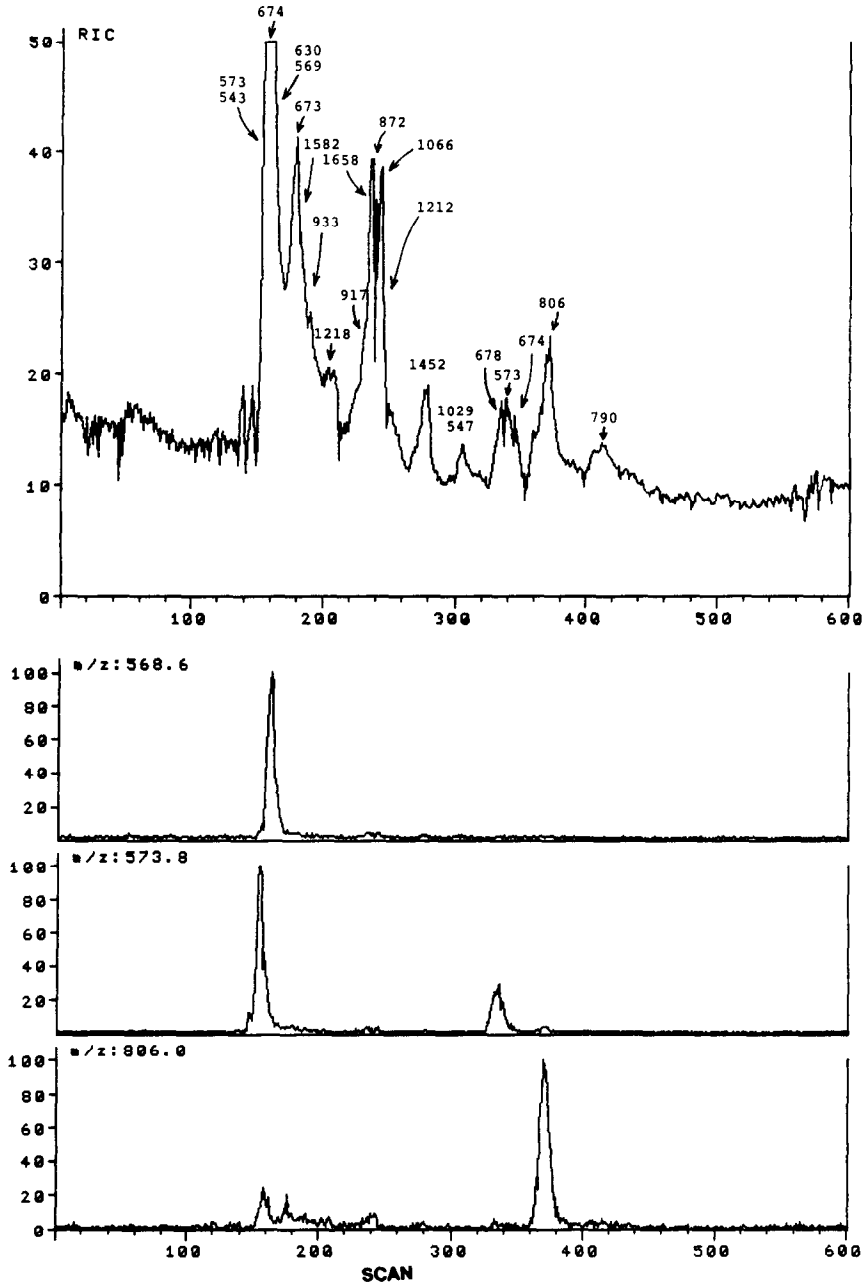

Fig. 10. The CZE–CF-FAB-MS analysis of the tryptic digest of 25 pmol of β-lactoglobulin A. (Top) the total ion recording using a scan range of m/z 500–2000, and (bottom) the selected ion recordings for three of the peptides.

panel several selected ion recordings. It is noted that the selected ion recording of m/z 573 shows two eluting peaks; the first is for the major tryptic fragment T7 and the second, unexpected in this analysis, for one or more peptides from several possible positions in the protein, produced by a contaminating protease. High-resolution separations were not used in this particular analysis because of the scan time required by the mass spectrometer (as discussed below). Nevertheless, specific peptides were easily identified in the mixture.

An important disadvantage of the use of a scanning detector such as a magnetic or quadrupole mass spectrometer is the fact that it cannot take full advantage of the extremely high-resolution separations achievable by CZE. For example, if one is scanning a decade of mass (300–3000 mass units), scan speeds of about 12–15 s would be required to get adequate signal-to-noise recordings for these spectra. This relatively lengthy scan time thus precludes the analysis of peaks in a separation process where the half-width of the peak emerging from the capillary is less than the scan time of the detector. Although there are integrating detectors and mass spectrometer analyzers that are becoming available that may be better suited for this purpose, these are, at the present time, either very expensive (array detectors) or are in development, such as the ion-trap detector. Despite this problem, the on-line combination of CZE with mass spectrometry provides a capability that will be invaluable for the structural analysis of proteins.

CONCLUSION

The chromatographic process limits the number of compounds entering the ion source of the mass spectrometer during the analysis. This has several important advantages. First, it can provide a temporal separation of compounds that have the same nominal mass or whose spectra have major ions in common. Second, it minimizes ion suppression effects, *i.e.*, the process whereby one or more compounds will not form ions as a result of the presence of other compounds in the mixture. Third, it allows the analyst to collect fractions and isolate purified compounds in the mixture for further study.

The two mass spectrometric ionization methods utilized in this work, CF-FAB and electrospray, have a number of features in common. They both have about the same sensitivity (5–50 pmol) when full-scan spectra are required for peptide digests analyzed by LC–MS methods and give optimal performance at flow-rates of about 5 μl/min. However, there are some important differences as well. Electrospray produces multiply-charged molecular species, an advantage that permits the analysis of molecules of significantly higher molecular weight than the upper mass limit of the spectrometer. CF-FAB gives high intensity $(M+H)^+$ ions, good MS–MS spectra, and tolerates a wide range of buffers with respect to their chemical diversity and volatility.

With respect to the use of microbore *versus* capillary bore LC columns, we were not able to discern significant differences in the analyses performed in this work. The identification of peptides in the mixtures could be accomplished using either column. The choice of one over the other may also be made based on other factors whose importance can vary depending on the task, *e.g.*, choice of flow-rates, use of a split or splitless interface, durability of the column, etc.

CZE–MS is potentially an extremely useful separation–detection device be-

cause its high-resolution capability can provide a new tool for the high sensitivity analysis needed for trace compound analysis. Nevertheless, the high-resolution process can also be a major problem if the mass spectrometer must scan a wide mass range. Currently, the CZE–MS combination is most effectively used for target compound analysis or in applications where only narrow mass ranges need to be scanned.

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