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Direct coupling of ionic high-performance liquid chromatography with electrospray ionization mass spectrometry utilizing a microdialysis junction interface

Chuanliang Liu*, Swati S. Verma

Genetics Institute, One Burtt Road, Andover, MA 01810, USA

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Abstract

A method for directly coupling ionic HPLC with electrospray ionization mass spectrometry (ESI-MS) utilizing a microdialysis junction interface is described. HPLC eluent was split postcolumn to allow a ~5–40 μl/min flow into the microdialysis assembly, which consists of a microbore microdialysis fiber and a larger concentric sheath tubing for the introduction of a counter-current dialysis buffer. The salt-containing sample was desalted on-line and transferred directly to the ESI source of a LCQ ion trap mass spectrometer. A sample flow-rate (inside the microdialysis fiber) of 10–20 μl/min was found optimal for both retaining HPLC resolution and achieving efficient on-line desalting. When performed at 50°C, the microdialysis system could provide complete on-line desalting for LC buffers containing up to 50 m*M* Tris–HCl and 1 *M* NaCl. The introduction of an organic sheath liquid with 2% acetic acid enhanced the ESI-MS detection sensitivity by as much as tenfold and a sensitivity in the low pmol range (initial LC injection before flow splitting) was achieved for insulin chain A. Successful on-line desalting was also achieved for a protein mixture consisting of ubiquitin, cytochrome *c* and lysozyme separated by cation-exchange chromatography and a good quality spectrum was obtained for each component. Limitations of the current system include the working pH range (4–11), the temperature tolerance (<60°C) and the molecular mass cut-off (~1000) imposed by the microdialysis fiber and the low sensitivity of the LCQ mass spectrometer for high molecular mass species.

Keywords: Microdialysis; Interfaces, LC–MS; Liquid chromatography–mass spectrometry; Proteins

1. Introduction

Electrospray ionization mass spectrometry (ESI-MS) has evolved as a powerful routine analytical technique for the analysis of biological molecules, and its application in biochemical and biomedical fields has been explored extensively [1–6]. The characteristic liquid flow requirement of ESI-MS makes it an ideal ionization method for HPLC detection by MS. A large volume of applications of reversed-phase HPLC–ESI-MS has demonstrated the many advantages of ESI-MS as a detection technique for HPLC [7–13]. However, the application of ESI-MS as a detection technique has been so far limited to reversed-phase HPLC or other HPLC systems utilizing only volatile buffers. Ionic HPLC such as ion-exchange, hydrophobic interaction and affinity chromatography has had little success in direct coupling with ESI-MS due to the incompatibility of

*Corresponding author. Tel.: +1-978-623-1588; fax: +1-978-623-2837; e-mail: cliu@genetics.com

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ESI-MS with the nonvolatile buffers commonly used in these chromatographic systems.

Ionic HPLC is widely used for the separation and purification of biological samples including proteins, peptides, nucleic acids and carbohydrates. When the preservation of biological activity is required, ionic HPLC is, in many situations, the only choice of separation technique. The development of protein-based therapeutics in the biotechnology industry demands a variety of ionic HPLC methods to isolate and purify target proteins from the complex cellular constituents. Monitoring the purification process presents an additional analytical challenge. In order to analyze the samples from ionic HPLC by ESI-MS, individual sample peaks have to be collected and desalted. Several commonly used desalting approaches include conventional dialysis, multiple buffer exchange using membrane cartridges [14,15], reversed-phase HPLC [16,17], hydrophobic interaction chromatography [18,19], ionic exchange beads or membranes [20–22] and size exclusion chromatography [23]. Although effective in most applications, these techniques are labor-intensive, require relatively large amounts of sample and can only be performed in the off-line mode. These drawbacks have resulted in significant increases in analysis time and cost. The multiple sample transfer steps increase the possibility of sample loss and could preclude analysis when only minute amounts of sample are available.

A technique capable of on-line desalting the eluent from ionic HPLC in real time should provide many advantages over the above mentioned methods. First, it allows direct ESI-MS analysis of sample components from ionic HPLC, eliminating additional sample transfer steps, simplifying analysis procedures and reducing analysis time and cost. Second, the separation resolution can be retained and individual sample components can be analyzed by ESI-MS, thus simplifying mass spectral interpretation. Thirdly, for samples of limited stability after the conventional slow desalting process (i.e. aggregate or precipitate after desalting), fast on-line desalting constitutes the only possible approach for desalting these samples for ESI-MS analysis [24]. Finally, it will allow easy automation of ionic HPLC–ESI-MS to increase analysis throughput, a feature that is widely implemented in reversed-phase HPLC–ESI-MS.

