Electrophoretic Profiling of Both RNA and Protein from a Single 250-pL Sample

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A novel approach is described that uses capillary electrophoresis (CE) to electrophoretically sample and separate both protein and RNA from a single injected plug of cell lysate. A 250-pL sample of lysate from Chinese hamster ovary cells (9.6×10^7 cells/mL) was hydrodynamically injected into a capillary containing a Tris-based aqueous buffer. This was followed by selective electrokinetic ejection of RNA from the lysate into water, yielding an effective cell concentration of RNA of 3000 cells/mL. The cellular components (e.g., proteins) retained in the capillary were separated and then detected with laser-induced fluorescence (LIF) using 275-nm excitation. The ejected/diluted sample was subsequently injected into a separate CE-LIF system, which utilized an entangled polymer sieving matrix and 543-nm excitation for the detection of ethidium bromide-labeled nucleic acids (i.e., RNA). Virtually no sample preparation is required other than simple washing and lysing of the cells isolated from culture. This combined approach can be easily modified for the detection of any analyte through adjustment of CE-LIF conditions. In addition, it provides an effective method for desalting cellular RNA samples having complex matrixes, which results in improved RNA injection efficiency and a 7600-fold effective signal enhancement over total lysate injection.

In the emerging field of proteomics, the ability to detect different classes of biomolecules, such as proteins and nucleic acids, in the same sample is of mounting interest. Quite often, such information might be beneficial in the diagnosis or prognosis of certain diseases or in the understanding of cellular function. Traditional methods of analysis can be performed using two separate platforms to yield the desired information from bulk biological samples. However, for typical applications, it is rare to have an unlimited amount of sample. Most of the time, only a small quantity is available, which necessitates a method for analysis that is not only sensitive but also practical for the use of ultralow volumes.

Although almost any analytical method can be used for independent measurements of proteins and nucleic acids, sensitive, simultaneous detection of these species is far more challenging, especially when sample volume is limited. There has been only one report, in which a mixture of standard proteins and isolated RNA was simultaneously analyzed using slab-gel electrophoresis.[1] However, this study still required a fairly large amount of sample. Approaches such as imaging methods, flow cytometry, and spectroscopy typically have had the most success overcoming the challenge of limited sample. For example, Chiu et al. used imaging with a radioactive label followed by a colorimetric probe to examine acetylcholine receptor (AChR) and the associated mRNA in cultured chick myoblasts.[2] Rowland, also using radioactivity measurements, monitored the incorporation of glucose-6-^{3}H in mouse tumor and reticuloendothelial tissue slices to assess RNA, DNA, and protein synthesis.[3] Olenev described a method for the simultaneous determination of DNA and proteins in fixed cells through the measurement of absorbance profiles following histochemical reactions with two different chromophores.[4] Similarly, Haag et al. accomplished differential tagging of DNA, RNA, and protein in histological sections with the use of a single carbocyanine dye.[5]

Flow cytometry, in conjunction with fluorescence labeling techniques, has been a predominate player in concurrent assessment of proteins and nucleic acids from cells. Several groups have performed simultaneous analysis of individual proteins (using FITC conjugates) and DNA (using propidium iodide); representative references are cited.[6,7] Crissman et al. reported differential staining of total DNA, RNA, and protein in cells using Hoechst 33342, pyronin Y, and FITC, respectively.[8] Triple-fluorescence flow cytometry was used by Corver et al. to simultaneously assess the expression of labeled surface antigens, intermediate filament antigens, and DNA in ovarian cancer cells.[9] Laser scanning cytometry, a slide-based method similar to flow cytometry, has been used by Pollice et al. for performing multiple measurements on cell samples.[10]

Other approaches for simultaneous measurement of protein and DNA from complex biological samples include IR and Raman

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spectroscopy. Liu et al. used IR spectroscopy for the quantitation of lipid, DNA, and protein from apoptotic cells.[11] Chiriboga et al. also used IR to analyze liver cells and tissue by utilizing selective enzymatic treatment with RNase or DNase to obtain spectra of DNA or RNA, respectively, with protein.[12] Resonance Raman spectroscopy has been used to measure proteins (via Trp and Tyr), DNA, and dUMP from the same sample.[13] A promising spectroscopic technology on the horizon is that of quantum dots, as reported recently for multicolor labeling of DNA oligomers.[14]

There have been volumes of literature describing the independent separations of proteins and nucleic acids using capillary electrophoresis (CE). However, examples of separating these biomolecules, from a single injected sample plug, are scarce. Krylov et al. reported a similar type of analysis, in which total DNA fluorescence was imaged from single cells and the recorded intensity used as a selection method for subsequent CE analysis of saccharide metabolites.[15] Since CE was not used for the separation of DNA, a nucleic acid profile was not obtained. Here we present a novel method based on CE, in which separations of both protein and nucleic acid cellular components are obtained from a single 250-pL injected sample.

