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Improving the Microdialysis Procedure for Electrospray Ionization Mass Spectrometry of Biological Samples

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Significant advances in the area of microdialysis which allowed more effective handling of small volumes (microliters) of samples, more efficient desalting and enhanced mass spectrometric detection sensitivity are described. The previously reported on-line coupling of microdialysis with electrospray ionization (ESI) mass spectrometry has been found to be highly effective; however, direct coupling requires relatively high sample flow rates ($\sim$2 µl min^{-1}) to obtain a stable ESI current compared with the flow rates of newer ESI sources (e.g. 'microspray,' 10–100 nl min^{-1}). To circumvent this major limitation imposed by the dimensions of currently available materials, the microdialysis procedure was modified to an off-line mode in order to avoid excessive sample consumption. A more than tenfold decrease in sample consumption was achieved using the off-line mode *vs* the on-line mode, which resulted in a similar quality spectrum. In addition, several other aspects of the microdialysis approach were altered to improve its performance further: (i) an increase in dialysis temperature was found to increase the desalting efficiency greatly and therefore improve the spectrum quality; (ii) the addition of piperidine and imidazole to the dialysis buffer solution resulted in a reduction of charge states and a further increase in detection sensitivity for DNA and (iii) use of low concentrations (0–2.5 mM NH_4OAc) of dialysis buffer shifted the DNA negative ions to higher charge states and produced a nearly tenfold increase in detection sensitivity and a slightly decreased desalting efficiency. Protocols for desalting different samples using microdialysis are discussed. © **1997 by John Wiley & Sons, Ltd.**

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INTRODUCTION

Electrospray ionization mass spectrometry (ESI-MS) has become an important technique for the analysis of biopolymers.[1–6] The multiple charging phenomenon allows fast, accurate and precise molecular mass measurement, identification of modifications and more detailed structural studies for very high-mass biopolymers using conventional (i.e. limited m/z) mass analyzers.[7–10] The mild ionization conditions in ESI also provide an approach for probing various noncovalent associations.[11–15] Nevertheless, sample matrix interference presents a major limitation on ESI-MS for analyzing biopolymers. Several groups have reported the interferences from the sample matrix during ESI-MS analyse of both proteins and DNA.[16–21] Because of the high affinity of the DNA phosphate backbone for sodium, a very low level of sodium ($\leq$1 mM) in a DNA sample can be problematic for obtaining reproducible mass spectra and accurate molecular mass measurements for small oligonucleotides and even much lower levels of sodium can preclude the analysis of larger oligonucleotides.

Several different approaches have been explored to reduce matrix effects and improve spectrum quality in ESI-MS in addition to conventional dialysis and chromatographic techniques. A general approach is multiple buffer exchanges using membrane filtration cartridges for sample clean-up of both proteins and DNA.[16] Emmet and Caprioli[17] reported the use of an on-line C_{18} cartridge for protein sample clean-up before ESI-MS analysis. Since cation adduction is ubiquitous in ESI-MS of DNA samples, efforts have focused on the removal of salt contamination in DNA samples. Stults and Marsters[18] reported a precipitation method using concentrated ammonium acetate to remove sodium adduction for oligonucleotides of up to 77-mer size, reducing sodium attachment to a single adduct. Nordhoff *et al.*[19] used a cation-exchange resin to trap cations in a DNA sample and significantly improved the matrix-assisted laser desorption/ionization (MALDI) mass spectrum quality. This method should be applicable for DNA sample clean-up for ESI-MS. Greig and Griffey[20] and Muddiman *et al.*[21] used organic bases (e.g. piperidine and imidazole) to suppress sodium adduction and reduce charge states for sensitivity enhancement.

Recently, we reported the use of an on-line microdialysis approach for sample clean-up for the ESI-MS of proteins and DNA, and demonstrated its broad applicability in biopolymer analysis.[22,23] Compared with existing sample clean-up techniques, microdialysis provides faster and more efficient matrix removal for both proteins and DNA. The purpose of this work was

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to demonstrate further the superiority of microdialysis over existing desalting techniques for rapidly desalting small volumes of biological samples, and to investigate the advantages of off-line microdialysis over the on-line mode in reducing sample consumption and increasing flexibility. The effects of dialysis temperature, composition of dialysis buffer and concentration of dialysis buffer were also investigated in order to increase the desalting efficiency and detection sensitivity further.