A few groups have been working extensively on the development of on-line desalting techniques for biological sample analysis by ESI-MS. Emmett and Caprioli have developed an on-line desalting technique using a C_{18} cartridge to remove the excessive salts before the sample was introduced into the mass spectrometer [25]. However, this approach cannot be performed in a continuous fashion, i.e., real time desalting for ionic HPLC separations; each sample peak has to be individually injected onto the cartridge and desalted. Opiteck al. [9] used a reversed-phase HPLC on-line with ion-exchange chromatography for further separating the sample components and desalting them for ESI-MS analysis. However, each peak from the ion-exchange chromatography has to be individually directed to the reversed-phase HPLC column and desalted. Thayer et al. demonstrated that a cation-exchange system can be used to desalt the eluent from high-pH anion-exchange chromatography and the recovered oligosaccharide samples can be analyzed directly by matrix-assisted laser desorption-time of flight (MALDI-TOF) MS [21]. Conboy and Henion [26] further reported the use of a similar micromembrane suppression system for on-line desalting of carbohydrate samples from high-performance anion-exchange chromatography for ion spray MS measurements. However, this method will likely have applicability only for high-pH anion-exchange chromatography with Na^+ (or other simple metal ions) being the major interfering species. So far, to our knowledge, no method has been reported to be generally applicable to desalt the eluent on-line from ionic chromatography in real time.

The two critical features of a useful on-line desalting technique include high desalting efficiency and low dead volume: a high desalting efficiency insures the complete desalting for continuous LC eluent and a low dead volume facilitates the retention of chromatographic resolution. While most of the current desalting techniques do not meet these two stringent requirements, a recent on-line microdialysis technique developed by Liu and coworkers [27–30] demonstrated great promise for on-line desalting for ionic HPLC. By employing a 200 μm I.D. hollow fiber microdialysis tube and a counter-current dialysis buffer flow, they achieved complete desalting (as indicated by ESI-MS) for a 5 μl/min continuous flow of protein and DNA samples in buffers with up to 1 *M* and 0.5 *M* NaCl, respectively.

In addition, they showed that an increase in dialysis temperature greatly increased the desalting efficiency of the microdialysis system. The high desalting efficiency and the low dead volume (~10–20 μl) of the microdialysis system closely match the requirements of an on-line desalting technique for ionic HPLC. In this paper, we report our results of using the microdialysis technique for on-line desalting of ionic HPLC eluents for ESI-MS analysis.

2. Experimental

2.1. Reagents

All the peptide and protein samples, as well as buffer components, were purchased from Sigma (St. Louis, MO, USA) and used as received. Water was purified using a Millipore (Bedford, MA, USA) water purification system.

2.2. Instruments

The HPLC system was a Waters (Milford, MA, USA) Alliance 2690 pump module equipped with a Waters 2487 dual-wavelength UV detector. MS was performed using a Finnigan (San Jose, CA, USA) LCQ ion trap mass spectrometer with an ESI ion source.

2.3. Methods

2.3.1. Flow injection–microdialysis–ESI-MS

The buffers of choice (as indicated in Section 3) were delivered at 1 ml/min directly to an Upchurch three-way needle valve (Oak Harbor, WA, USA) for flow splitting. Different flow-rates (~5–40 μl/min, as measured manually by a 50-μl syringe) were split into one end of the microdialysis system, with the remaining solution going to the Waters 2487 UV detector. The microdialysis system (Fig. 1) was similar to that described in the literature [27,28], except that the length of the larger fused-silica tubing (430 μm O.D., 320 μm I.D., which was used to connect the microdialysis fiber with the smaller fused-silica tubing) was reduced from 3 cm to 1.5 cm. This further reduced the dead volume of the microdialysis system. Repetitive injection of 50 μl of 0.2 mg/ml insulin chain A in 20 m*M* NH_4OAc was performed through a built-in autosampler of the Waters Alliance system. A solution of 20 m*M* NH_4OAc was used as the dialysis buffer for all experiments and the flow-rate was maintained at ~0.4 ml/min by a simple vertical displacement of the buffer reservoir over the buffer outlet by ~15 cm (no mechanical pump was used). The other end of the microdialysis assembly was directly coupled to the ESI source of the LCQ instrument without any modification. For microdialysis experiments performed at elevated temperature, the microdialysis assembly was put inside a stirred 1.7-l water-bath, the temperature of which was controlled by an

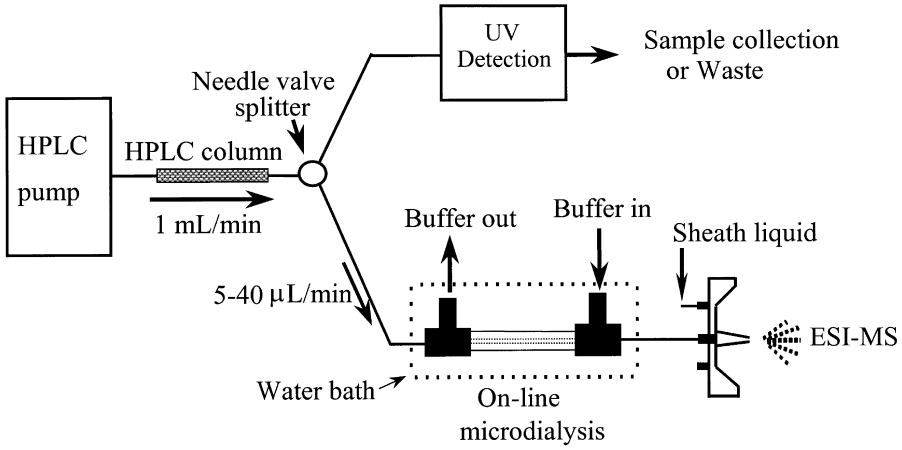

Fig. 1. Schematic of the ionic HPLC–on-line microdialysis–ESI-MS system.

external heating device to 50±0.5°C. Typical ESI conditions are: ionization voltage, 4 kV; heated capillary temperature, 200°C; number of microscans/scan, 3; maximum injection time, 200 ms; sheath gas, 80 units. In some experiments, a sheath liquid consisting of 2% acetic acid in methanol was introduced at 10 μl/min through the sheath liquid inlet of the LCQ ESI source for sensitivity enhancement.