EXPERIMENTAL SECTION

Chemicals and Reagents. Tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl), tris(hydroxymethyl)aminomethane (Tris), sodium dodecyl sulfate (SDS), and mannitol were from Sigma (St. Louis, MO). Ethylenediaminetetraacetic acid disodium salt (EDTA) and 0.25% trypsin−EDTA were from Life Technologies (Gaithersburg, MD). Poly(vinylpyrrollidone) (PVP), molecular weight 1 000 000, was obtained from Polysciences Inc. (Warrington, PA). Hydroxypropylmethylcellulose (HPMC), having a molecular weight of 10 000, was purchased from Aldrich (Milwaukee, WI). Ethidium bromide was from Molecular Probes (Eugene, OR). ΦX174/*Hae*III DNA, consisting of 11 double-stranded fragments (72, 118, 194, 234, 271, 281, 310, 603, 872, 1078, and 1353 bp), was obtained from Promega (Madison, WI). Deionized water (resistance, >18 MΩ) was obtained from a Milli-Q System (Bedford, MA). All other chemicals were purchased from Fisher Scientific (Fairlawn, NJ).

Cell Preparation. The Chinese hamster ovary (CHO-K1) cell line, obtained from American Type Culture Collection (ATCC, Manassas, VA), was used to prepare the cell lysate. The cells were cultured in F-12K medium (ATCC), containing 2 mM L-glutamine and 1.5 g/L sodium bicarbonate. The medium was supplemented with 10% fetal bovine serum (Fisher), 0.18 μg/mL streptomycin, and 0.18 IU/mL penicillin (Life Technologies). The cells were grown in an incubator, which maintained a humid atmosphere at 37 °C and 5% CO_2.

Lysate Preparation. After the cell medium was removed from the flask, 4 mL of trypsin−EDTA was added and the cells were placed in the incubator for 5 min. The trypsin was then removed by centrifugation at 1000*g* for 2 min. The resulting cell pellet was washed four times with 1-mL aliquots of phosphate-buffered saline

(PBS, containing 9 g/L sodium chloride, 5 mM D-glucose, 1 mM sodium phosphate monobasic, and 3 mM sodium phosphate dibasic; pH was adjusted to 7.4 with 2 M sodium hydroxide). After the final wash, the cells were suspended in 1 mL of PBS and counted using a Brightline hemacytometer. A 400-μL aliquot of the cell suspension was centrifuged for 2 min at 1000*g* to remove the PBS. 50 μL of 1% (w/v) SDS in water was added to the cell pellet, and the resulting lysate was allowed to sit at room temperature for 30 min to ensure complete lysis.

Capillary Electrophoresis-Laser-Induced Fluorescence (CE-LIF). The experiments were performed on two laboratory-built CE-LIF systems. One setup utilized 275-nm excitation for the detection of proteins, and the other utilized 543-nm excitation for the detection of nucleic acids. A DT 2804 data board was used to digitize the data. Electropherograms were generated using ChromPerfect software (Justice Innovations, Mountain View, CA), converted to ASCII, and replotted using Excel.

Protein Detection. The CE-LIF system used for the detection of cellular proteins has been previously described.[16] Separations were performed using 40-cm bare fused-silica capillaries (23-μm i.d., 360-μm o.d.; Polymicro Technologies, Phoenix, AZ). A detection window was created 25 cm from the inlet. The separation buffer consisted of 5 mM Tris-HCl and 0.2 mM $CaCl_2$, adjusted to pH 8.0 using 2 M NaOH. To drive the electrophoresis, a high-voltage power supply (Glassman High Voltage, Whitehouse Station, NJ) was used to apply a potential of +9.9 kV to the inlet; the outlet was grounded. All samples were hydrodynamically injected by raising the inlet 10 cm relative to the outlet for 15 s (injection volume, ~250 pL). The 275-nm line of an argon ion laser (Coherent Innova Sabre TSM, Santa Clara, CA), isolated with a fused-silica prism, provided an excitation source. The laser was focused on the detection window using a fused-silica lens (1-cm focal length). Fluorescence was collected and passed through a spatial filter and a UG-1 filter (Schott Scientific Glass, Parkersburg, WV) and was focused onto a photomultiplier tube (931B; Hamamatsu, Bridgewater, NJ).