EXPERIMENTAL

Materials

Polydeoxynucleotide $d(pT)_{18}$ was purchased from Sigma (St Louis, MO, USA). A sample containing two complimentary single-stranded 17-mer oligonucleotides (strand A, 5′-TGAAAGAGGAACTTGGT-3′; strand B, 3′-ACTTTCTCCTTGAACCA-5′) was a gift from Dr Paul Morin (University of Ontario and Ontario Cancer Institute, Toronto, ON, Canada). All other reagents were of analytical grade or better from Sigma and were used as received.

Methods

Microdialysis system. The on-line microdialysis system was the same as described previously.[22,23] Briefly, a regenerated cellulose hollow fiber was used as a dialysis tube. A counter-current dialysis buffer flow, relative to the sample flow inside the dialysis tube, was continuously introduced through the annular space between the dialysis tube (200 μm i.d., 216 μm o.d.) and a larger (1 mm i.d.) concentric Teflon tube sheathing the dialysis tube. The sample was injected into the dialysis tube by a syringe pump and the dialyzed sample was directly electrosprayed through a micro-electrospray source (50 μm i.d. fused-silica capillary) coupled to the other end of the dialysis tube. For off-line microdialysis, the micro-electrospray source was disconnected from the dialysis tube and replaced with a microcentrifuge tube for sample collection. The sample flow rate inside the microdialysis tube (for both on-line and off-line modes) was 3 μl min^{-1} and the dialysis buffer flow rate was 500 μl min^{-1}. In more recent experiments, the dialysis buffer was introduced solely by gravity (i.e. vertical displacement of the buffer reservoir over the buffer outlet by ~20 cm). Although the buffer flow rate might change slightly during the dialysis process, the dialysis efficiency was not affected, as demonstrated earlier.[22] This eliminates the requirement for a solvent pump and further increases the flexibility of the microdialysis technique. For microdialysis at 50 °C (the temperature of the water-bath), the microdialysis assembly was put inside a stirred 1.7 L water-bath, the temperature of which was controlled by an external stirrer heating device controlling temperature to ±0.5 °C. The water-bath was stirred to ensure a homogeneous temperature inside.

Sample preparation. The following samples were prepared: (i) 10 μM apomyoglobin in 10 mM NH_4OAc and 1.5 M NaCl; (ii) 3 μM 17-mer oligonucleotides in 10 mM NH_4OAc and 150 mM NaCl; and (iii) 60 μM $d(pT)_{18}$ in 10 mM NH_4OAc and 100 mM NaCl. The oligonucleotide samples containing organic bases were prepared using the above oligonucleotide samples and stock solutions of 100 mM piperidine and 100 mM imidazole with the final concentrations indicated in the text.

For multiple buffer exchange experiments, 50 μl of 60 μM $d(pT)_{18}$ in 10 mM NH_4OAc and 100 mM NaCl were diluted with 10 mM NH_4OAc to 500 μl in a Centricon-3 membrane cartridge (Amicon, Beverly, MA, USA) and the final solution was centrifuged at 7000 rpm (4000 rcf units) for 45 min. The retentate was further diluted with the same buffer and centrifuged. This process was repeated four more times and the final retentate (~50 μl) was collected for MS analysis.

Mass spectrometry

A Finnigan (San Jose, CA, USA) TSQ 7000 triple quadrupole mass spectrometer was used for all MS analyses. The microspray-ESI source was constructed from 50 μM i.d. fused-silica tubing using the procedure described by Gale and Smith.[24] A typical electrospray ionization voltage was −2.0 to −2.3 kV. The inlet capillary temperature was 120 °C for the 17-mer oligonucleotides and 160 °C for all the other samples. A coaxial SF_6 gas flow around the ESI emitter was used to suppress corona discharge. For direct infusion experiments, the sample flow rate was 0.3 μl min^{-1}, and for on-line microdialysis with ESI-MS, 3 μl min^{-1} was used. The spectra were obtained by signal averaging for 2 min at a scan rate of 3 s per scan. The m/z scan ranges are indicated in the Results and Discussion section.