2.3.2. Cation-exchange–microdialysis–ESI-MS

A mixture of 0.2 mg/ml ubiquitin, 4 mg/ml cytochrome *c* and 4 mg/ml lysozyme in 50 m*M* Tris–HCl was injected onto a PerSeptive Biosystems (Framingham, MA, USA) Porous S/H cation-exchange column (50×4.6 mm). The samples were eluted using a 100% A, 0% B to 40% A, 60% B gradient in 30 min, while A is 50 m*M*, pH 6.2 Tris–HCl and B is 50 m*M*, pH 6.2 Tris–HCl with 500 m*M* NaCl. The LC eluent was split (with 15 μl/min going to the microdialysis system), desalted by microdialysis using 2 m*M* NH_4OAc as dialysis buffer and analyzed by ESI-MS as described above.

3. Results and discussion

3.1. The effects of microdialysis flow-rate on peak broadening

As demonstrated by Liu et al. [27], a lower sample flow-rate will result in a higher microdialysis desalting efficiency. While the flow-rate can be changed without much limitation for off-line desalting or on-line continuous sample infusion, real time on-line desalting for ionic HPLC requires a certain flow-rate to minimize peak broadening due to the dead volume of the microdialysis assembly. With the length reduction of the large fused-silica tubing, the dead volume of the microdialysis system (including the volume of the large fused-silica tubing and the microdialysis fiber) measured to be around 15–20 μl. If a flow-rate of 5 μl/min is used (as reported in the literature [26]), significant peak broadening will be expected. A higher flow-rate will benefit the resolution, however, it will greatly increase the desalting load of the microdialysis system, and incomplete desalting could occur. Therefore, an optimal flow-rate or flow-rate range which allows complete desalting while maintaining HPLC resolution needs to be established.

A flow injection analysis was used to establish the optimal flow-rate using 20 m*M* NH_4OAc as the LC mobile phase. Four repetitive injections of 0.2 mg/ml insulin chain A were performed using the autosampler with a 2-min interval and different flow-rates were split to the microdialysis system. Fig. 2 compares the UV chromatogram and ESI-MS total ion current chromatograms at different microdialysis flow-rates. The baseline peak width was 0.5, 0.6, 1 and 1.5 min for a microdialysis flow-rate of 40 μl/min, 20 μl/min, 10 μl/min and 5 μl/min,

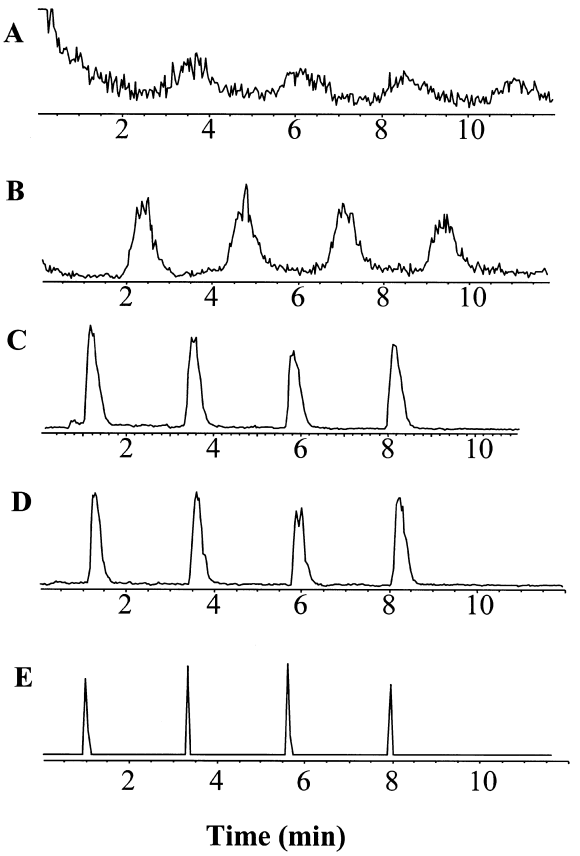

Fig. 2. The effects of microdialysis flow-rate on peak broadening. LC buffer: 20 m*M* NH_4OAc; LC flow-rate: 1 ml/min; dialysis buffer: 20 m*M* NH_4OAc. The ESI total ion current chromatogram for four repetitive injections at a microdialysis flow-rate of: (A) 5 μl/min; (B) 10 μl/min; (C) 20 μl/min and (D) 40 μl/min. (E) Corresponding UV chromatogram.

respectively in the ESI-MS chromatogram, while a peak width of 0.2 min was observed in the UV chromatogram. Apparently, peak broadening deteriorates with a decrease in microdialysis flow-rate; and HPLC resolution will be largely lost for a microdialysis flow-rate of <5 μl/min. Considering that the peaks from the ionic HPLC system tend to be relatively wide, i.e., half a minute to a few minutes, further broadening the peak by another ~0.5–1 min by microdialysis should not present a major setback on resolution. We therefore chose a flow-rate between 10–20 μl/min as the optimal flow-rate for on-line microdialysis, with the actual flow-rate being adjusted according to individual sample desalting needs. Notice that a decrease in detection sensitivity was also observed with a decrease in sample flow-rate presumably due to peak broadening.