Nucleic Acid Detection. The CE-LIF system used for the detection of RNA has been described previously.[17,18] Briefly, 40-cm (25-cm effective length) fused-silica capillaries (50-μm i.d., 360-μm o.d.) from Polymicro were used for separations. The sieving matrix used for the separation of RNA has been described previously[17] and consisted of 1% HPMC, 0.5% PVP, and 6% mannitol dissolved in TBE buffer (100 mM Tris−100 mM boric acid−2 mM EDTA, pH 8.3) and supplemented with 0.5 μg/mL ethidium bromide. A potential of +7.8 kV was applied to the outlet to drive the electrophoresis; the inlet was grounded. All nucleic acid samples were electrokinetically injected for either 2 or 5 s at 7.8 kV. A 543-nm green helium−neon laser (4 mW; JDS Uniphase Inc., Manteca, CA) provided the excitation source. The laser was focused on the detection window using a 1-cm focal length lens. Fluorescence was collected through a 10× microscope objective (Melles Griot, Irvine, CA), passed through a spatial filter and an RG-610 filter (Schott) and focused onto the photomultiplier tube (R928; Hamamatsu).

Sampling Protocol. A flowchart illustrating the protein/nucleic acid sampling protocol is shown in Figure 1. A plug of

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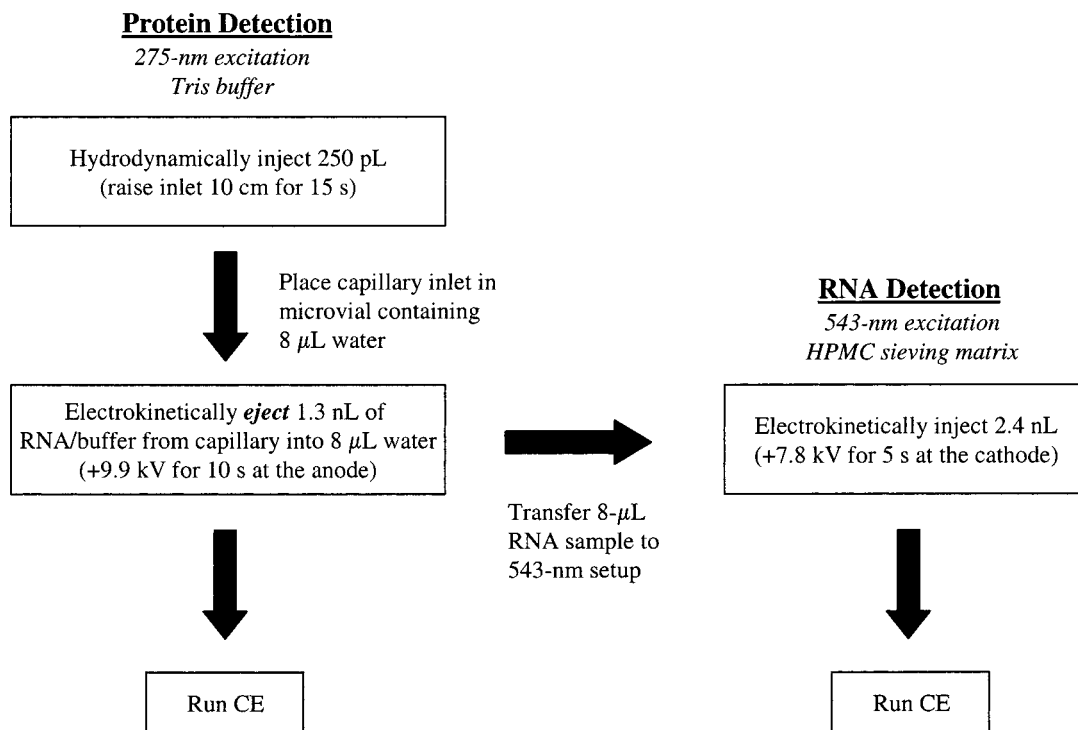Hydrodynamically inject 250 pL
(raise inlet 10 cm for 15 s)