RESULTS AND DISCUSSION

Comparison of the microdialysis approach with other desalting methods

Removal of matrix (e.g. high concentrations of salts) in biological samples has been a mandatory but difficult process for ESI-MS, particularly for DNA where even very low levels of sodium are problematic. One common approach is the multiple buffer exchange method using a membrane cartridge.[16] The salt-containing sample is repeatedly diluted with ammonium acetate buffer and concentrated by centrifugation, resulting in a decreased salt concentration. This process, although simple in nature, typically requires several hours and significant sample losses often occur; this greatly limits its application when only limited sample is available. The desalting efficiency of this technique is very low for DNA samples owing to the high affinity of DNA molecules for sodium, and significant cation adduct ions are still present even after more than five cycles of buffer exchange. In addition to multiple buffer exchange, the recently introduced organic base (typically 25 mM piperidine and 25 mM imidazole) addition method has been used to reduce the cation adduction in ESI-MS of oligonucleotide samples,[20,21] but the salt concentration tolerance of this method is relatively low (<10 mM). Further compensating for high salt concentration by addition of higher concentrations of

organic bases (>50 mM) resulted in a greatly decreased detection sensitivity. Another common practice for desalting is the ammonium acetate precipitation method.[18] This method requires a relatively large amount of sample and can result in significant sample loss, especially for smaller oligonucleotides. It has also been shown to be less effective for desalting of larger (≥60-mer in size) DNA or RNA molecules.[18]

Compared with these methods, the microdialysis approach has been demonstrated to be a faster and more efficient desalting technique.[22,23] In order to demonstrate further the superiority of a microdialysis over the other desalting techniques, a sample containing 60 μM $d(pT)_{18}$ in 10 mM NH_4OAc and 100 mM NaCl was desalted by three different techniques (multiple buffer exchange, organic base addition and on-line microdialysis) followed by ESI-MS analysis. A dramatic difference in desalting efficiency and subsequent mass spectrum quality was observed. Figure 1(A) shows the mass spectrum of 60 μM $d(pT)_{18}$ in 10 mM NH_4OAc and 100 mM NaCl after five cycles of buffer exchange (~4 h) against 10 mM NH_4OAc. No analytically useful signal was observed, which could be attributed to the combining effects of sample loss and incomplete desalting. When piperidine and imidazole (25 mM each) were added to the original sample and the sample was analyzed by ESI-MS, a greatly suppressed background signal and some minor peaks were observed [Fig. 1(B)], but the detection sensitivity and the signal-to-noise ratio (S/N) were low and an accurate mass measurement was not obtainable. Note that after on-line microdialysis of the same original sample, sodium adducts were completely removed from the mass spectrum and peaks corresponding to different charge states were observed in high abundance [Fig. 1(C)]. An accurate molecular mass measurement was readily obtained, allowing further structural studies to be conducted.

From the data presented in Fig. 1 and other significant experiments (data not shown), the on-line microdialysis approach is demonstrated to offer several distinct advantages. First, the total analysis time for obtaining a good-quality spectrum in on-line microdialysis is ~5 min. Thus, the desalting speed of microdialysis is comparable to the organic base addition method and over an order of magnitude faster than multiple buffer exchange and precipitation techniques. Second, the salt concentration tolerance in on-line microdialysis (1.3 M NaCl for proteins and 0.5 M NaCl for oligonucleotides using the current microdialysis design) is at least an order of magnitude higher than that in the other methods. For multiple buffer exchanges, a higher salt concentration may be compensated by additional dilution and concentration cycles, but a longer desalting time and more sample losses are unavoidable. Third, the sample volume requirement for on-line microdialysis (~15–20 μl) is comparable to that of the organic base addition method, but much smaller than that for multiple buffer exchange and precipitation methods.