3.2. Desalting efficiency of the microdialysis system

Although preliminary results on the desalting efficiency of the microdialysis system have been reported by Liu and coworkers [27,28], reexamination of the microdialysis system using the current configuration is necessary for assessing its usefulness in on-line desalting for ionic HPLC. The same flow injection scheme as described in Section 2 was used. Using the same insulin chain A sample, the desalting efficiency was evaluated by running different concentrations of LC buffer. When a buffer containing 3 m*M* Tris–HCl and 25 m*M* NaCl was used as the LC mobile phase, no useful ESI mass spectrum would be expected without on-line desalting. However, as shown in Fig. 3, complete desalting was achieved after the sample passed through the microdialysis

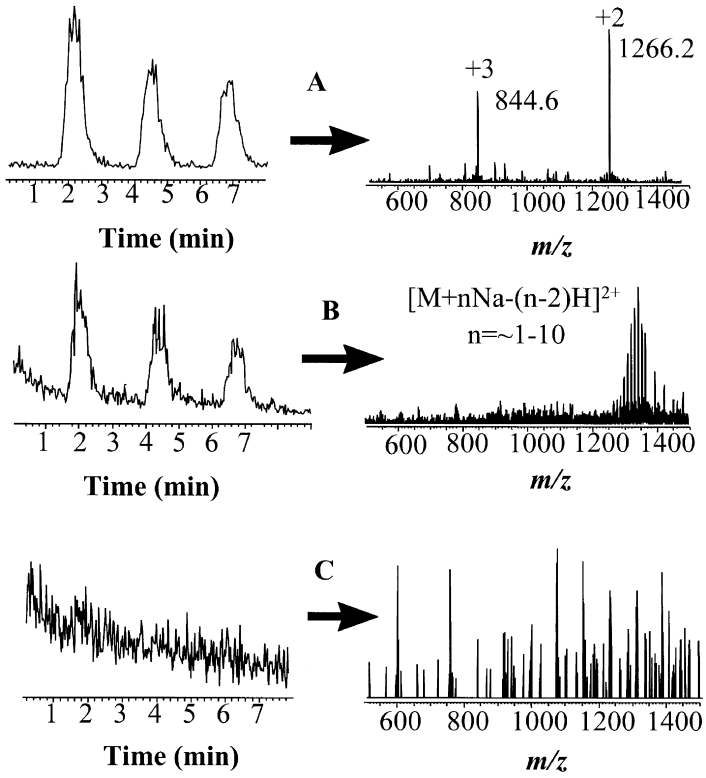

Fig. 3. Desalting efficiency of the microdialysis system at room temperature. LC flow-rate: 1 ml/min; microdialysis flow-rate: 10 μl/min. The ESI total ion current chromatogram for three repetitive injections and corresponding mass spectrum of insulin chain A with a LC buffer: (A) 3 m*M* Tris–HCl and 25 m*M* NaCl; (B) 5 m*M* Tris–HCl and 50 m*M* NaCl and (C) 10 m*M* Tris–HCl and 100 m*M* NaCl.

system at a flow-rate of ~10 μl/min at room temperature (Fig. 3A). A high quality spectrum was obtained; showing distinct doubly (m/z 1266.2) and triply (m/z 844.6) charged molecular ions of insulin chain A. Significant Na^+ adducts were observed along with a reduction in sensitivity when the buffer concentration was increased to 5 m*M* Tris–HCl and 50 m*M* NaCl (Fig. 3B), indicating a low desalting capacity of the current configuration. When the concentration of buffer was further increased to 10 m*M* Tris–HCl and 100 m*M* NaCl, no useful signal was observed (Fig. 3C). Clearly, with this configuration, the microdialysis system will have limited applicability in on-line desalting for ionic HPLC; since most of the ionic HPLC systems use buffers with higher salt concentrations.

As reported by Liu et al., an elevated temperature in microdialysis can greatly increase the desalting efficiency. It is therefore expected that an increase in microdialysis temperature would also enhance the desalting efficiency for the current system. As demonstrated in Fig. 4, when the microdialysis was performed at 50°C (the temperature of the water bath; actual dialysis temperature should be lower than 50°C), much higher desalting efficiency was achieved. Complete desalting was obtained even for a buffer containing 50 m*M* Tris–HCl and 1 *M* NaCl. With such a high desalting capacity, we believe that microdialysis will be widely applicable for various ionic HPLC systems.