Place capillary inlet in
microvial containing
8 μL water

RNA Detection
543-nm excitation
HPMC sieving matrix

Electrokinetically *eject* 1.3 nL of
RNA/buffer from capillary into 8 μL water
(+9.9 kV for 10 s at the anode)

Transfer 8-μL
RNA sample to
543-nm setup

Electrokinetically inject 2.4 nL
(+7.8 kV for 5 s at the cathode)

Run CE

Run CE

Figure 1. Flowchart illustrating method for obtaining protein and RNA profiles from a 250-pL injection of CHO cell lysate.

lysate (∼250 pL) was injected hydrodynamically into the 23-μm capillary of the 275-nm system. The capillary inlet was placed into an 8-μL aliquot of water, and RNA was electrokinetically ejected from the capillary into the water by applying a potential of +9.9 kV to the inlet for 10 s. The capillary was returned to the inlet buffer vial and electrophoresis was commenced. The water sample containing RNA was mixed briefly with a pipet and transferred to the 543-nm system. The sample was electrokinetically injected for 5 s and electrophoresis was initiated.

Volume Calculations. Volumes were estimated for hydrodynamic injections, electrokinetic injections, and electrokinetic ejections. The Poiseuille equation (eq 1) was used to approximate the volumes of hydrodynamic injections. The dilute Tris buffer allowed parameters for H_2O to be used in our calculation, which are indicated below.

$$V_{inj} = \left[\frac{\pi \rho g}{8\eta}\right] \frac{hr^4 t}{1000 L_{tot}} \qquad (1)$$

V_{inj} is volume injected (L), ρ is density (1.0 g/cm³), η is viscosity (1×10^{-2} g cm^{-1} s^{-1}), g is gravitational force (9.8×10^2 cm/s²), h is height difference between the capillary inlet and outlet (cm), t is injection time (s), r is capillary radius (cm), and L_{tot} is total capillary length (cm). Using these parameters, our estimated hydrodynamic injection volume is 250 pL. Volumes derived electrokinetically were estimated using the average analyte velocities from individual electropherograms.

RESULTS

DNA Standard Experiments. Proteins and nucleic acids typically migrate in opposite directions under the same applied electric field in the presence of electroosmotic flow (EOF). This
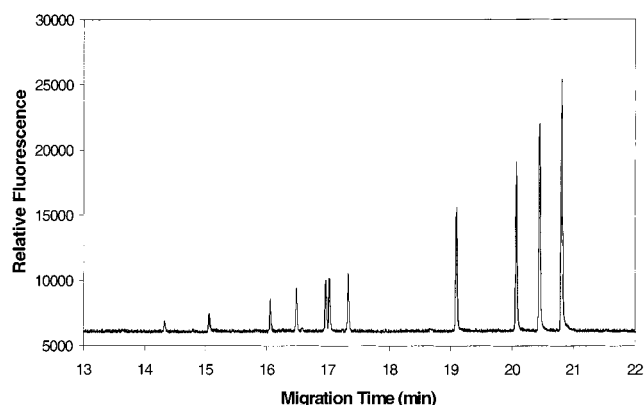

Figure 2. Electropherogram of ΦX174/*Hae*III DNA fragments. A 91 μg/mL sample was electrokinetically injected into a 40 cm × 23 μm i.d. (360 μm o.d.) capillary filled with 5 mM Tris-HCl−0.2 mM CaCl₂ buffer using reverse polarity. The polarity was then switched, and the contents of the capillary were electrokinetically ejected into a 2.5-μL aliquot of water. Electrokinetic injection for 5 s at −9.9 kV; electrokinetic ejection for 10 s at +9.9 kV. The ejected sample was subsequently analyzed by CE-LIF. Excitation at 543 nm using a green helium−neon laser; electrokinetic injection for 5 s at −7.8 kV; electrophoresis voltage, −7.8 kV. Separation conditions are outlined in the Experimental Section.