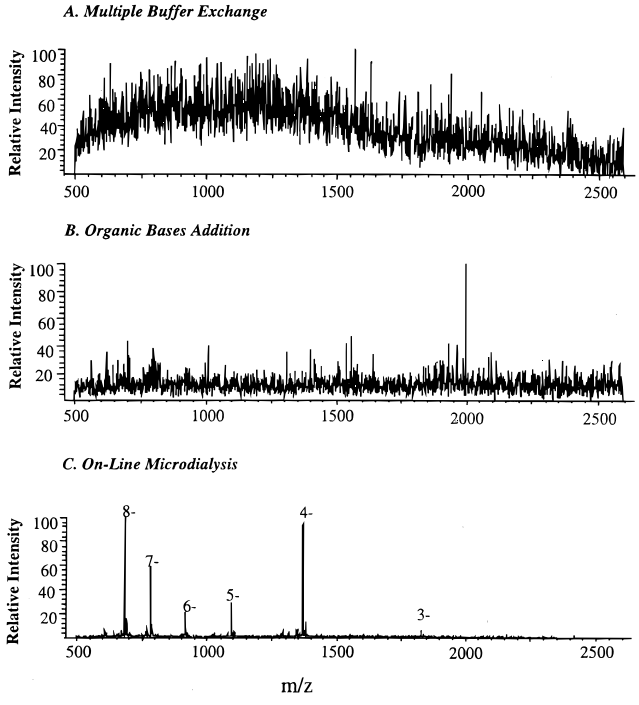

Figure 1. Comparison of desalting efficiencies of three oligonucleotide desalting techniques using a sample containing 60 μM $d(pT)_{18}$ in 10 mM NH_4OAc and 100 mM NaCl. The mass spectra were obtained (A) after five cycles of buffer exchange against 10 mM NH_4OAc using a Centricon-3 membrane cartridge, (B) after addition of piperidine and imidazole to the sample (25 mM final concentration of each base) and (C) after on-line microdialysis against 10 mM NH_4OAc.

Off-line microdialysis followed by microspray ESI-MS can reduce sample consumption and increase microdialysis flexibility

Although on-line microdialysis has been shown to be rapid and effective, a major limitation is the relatively high sample flow rate requirement. A minimum sample flow rate of ~2 μl min^{-1} was required to obtain a stable ESI current owing to the solvent diversion across the dialysis membrane, as discussed previously.[22] This flow rate is about 10 times and 100 times higher than that used for microspray (0.1–0.3 μl min^{-1}) and 'nanospray' (20–40 nl min^{-1}), respectively.[24,25]

In order to circumvent this limitation, we modified the on-line system to off-line sample clean-up by replacing the micro-electrosprayer with a microcentrifuge tube for sample collection. This design preserves the fast and efficient desalting offered by microdialysis, in addition to allowing the analysis of the dialyzed sample by micro-electrospray or nano-electrospray, greatly reducing the sample consumption. Figure 2 compares the mass spectra of the same $d(pT)_{18}$ sample as shown in Fig. 1 after on-line and off-line microdialysis. As expected, no significant difference was found between the two spectra. Note that, however, the sample consumed in Fig. 2(A) was 360 pmol, whereas that in Fig. 2(B) was only 36 pmol. Based on the signal intensities in Fig. 2, it can be concluded that a 14-fold decrease in sample consumption was achieved by using off-line microdialysis followed by microspray ESI-MS analysis for obtaining a similar quality spectrum. It is important to note that we did not attempt to determine detection limits in this comparison, and it is obvious that useful spectra are obtainable from much smaller sample sizes than used here.

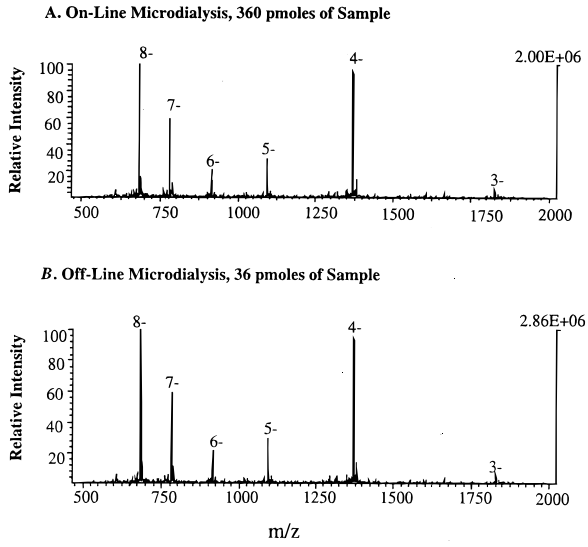

Figure 2. Comparison of on-line *vs*. off-line microdialysis with respect to sample consumption. The mass spectrum of 60 μM $d(pT)_{18}$ in 10 mM NH_4OAc and 100 mM NaCl was obtained (A) after on-line microdialysis against 10 mM NH_4OAc (the sample consumed in obtaining this spectrum was 360 pmol) and (B) from direct infusion micro-electrospray after off-line microdialysis against 10 mM NH_4OAc (the total sample consumed in obtaining this spectrum was 36 pmol).