Note that there is a small shift in elution time and decrease in sensitivity with higher buffer concentrations (Fig. 4B, C). Although the reasons of which are currently unclear, it is possible that with a high

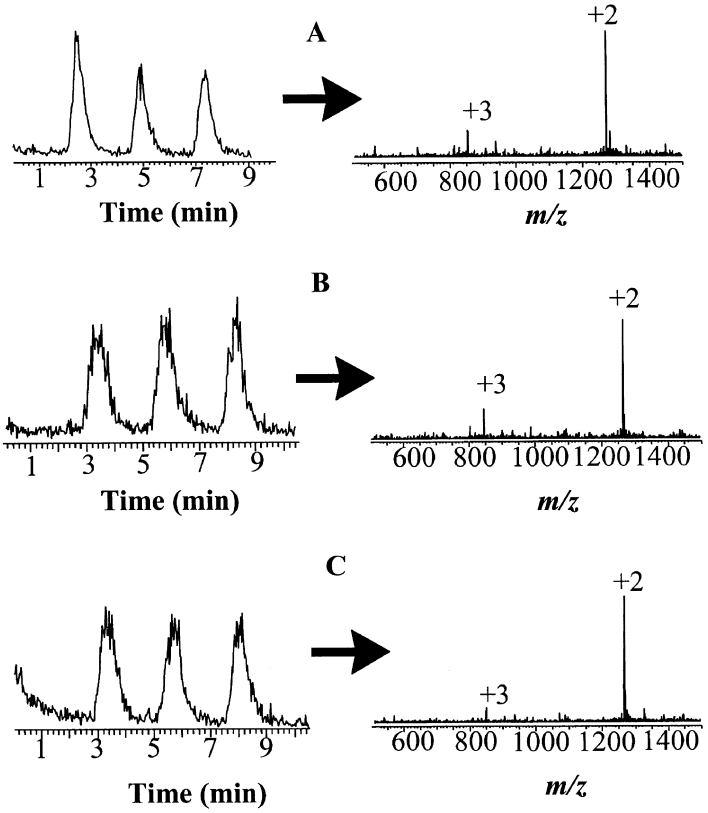

Fig. 4. Desalting efficiency of the microdialysis system at 50°C. LC flow-rate: 1 ml/min; microdialysis flow-rate: 10 μl/min. The ESI total ion current chromatogram for three repetitive injections and corresponding mass spectrum of insulin chain A with a LC buffer: (A) 5 m*M* Tris–HCl and 100 m*M* NaCl; (B) 25 m*M* Tris–HCl and 500 m*M* NaCl and (C) 50 m*M* Tris–HCl and 1 *M* NaCl.

salt concentration, the diversion of sample flow through the wall of the microdialysis fiber increased due to the higher viscosity of the sample before complete desalting; and therefore the flow-rate eluting out of the microdialysis system was reduced. A lower flow-rate resulted in later elution and lower sensitivity due to peak broadening. A study is currently underway in our laboratory to further confirm this hypothesis.

3.3. The effects of microdialysis flow-rate on desalting efficiency

As mentioned earlier, the microdialysis flow-rate not only affects the peak resolution, but also governs the desalting efficiency because it determines the residence time of the samples inside the microdialysis fiber. A higher flow-rate will result in short sample residence time, therefore lower desalting efficiency; likewise, a lower flow-rate will allow longer sample dialysis time and higher desalting efficiency. This is clearly demonstrated in Fig. 5, where the flow-rate greatly changed the desalting performance of the microdialysis system. When the microdialysis was performed at 50°C, complete desalting was obtained for a sample flow-rate of 10 μl/min (Fig. 5A). At a flow-rate of 15 μl/min, distinct sample peaks could still be observed, although at a lower sensitivity, however, significant Na^+ adduction was observed in the mass spectrum (Fig. 5B). Further increasing the flow-rate to 20 μl/min resulted in the disappearance of all sample peaks and MS analysis was precluded (Fig. 5C). These results suggest that the microdialysis flow-rate should be determined based on the buffer contents of