difference in analyte migration is due to the net cathodic migration of proteins and the anodic migration of nucleic acids, which are highly negatively charged. On the basis of this differential migration, it should be possible to specifically remove the nucleic acids from an injected plug, while retaining the proteins from said sample for CE-LIF analysis. A ΦX174/*Hae*III DNA standard was used to initially test the feasibility of electrokinetically ejecting DNA from a previously injected sample aliquot. Figure 2 shows a representative electropherogram of a DNA standard electro-
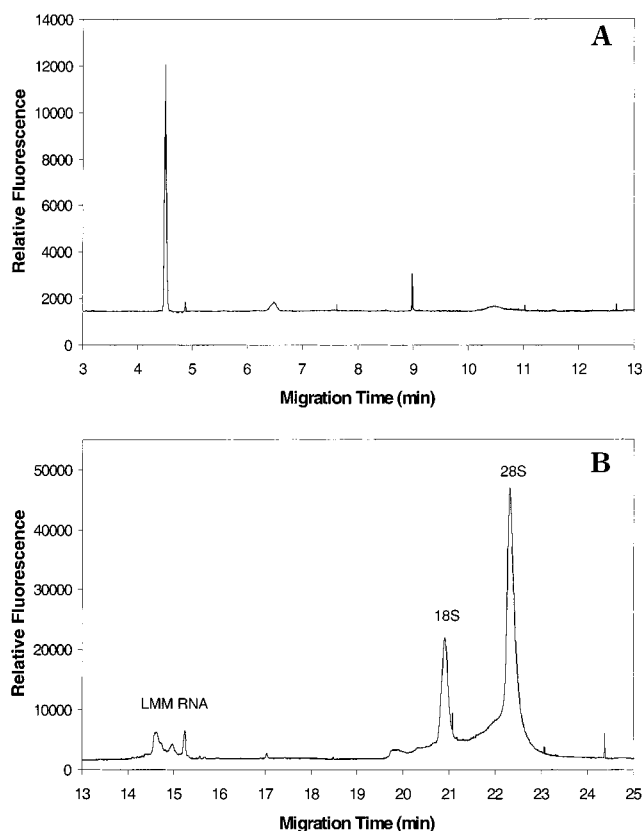
Figure 3. (A) Electropherogram of retained CHO cell lysate components following electrokinetic ejection of RNA. Excitation at 275 using an argon ion laser; hydrodynamic injection for 15 s by raising the inlet 10 cm relative to the outlet; electrokinetic RNA ejection for 10 s at +9.9 kV into an 8-μL aliquot of water; electrophoresis voltage: +9.9 kV. Separation conditions are outlined in the Experimental Section. (B) Electropherogram of ejected RNA sample. 543-nm excitation; PMT bias voltage, −808.4 V; All other CE-LIF conditions were the same as in Figure 2.

kinetically *ejected* from a separate capillary into a sample vial prior to CE-LIF. Five hundred ± sixty picoliters of a 91 μg/mL DNA sample (in water) was electrokinetically injected into and immediately ejected from a 23-μm capillary into a 2.5-μL aliquot of water. This DNA sample (~18 ng/mL after dilution) was analyzed by CE-LIF with the 543-nm system. The observed peak pattern is typical for this DNA standard. The reproducibility of the DNA injection/ejection procedure was also assessed by examining the signal-to-noise ratios (S/N) following CE analysis of the ejected DNA. The relative standard deviation (RSD) of the S/N for all DNA peaks fell between 7 and 12% ($n = 3$). The result of this simulation indicates that nucleic acids can be successfully ejected from a bare capillary with good reproducibility using a normal-polarity configuration.

Electrokinetic RNA Ejection. The electrokinetic sampling technique was applied to a cell lysate (9.6 × 10⁷ cells/mL), and the electropherograms are shown in Figure 3. First, 250 pL of the lysate was injected hydrodynamically into the protein capillary. Following the injection and prior to electrophoresis, ~1.3 ± 0.2 nL of solution was electrokinetically ejected into an aliquot of water and saved for RNA analysis. The protein profile for the 250-pL aliquot is shown in Figure 3A. Three peaks are seen, at 4.50 (S/N ~ 530), 6.48 (S/N ~ 20), and 10.50 min (S/N ~ 12). Specific

identification has not been performed as of this time; however, we are reasonably confident that these peaks indicate intrinsic fluorescence of peptides or proteins, which contain tryptophan or tyrosine amino acid residues susceptible to UV excitation.[16,19−22] While several types of biomolecules exhibit intrinsic fluorescence, we believe these species do not contribute substantially to the UV peak profile. Common cellular biogenic amines excited at 275 nm, including catecholamines[23,24] or serotonin,[25] while prevalent in neuronal-type cells (e.g., chromaffin cells) or mast cells would probably not be found in CHO cells. The small amines that have been identified in CHO cells are nonfluorescent, such as spermine, spermidine, and putrescine.[26] Although free Trp or Tyr would fluoresce and might exist in the cytoplasm, we have not found any reports that describe these amino acids in high abundance. We maintain there could be a small contribution from small molecules in the UV profile. However, considering the relative abundance of cellular proteins, we believe these peaks are most likely due to proteins.