Another advantage of off-line scheme, as we have experienced in recent studies, is that much lower sample flow rates (0.3–1.0 μl min^{-1}) can be used if a longer dialysis time is needed for desalting of samples with complicated matrices (a typical example of such a matrix contains 0.1 M Tris–HCl, 0.5 M NaCl, 10% glycerol and 1–2 mM dithiothreitol and other preservatives often added to biological samples). Although lower sample flow rates did not generate a stable ESI current in the on-line mode with our present ESI source, samples can be cleaned up using the dialysis system. Indeed, nearly quantitative recoveries were achieved when 10 μl of 30 μM apomyoglobin was dialyzed at a sample flow rate of 0.5 μl min^{-1} (using a BCA protein assay method). Currently, ESI-MS analysis of ~10 μl of protein or DNA sample in a complicated matrix resorting to off-line microdialysis desalting has been routinely successful in our laboratory. Research involving small sample volumes had been extremely difficult, if not impossible, before the introduction of microdialysis. We anticipate that, complementary to the on-line microdialysis scheme, the flexibility of the off-line approach will further extend the applicability of microdialysis in ESI-MS and other related studies involving biopolymers.

Higher temperature greatly increases the dialysis efficiency and improves the spectrum quality

Since dialysis is a diffusion-controlled process, an increase in dialysis temperature is expected to increase further the diffusion rates of salt components (relative to large biopolymers), resulting in a higher dialysis efficiency. An additional advantage of higher temperatures is that small ions more closely associated with biopolymers in solution (i.e. 'condensed' counter ions) will also be less strongly associated, allowing more rapid exchange and removal. Figure 3 compares the mass spectra of 10 μM apomyoglobin in 1.5 M NaCl after on-line microdialysis at (A) room temperature and (B) 50 °C. At room temperature, even with the efficient desalting provided by microdialysis, the spectrum showed only a few charge states with low sensitivity, and significant sodium adduction was evident [Fig. 3(a)]. When the microdialysis was performed at 50 °C, the spectrum quality was greatly improved [Fig. 3(B)]. Multiple (and higher) charge states were observed with high abundance and sodium adduction was minimal [Fig. 3(B)]. An accurate molecular mass was determined despite the presence of some residual sodium adduction, with at least a five fold increase in S/N. When the highest tolerable NaCl concentration (which we arbitrarily define as that producing a spectrum with the most intense peaks arise from intact molecular ions) was used to assess the dialysis efficiency, this concentration was increased from 1.3 M at room temperature to 2 M at 50 °C. When the NaCl concentration was further increased to 2.67 M, a similar spectrum to that in Fig. 3(B) was obtained with the major molecular ions corresponding to intact molecule with only one sodium attached (data not shown), and an accurate molecular mass was still readily determined. This indicated that a higher dialysis temperature should be advantageous for addressing more complex and impure samples provided that the biopolymers are stable (e.g. do not aggregate) at the elevated temperature. (Note that the suggested maximum operating temperature for the microdialysis fiber is 60 °C.)

Addition of organic bases in dialysis buffer reduces charge states and further increases detection sensitivity for oligonucleotide samples

A large number of inorganic or organic additives (or modifiers) have been widely used in ESI-MS for sensitivity enhancement. While essentially an infinite number