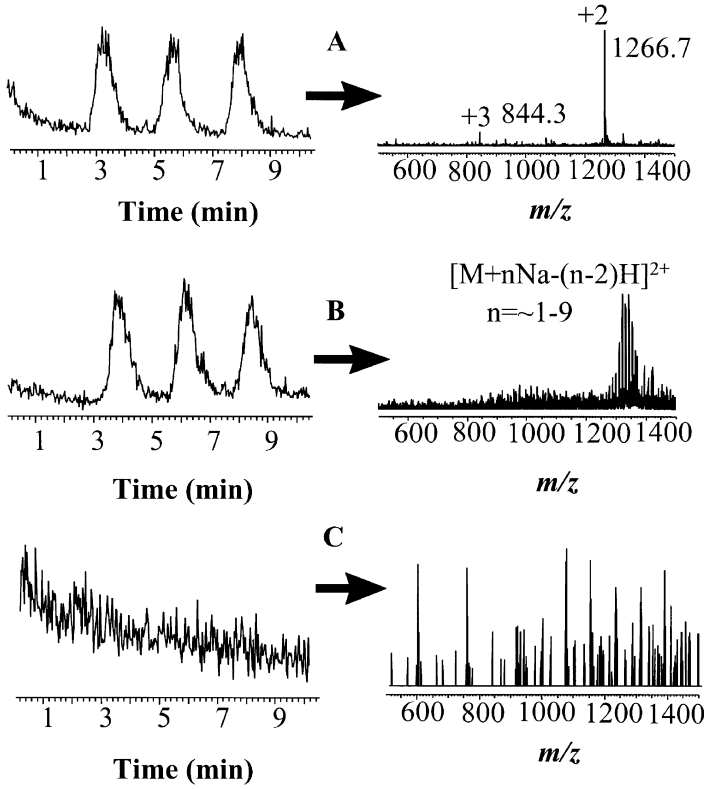

Fig. 5. The effects of microdialysis flow-rate on desalting efficiency. LC buffer: 50 m*M* Tris–HCl and 1 *M* NaCl; LC flow-rate: 1 ml/min; microdialysis temperature: 50°C. The ESI total ion current chromatogram for three repetitive injections and corresponding mass spectrum of insulin chain A at a microdialysis flow-rate of: (A) 10 μl/min; (B) 15 μl/min and (C) 20 μl/min.

the sample in order to both achieve complete desalting and retain highest possible resolution.

3.4. Sensitivity enhancement by the addition of a sheath liquid

It is well known that organic solvent with 1–2% volatile acid constitutes the best solvent for sample preparation in positive ESI-MS analysis. However, such a solvent system could not be used in microdialysis for two reasons: the low dialysis efficiency in organic solvent and the limited pH tolerance of the microdialysis fiber (working pH range: 4–11). In order to both retain the microdialysis efficiency and increase ESI-MS sensitivity, a sheath liquid consisting of 2% acetic acid in methanol was introduced to the ESI source at 10 μl/min using the LCQ built-in syringe pump for sensitivity enhancement. As expected, a ~tenfold increase in sensitivity was observed in the total ion current chromatogram, along with a much improved mass spectrum (Fig. 6). A detection sensitivity of 10–50 pmol in terms of initial LC injection amount could be readily achieved for insulin chain A at a microdialysis flow-rate of 10 μl/min with the incorporation of the sheath liquid. The increase in sensitivity with the addition of sheath liquid further extends the applicability of microdialysis for on-line desalting. An increase in sensitivity allows the use of lower sample flow-rates (e.g. 5 μl/min in this case) to achieve complete desalting for samples with high salt contents without sacrificing sensitivity. Similarly, desalting for buffer components of higher molecular masses [e.g. Tris, 2-(N-morpholino)ethanesulfonic acid and other similar species] is much less efficient than that for Na^+ due to their relative low diffusion rates. A low sample flow-rate is essential for complete desalting of these buffer components.

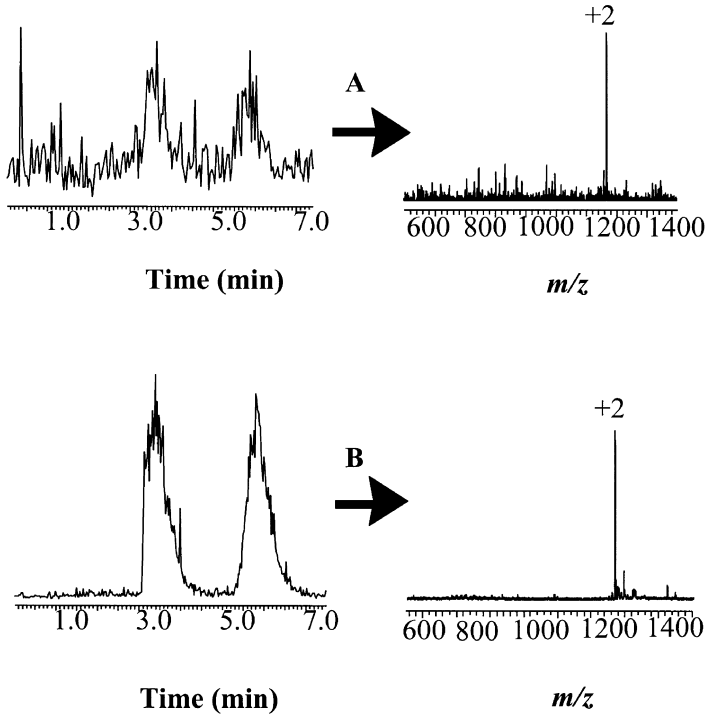

Fig. 6. The effects of sheath liquid on ESI detection sensitivity. LC buffer: 25 m*M* Tris–HCl and 500 m*M* NaCl; microdialysis flow-rate: 5 μl/min; microdialysis temperature: 50°C. The ESI total ion current chromatogram for two repetitive injections and corresponding mass spectrum of insulin chain A when (A) no sheath liquid was used and (B) 10 μl/min of methanol with 2% acetic acid was used as sheath liquid.