The electropherogram of the corresponding RNA profile for the same 250-pL sample is shown in Figure 3B. A cluster of peaks from 14.61 to 15.25 min and two intense peaks at 20.90 (S/N ~ 340) and 22.32 min (S/N ~ 840) are seen. This RNA peak pattern is consistent with that seen previously in single cells[17,18,27] and is primarily due to ribosomal RNA (rRNA). Prior experiments[27] confirm that the peaks at 20.90 and 22.32 min result from 18S and 28S rRNA, respectively, and the cluster of smaller peaks corresponds to low molecular mass (LMM) RNA fragments of ≤155 bases.

To verify that the peaks from a previously ejected sample are not anomalous, a control experiment was performed in which only the buffer from the 23-μm capillary (in the 275-nm system) was electrokinetically ejected for 10 s into 8 μL of water. Subsequent electrokinetic injection (5 s) of this sample into the 543-nm system resulted in no peaks, thus confirming the integrity of this method.

Injection without Electrokinetic RNA Sampling. To validate the sampling technique (i.e., RNA ejection and protein retention), the lysate sample was injected into each CE-LIF system. These electropherograms are shown in Figure 4. The protein profile in Figure 4A is similar to that seen in Figure 3A, with the most noticeable difference being an extra peak at 8.92 min. In addition, the broad peaks at ~10 min are 13× more intense than the corresponding peaks in Figure 3A. The RNA profile shown in Figure 4B (5-s injection) is consistent with its counterpart shown in Figure 3B. To keep the RNA peaks on scale, it became necessary to lower the PMT bias voltage (Figure 4B). Simple dilution was not a favorable option since it would alter the sample matrix. Reducing the injection time presented its own challenges as well, due to the injection bias toward smaller molecules, which is inherent to electrokinetic injections.[28]

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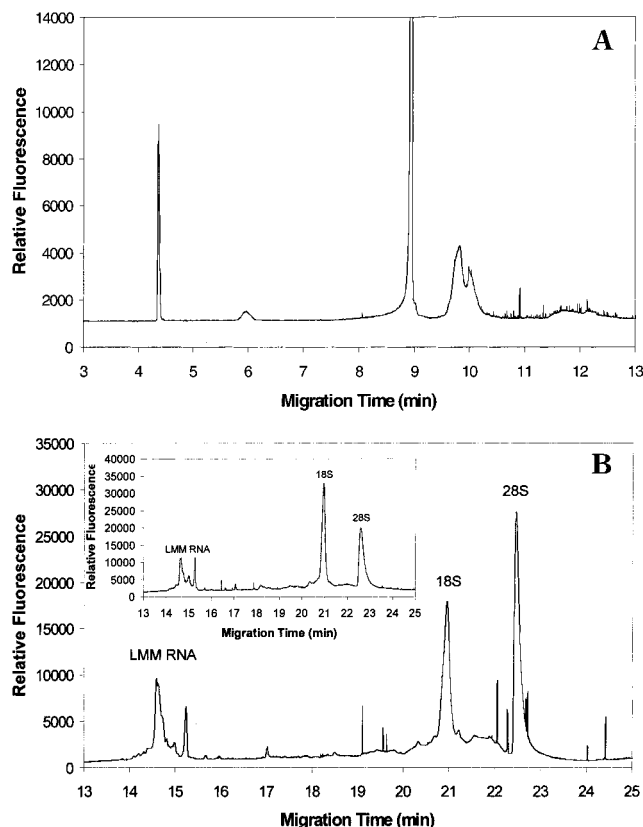(27) Han, F.; Lillard, S. J. *Anal. Biochem.* **2002**, *302*, 136−143.

Figure 4. Electropherograms of CHO cell lysate directly injected into each CE-LIF system. (A) 275-nm excitation; No RNA ejection. CE-LIF conditions were the same as in Figure 3A. (B) 543-nm excitation; PMT bias voltage, −729.1 V; All other CE-LIF conditions were the same as in Figure 2. Figure 4B inset shows a lysate electropherogram generated using a 2-s injection time and a PMT bias voltage of −808.4 V. All other CE-LIF conditions were the same as in Figure 2.