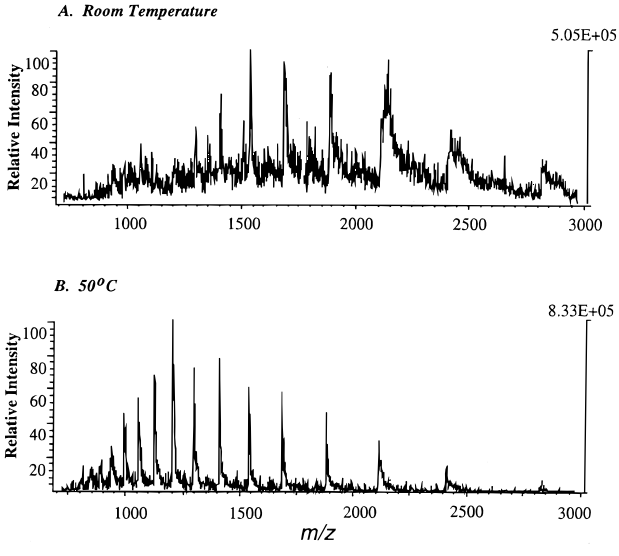

Figure 3. Effects of dialysis temperature on microdialysis efficiency and spectrum quality. The mass spectrum of 10 μM apomyoglobin in 10 mM NH_4OAc and 1.5 M NaCl was obtained after on-line microdialysis (A) at room temperature and (B) at 50 °C. Note the evident reduction in sodium adduction and improvement in spectrum quality.

of composition and concentration of additives could be investigated, several have been shown to be effective in most cases studied. In particular, Greig and Griffey[20] and Muddiman *et al.*[21] reported that the addition of piperidine and imidazole to DNA samples significantly suppressed sodium adduct ions and improve S/N. Muddiman *et al.*[21] also studied the charge state reduction of oligonucleotide negative ions by imidazole for sensitivity enhancement. As a model system to investigate the potential advantages of combining additives with microdialysis, piperidine and imidazole were added to the dialysis buffer (i.e. 10 mM NH_4OAc and 25 mM of each base) for DNA sample desalting. A sample consisting of two complimentary 17-mer oligonucleotides (see Materials for sequences) were analyzed by on-line microdialysis. A low inlet capillary temperature (120 °C) was used to facilitate the detection of double-stranded (duplex) oligonucleotides. [Figure 4(A)] shows the mass spectrum obtained using 10 mM NH_4OAc as the dialysis buffer. Charge states corresponding to both the individual strands and the duplex form were observed. Within the S/N limit of these data, no sodium adduction was apparent. After addition of piperidine and imidazole to the dialysis buffer, a much simpler spectrum was obtained [Fig. 4(B)]. Interestingly, only one charge state was observed for both the individual strands and the duplex form along with a shift to higher m/z (lower charging). A five-fold increase in S/N compared with the signal in Fig. 4(A) was also achieved. These results demonstrated a further increase in sensitivity due to the addition of piperidine and imidazole to the dialysis buffer. It is expected, therefore, that other additives can also be incorporated in the dialysis buffer to improve ESI-MS analysis for specific analytes.

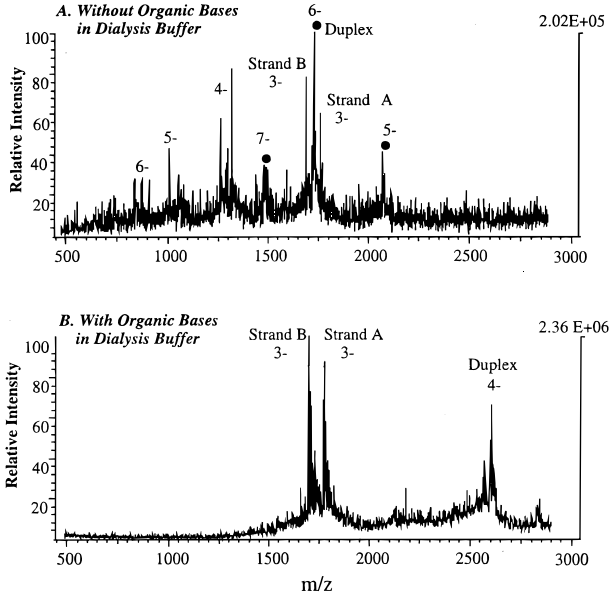

Figure 4. Effects of incorporation of piperidine and imidazole in the dialysis buffer on DNA negative ions. The mass spectrum of 17-mer oligonucleotides in 10 mM NH_4OAc and 150 mM NaCl was obtained after on-line microdialysis (A) using 10 mM NH_4OAc as dialysis buffer (peaks marked with dots arise from double-stranded DNA and (B) using 10 mM NH_4OAc, 25 mM piperidine and 25 mM imidazole as the dialysis buffer. Note the charge state reduction and sensitivity enhancement.