Although LC resolution may be sacrificed to some extent, the ability of MS to detect partially overlapping peaks should ease the requirement on resolution and peak identification should not be affected. A higher sensitivity will also facilitate the detection of minor components from ionic HPLC and provide additional sample information that might otherwise be lost. The composition and concentration of the sheath liquid can also be changed according to the nature of the analytes and ESI ionization mode. For example, piperidine and imidazole can be used in the sheath liquid for sensitivity enhancement for negative ESI-MS analysis of DNA samples after on-line microdialysis [28,31]. The ability to incorporate different sheath liquid independent of microdialysis process in the ESI source for sensitivity enhancement should greatly increase the flexibility and robustness of microdialysis as an on-line desalting technique for ionic HPLC.

3.5. Microdialysis on-line desalting for cation-exchange HPLC of proteins

In order to evaluate the performance of microdialysis as an on-line desalting technique for actual ionic HPLC separations, a protein mixture consisting of 0.2 mg/ml ubiquitin, 4 mg/ml cytochrome *c* and 4 mg/ml lysozyme was separated by a Porous S/H cation-exchange column and analyzed by ESI-MS after on-line desalting using microdialysis. As shown in Fig. 7, three peaks were observed from both UV and ESI-MS chromatograms (Fig. 7A and B). While peak broadening in the ESI-MS chromatogram is apparent for the ubiquitin

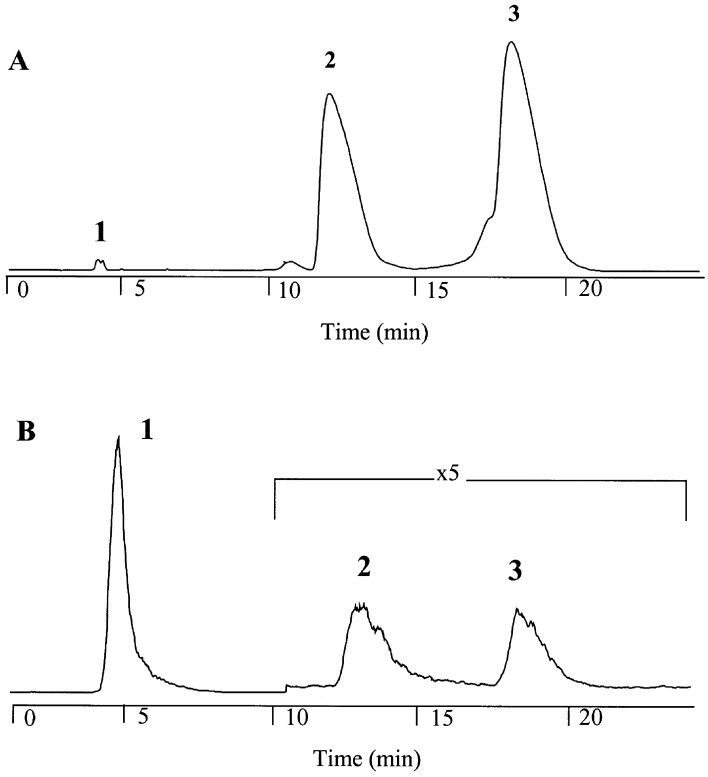

Fig. 7. Comparison of the UV chromatogram of the protein mixture after separation by cation-exchange chromatography with its corresponding ESI total ion current chromatogram after on-line microdialysis desalting. (A) UV chromatogram; (B) ESI total ion current chromatogram. The identities of the peaks are: 1, ubiquitin; 2, cytochrome *c*; and 3, lysozyme. The signal intensity between 11–25 min for cytochrome *c* and lysozyme has been amplified by 5×.

peak (peak 1), loss of resolution for the other two peaks (cytochrome *c* and lysozyme) was minimal. This confirmed our speculation that further peak broadening due to microdialysis does not adversely affect HPLC resolution for relatively broad peaks. More importantly, a very clean and informative spectrum was obtained from each peak, allowing their easy identification (Fig. 8). Complete desalting was achieved for all peaks and no Na^+ adduct peak was apparent in the spectra.

It is important to note that the LCQ ion trap mass spectrometer has a much lower sensitivity for proteins with a molecular mass over 10 000 than smaller proteins and peptides. This is reflected in the relative peak heights of the three samples in the chromatograms (Fig. 7A and B). In the UV chromatogram, the ubiquitin peak was very small compared with those of cytochrome *c* and lysozyme due to its lower concentration (0.2 mg/ml for ubiquitin vs. 4 mg/ml for cytochrome *c* and lysozyme). However, in the ESI-MS chromatogram, ubiquitin was the most intense peak, while both cytochrome *c* and lysozyme produced only a low intensity peak; consistent with the sensitivity dependence of LCQ on sample molecular mass. Therefore, both cytochrome *c* and lysozyme were prepared at such a high concentration (i.e. 4 mg/ml) to insure their detection by LCQ. There are some residual cytochrome *c* peaks present in the lysozyme spectrum (Fig. 8C). This might be due to the high concentration of cytochrome *c*, causing nonspecific sample adsorption to the fused-silica tubing or microdialysis fiber of the microdialysis assembly. These limitations can be easily overcome by the use of lower sample concentrations and other mass analyzers, e.g., quadrupole, time-of-flight, Sector or Fourier-transform ion cyclotron