The effect of a shortened injection time is evidenced in the inset of Figure 4B. This electropherogram was recorded with the same PMT bias voltage as for Figure 3B, following a 2-s injection time. Although the 18S peak in Figure 4B inset is of the expected intensity (i.e., the ratio of 18S to LMM RNA peak intensity is the same as in the dual sampling assay), the 28S peak is much less intense than it should be. The 2-s injection time is not long enough to allow complete electrokinetic introduction of the larger RNA fragment (i.e., 28S), since under the same electric field strength, larger molecules migrate slower than smaller ones. However, Figure 4B inset allowed a direct comparison of S/N with the ejected RNA electropherogram (Figure 3B).

DISCUSSION

Effects of RNA Ejection on Protein Migration. As mentioned previously, the electropherograms shown in Figures 3A and 4A differ slightly. Specifically, one of the protein peak heights is drastically reduced and another peak is completely absent following RNA ejection. In an attempt to retain these peaks, the RNA ejection time was reduced from 10 to 5 s. The protein profile following 5-s RNA sampling, seen in Figure 5A, is identical to that in Figure 4A, indicating retention of the two protein peaks.
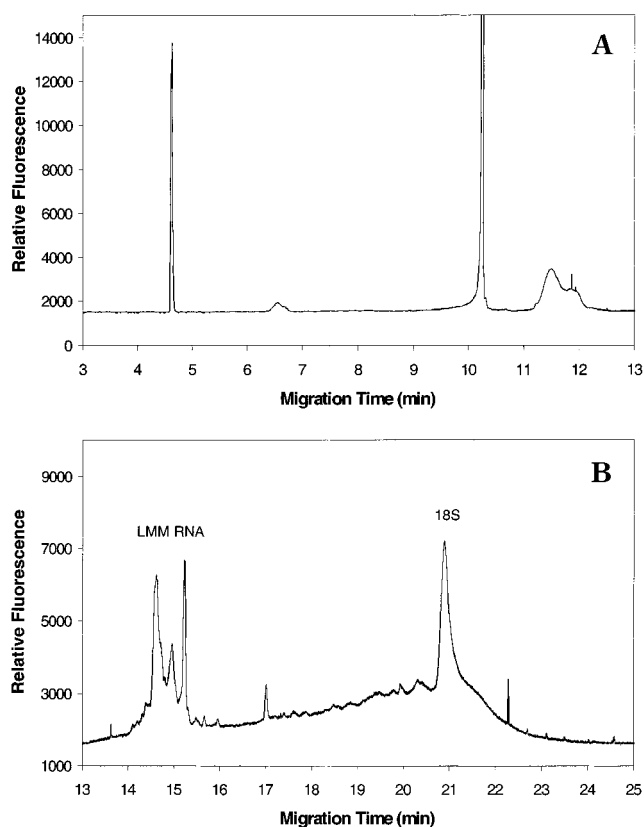
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Figure 5. (A) Electropherogram of retained CHO cell lysate components following electrokinetic ejection of RNA. 275-nm excitation; electrokinetic RNA ejection for 5 s at +9.9 kV into a 4-μL aliquot of water. All other CE-LIF conditions were the same as in Figure 3A. (B) Electropherogram of ejected RNA sample using 543-nm excitation. All other CE-LIF conditions were the same as in Figure 2.

However, this sampling time was not sufficient to eject all RNA fragments from the 250-pL sample as seen in Figure 5B. The relative intensity of the LMM RNA is much higher than seen in a typical RNA profile, while that of the 18S peak is much lower. The 28S peak is completely absent. This indicates that the 5-s sampling time is insufficient for complete ejection of the slower moving 18S and 28S fragments. It is clear that for this type of analysis the ejection time must be carefully coordinated with the separation conditions so that all peaks of interest are retained. In our experiments, we elected to preserve the integrity of the known RNA profile in lieu of the protein profile.

The loss of the later-migrating protein peaks may have been caused by complexation of those particular proteins with the SDS (1%) present in the cell lysate. This would confer upon them a significant negative charge, which could result in outward migration during the 10-s ejection. Peak loss is observed when the sample is ejected into water; however, as expected, the peaks are retained during ejection into running buffer. When the concentration of SDS is reduced from 1 to 0.5%, the peaks are partially retained (indicating complexation to a lesser extent), even after a 20-s ejection. Unfortunately, under lower SDS concentrations, the stability of the RNA is significantly compromised. The use of a 0.5% SDS solution for lysis resulted in appreciable degradation of the 18S and 28S fragments within ~1 h. The 18S and 28S peaks are not seen at all when the SDS concentration is less than 0.5%, even if the sample is injected within 5 min of lysis. We believe