It can be seen from Fig. 4(B) that significant sodium adduction was observed after the addition of piperidine and imidazole. One possible reason for the difference in sodium adduction between Fig. 4(A) and (B) is that before the addition of piperidine and imidazole, the S/N was low, and therefore the sodium adduct peaks are less evident. After the addition of piperidine and imidazole, the S/N was improved by more than five-fold and sodium adduct peaks could be readily observed. Alternatively, this observation might also be explained by a decrease in desalting efficiency of microdialysis due to the association of sodium with piperidine and imidazole. When sodium is associated with either piperidine or imidazole, its diffusion across the membrane will be slowed, resulting in a relatively lower desalting efficiency. To avoid this problem, we suggest adding piperidine and imidazole after the microdialysis step. Similarly, if any other additives interfere with the microdialysis procedure, they can be added after microdialysis in order both to maintain the desalting efficiency of microdialysis and to incorporate the sensitivity enhancement capability of these additives.

Use of low-concentration (0–2.5 mM NH_4OAc) dialysis buffer shifts the DNA negative ions to higher charge states and results in a tenfold increased in detection sensitivity and a slightly decreased desalting efficiency

Previous experience has indicated that DNA samples, when dissolved in <5 mM NH_4OAc and analyzed by ESI-MS, produced molecular negative ions with higher charge states and more sodium adduction than samples dissolved in >10 mM NH_4OAc. Since the sodium adduction arose from the salt contamination, the use of microdialysis is expected to eliminate or reduce sodium adduction significantly. The shift of molecular ions to higher charge states suggests that low concentrations of buffer should favor DNA analysis: the lower m/z values will extend the size of DNA molecules amenable to MS, and an increase in sensitivity is expected owing to the more efficient detection of mass spectrometers for ions at lower m/z values. While earlier studies of oligonucleotides were hindered by the extensive sodium adduction, we sought to explore the advantages of low buffer concentrations by the use of microdialysis. A sample containing 60 μM $d(pT)_{18}$ in 10 mM NH_4OAc and 100 mM NaCl was dialyzed against different concentrations of buffer and analyzed on-line by ESI-MS. Figure 5 compares the mass spectra of this sample after on-line microdialysis using three different dialysis buffers: H_2O, 2.5 mM NH_4OAc and 10 mM NH_4OAc. When pure H_2O was used as the dialysis buffer, desalting was incomplete, as indicated by the sodium adduct peaks [Fig. 5(A)]. The main charge states were 7−, 8− and 9−. When the dialysis buffer was changed to 2.5 mM NH_4OAc, greatly improved desalting was achieved with negligible sodium adduction. The observed charge states shifted slightly towards lower values and the sensitivity was nearly unchanged [Fig. 5(B)]. A further increase in dialysis buffer concentration to 10 mM more effectively removed the residual sodium adducts [Fig. 5(C)], but a nearly tenfold decrease in sensitivity compared with using 2.5 mM NH_4OAc as dialysis buffer was

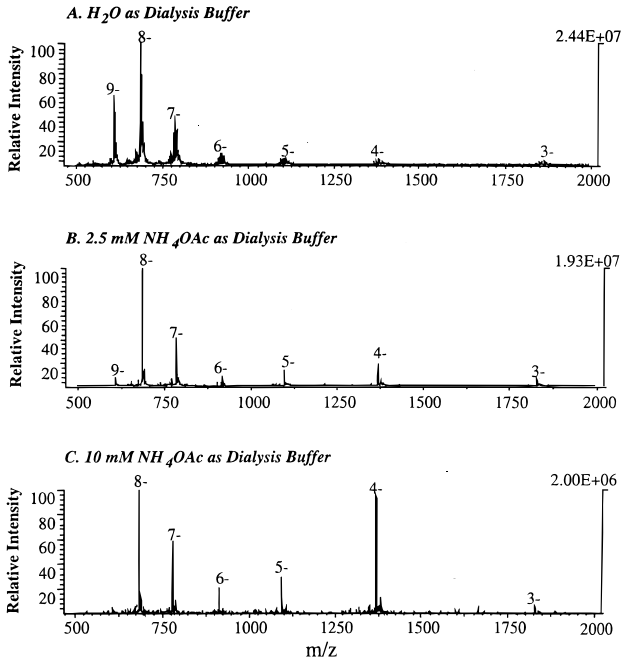

Figure 5. Effect of dialysis buffer concentration on DNA negative ions. The mass spectrum of 60 μM $d(pT)_{18}$ in 10 mM NH_4OAc and 100 mM NaCl was obtained after on-line microdialysis using (A) H_2O, (B) 2.5 mM NH_4OAc and (C) 10 mM NH_4OAc as the dialysis buffer. Note the charge state shift, detection sensitivity enhancement and desalting efficiency change; see text for further explanation.