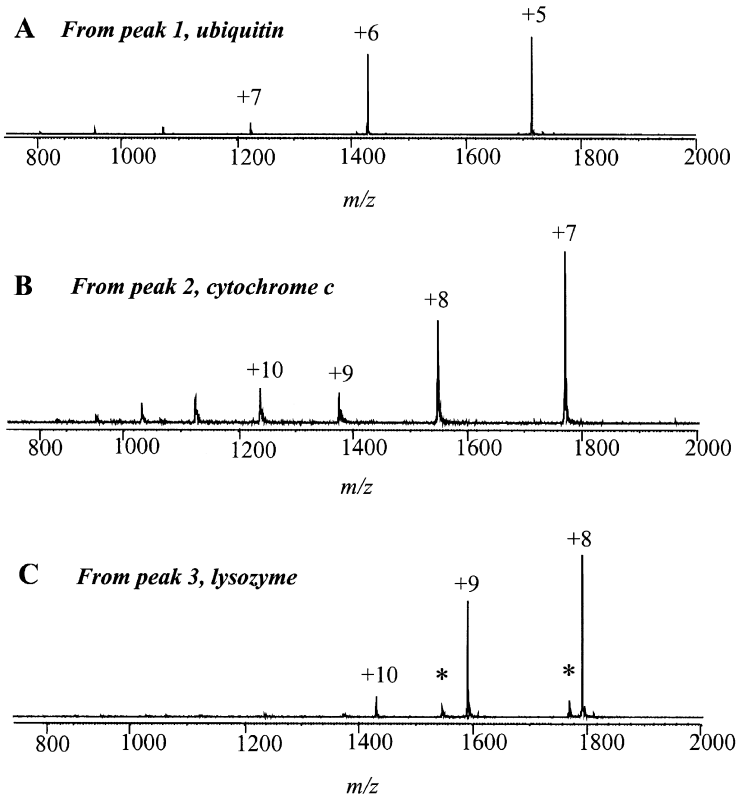

Fig. 8. The ESI mass spectrum of each of the three components of the mixture represented by the corresponding peaks from cation-exchange chromatography (Fig. 7) after on-line microdialysis. (A) Peak 1, ubiquitin; (B) Peak 2, cytochrome *c* and (C) Peak 3, lysozyme. Ions labeled ''*'' in (C) indicate contaminating cytochrome *c* ions.

resonance which have a much less degree of mass discrimination in detection.

In addition to using high protein concentrations, low dialysis buffer concentration (i.e. 2 m*M* NH_4OAc) was also used to further increase ESI-MS detection sensitivity. As demonstrated by Tang and Kebarle [32] and Liu et al. [28], a lower buffer concentration (<5 m*M* NH_4OAc) would result in higher sensitivity presumably due to a higher analyte ion density in the ESI charge droplets. Using 20 m*M* NH_4OAc as a dialysis buffer, no peaks arising from cytochrome *c* and lysozyme could be detected; while two distinct peaks (Fig. 7B) were observed when 2 m*M* NH_4OAc was used instead. The addition of a sheath liquid as mentioned above did increase the sample signal, however, noise level also increased and the signal-to-noise ratio did not improve. This is likely due to the mass discrimination of LCQ to high molecular mass proteins as well.

Although we used only peptides and proteins as samples, the current system should be generally applicable to other biomolecules, such as nucleic acids and carbohydrates, as suggested by off-line microdialysis studies [27,28]. The noncovalent association between buffer components and biomolecules differs considerably from species to species; therefore, the microdialysis flow-rate should be determined based on the nature of sample and salt concentration. Nucleic acids have a much higher affinity for cations than proteins and carbohydrates; a further compromise between desalting and resolution has to be established so that a lower sample flow-rate can be used to insure complete desalting. In addition, the current availability of microdialysis fiber limits the samples amenable to this technique. Although the nominal molecular mass cut-off of the dialysis fiber is 13 000, molecules of much lower molecular mass can still be retained due to the short residence time of the samples inside the dialysis fiber [27]. However, samples of molecular mass <1000 will likely have a lower recovery after microdialysis and might not be detectable by ESI-MS.

4. Conclusions

In this work, direct coupling of ionic HPLC with ESI-MS using a microdialysis interface is presented. A sample flow-rate of 10–20 μl/min was found optimal to both retaining reasonable resolution and insuring complete desalting. By performing the microdialysis at 50°C, the current configuration demonstrated sufficient efficiency for on-line desalting of the eluent from most ionic HPLC systems. The introduction of an organic sheath liquid containing a low percentage of volatile acids proved to enhance positive ESI-MS detection sensitivity by as much as tenfold for peptides and further increased the robustness and flexibility of the on-line microdialysis desalting system. Similar additives should be applicable for other biomolecules such as nucleic acids and negative ESI-MS. Complete on-line desalting of peptides and proteins was achieved in both flow injection analysis and cation-exchange chromatography, while sufficient HPLC resolution was also retained. The results presented here constitute the first real time on-line desalting technique that could be generally applicable to various ionic HPLC separations and a variety of biomolecules.

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