that the stability achieved with 1% SDS results from denaturation and partial inactivation of endogenous RNases, which would otherwise degrade the RNA. Various nonionic and cationic detergents, including Tween 20, Triton-X 100, and polyoxyethylene 10 lauryl ether, were examined.[29,30] However, complete lysis was not achieved with any of these detergents. Freeze−thaw lysis, in which cells are frozen at −70 °C and subsequently thawed, has also been used for cell disruption; however, it is not effective for rRNA analysis. The 18S and 28S rRNA peaks are not seen using freeze−thaw lysis, possibly due to insufficient solubilization of the ribosome.[31]

Salt Effects. It is well documented that a high-salt matrix interferes with the electrokinetic injection of nucleic acids into polymer matrixes. Although desalting is relatively easy when dealing with large sample volumes, it becomes more difficult when using ultrasmall volumes such as with CE. Xue et al. have demonstrated an on-line method using CE for purifying DNA sequencing products.[32] In a similar manner, we have utilized an inherent desalting effect observed during electrokinetic ejection of RNA. This curious byproduct is evident upon closer examination of the electropherograms in Figure 3B (RNA sampling from the 250-pL injection) and Figure 4B inset (total (concentrated) lysate injection of 2.4 ± 0.4 nL). The initial concentration of the lysate was 9.6×10^7 cells/mL, and 250 pL of this lysate was injected into the 23-μm capillary for determination of the protein profile. As a conservative approximation, if all of the RNA from the 250-pL injection was ejected from the capillary into the 8-μL aliquot of water, then the concentration of the ejected RNA would be equivalent to that in a 3000 cells/mL lysate. This is 32 000-fold more dilute that the initial lysate solution. Subsequently, 2.4 ± 0.4 nL of this dilute (3000 cells/mL) sample was electrokinetically injected for 5 s to obtain the RNA profile. The 18S peak in that electropherogram (Figure 3B) has a S/N of ~340. When the concentrated lysate (9.6×10^7 cells/mL) is directly injected into the RNA system for 2 s (an injection volume of ~1 ± 0.2 nL), a S/N of 580 is seen for the 18S peak, which is 1.7 times larger than for the dilute injection. This intensity factor is corrected to 4.2 after accounting for the difference in injection volumes. Adjusting the initial 32 000× dilution by the intensity factor of 4.2 results in a normalized enhancement of 7600×; i.e., the original lysate and an ejected RNA sample 7600-fold more dilute would produce equivalent signals.

The basis for this phenomenon returns to the nature of the bias toward small molecules during electrokinetic injection. The lysate contains more than enough RNA to allow detection; however, due to the injection bias, the sample plug itself consists primarily of SDS (1% or ~35 mM) along with ions and other small molecules from the cells. The electrokinetically sampled RNA solution is in water with minimal ionic contamination, since EOF present in the protein capillary presumably swept the majority of the small molecules and ions in the direction opposite from the RNA. Thus, the process of electrokinetic sampling provides quick and effective RNA cleanup from a complex biological matrix. Without the competition from the smaller species,[33,34] RNA is injected more efficiently, resulting in the intense signals seen in Figure 3B. This is further confirmed by ejecting the RNA into 2.5 μL of 0.5% (~17 mM) SDS solution instead of water. When that sample is summarily injected for RNA analysis, the resulting RNA peaks are diffuse and poorly resolved.

The injection/ejection of the DNA standard, shown in Figure 2, also supports this hypothesis. No enhancement effect is observed when the electropherogram from an ejected (hence diluted) DNA sample of 18 ng/mL is compared to that from a direct injection of a 20 ng/mL sample. This is due to the absence of salt ions in the water matrixes of both the original (20 ng/mL) DNA standard and the ejected (18 ng/mL) DNA sample.

CONCLUSIONS

Our CE-based method, allowing both RNA and proteins to be electrokinetically sampled and distinguished from a single 250-pL injected cell lysate, represents a novel advancement toward simultaneous monitoring of different classes of biomolecules from ultrasmall volumes. A significant feature of this technique is the inherent desalting effect present during electrokinetic sampling. This simple and automatic procedure for RNA cleanup results in a net 7600-fold enhancement. This method can be applied to nearly any protein or nucleic acid component of interest, by simply modifying the experimental conditions of the two separate electrophoresis systems.

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