observed. The charge states shifted significantly to lower values, and the decreased sensitivity is probably associated with the decreased transmission efficiency of the mass spectrometer at higher m/z.

It is apparent that despite the high desalting efficiency of microdialysis, the strongly associated sodium ions are not rapidly removed by dialyzing against pure H_2O. Cation exchange with NH_4^+ at relatively higher buffer concentrations is required for effective sodium removal. The ion pairing between sodium ion and the phosphate group in the oligonucleotide might account for the charge state shift. At high salt concentrations, a greater number of phosphate groups are ion paired by sodium ions in solution and upon transfer into the gas phase. When the salt concentration decreases, more phosphate groups will be left 'unprotected' and become charge-carrying sites. Therefore, higher charge states are expected for low buffer concentrations. The sensitivity enhancement at lower buffer concentrations could be partially due to the more efficient MS detection for lower m/z ions, as mentioned earlier. Another reason may be attributed to the smaller number of charge-carrying buffer species in solution when the buffer concentration is low, and consequently an increase in transfer efficiency of sample ion from solution to the gas phase.[26,27] These results suggest that low dialysis buffer concentrations (~2.5 mM NH_4OAc) should be used in microdialysis of DNA samples for studies where higher buffer concentrations are not required, such as for single-stranded DNA.

Based on these experiments, we found that off-line microdialysis using 2.5 mM NH_4OAc as dialysis buffer followed by the addition of piperidine and imidazole (25 mM final concentration of each base) provided the best combination thus far for the extremely challenging DNA sample clean-up problem for ESI-MS. The application of this technique for PCR products clean-up has also been demonstrated elsewhere.[28] While the purpose of using low buffer concentrations was to increase sensitivity and extend oligonucleotide size amenable to MS, we observed the 'melting' (i.e. loss) of duplex conformation in solution during microdialysis of PCR products using 2.5 mM NH_4OAc as dialysis buffer. This observation was qualitatively consistent with the correlation of DNA melting with solution salt concentrations.[29] Since the salt concentration in the DNA samples after microdialysis was only 2.5 mM NH_4OAc, the PCR products we studied should have melting temperatures lower than room temperature after microdialysis. The advantages of using microdialysis to denature double-stranded DNA in solution and analyzing single-stranded DNA are discussed elsewhere.[28] When the double-stranded DNA conformation is essential (e.g. in studies of non-covalent complexes with duplex DNA), higher dialysis buffer concentrations (>10 mM NH_4OAc) should be used.

CONCLUSIONS

We have described new aspects of the use of microdialysis for the ESI-MS of biological samples. The microdialysis approach was further demonstrated to be superior to existing desalting techniques in desalting speed, desalting efficiency and minimal sample volume requirement. Off-line microdialysis was shown to reduce sample consumption more than tenfold *vs.* the on-line mode for producing a similar quality spectrum, given the present flow rate considerations with our ESI source. The sample flow rate in the off-line mode can be decreased to allow a longer dialysis time for desalting of samples with complicated matrices, without the flow rate limitations encountered in the on-line mode. Elevated temperature was also shown to increase the dialysis efficiency significantly and improve the spectrum quality. Addition of piperidine and imidazole to the dialysis buffer reduced DNA negative ion charge states and further enhanced the detection sensitivity. The use of low (0–2.5 mM NH_4OAc) concentrations of dialysis buffer shifted the DNA negative ions to higher charge states, resulting in a tenfold increase in sensitivity and a slightly decreased desalting efficiency. Microdialysis protocols for different samples have been discussed. These improvements serve to extend the applicability of microdialysis in biopolymer analysis using ESI-MS and other methods where rapid and effective desalting is essential.

Acknowledgements

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