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Continuous Cell Introduction for the Analysis of Individual Cells by Capillary Electrophoresis

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Instrumentation for high-throughput analysis of single cells by capillary electrophoresis is described. A flow-based interface that uses electroosmotic flow (EOF) provides continuous injection of intact cells through an introduction capillary into a cell lysis junction and migration of the resulting cell lysate through a separation capillary for analysis. Specifically, two capillaries were coupled together with 5-mm-long Teflon tubing to create a ∼5-μm gap, and the junction was immersed in a buffer reservoir. High voltage was applied across both capillaries so that cells were continuously pumped into the first capillary by EOF. Individual cells were lysed on-column at the junction without detergents, presumably owing to mechanical disruption caused by a dramatic change in flow properties at the gap. After each cell was lysed at the junction, the major proteins hemoglobin and carbonic anhydrase were separated by capillary electrophoresis and the resultant analyte zones were detected by laser-induced native fluorescence using 275-nm excitation. The detection limits of hemoglobin and carbonic anhydrase were 37 and 1.6 amol, respectively, which correlate well with the literature. The instrumentation was evaluated with intact red blood cells. The averaged time for complete analysis (i.e., continuous injection, lysis, separation, and detection) of one human erythrocyte was less than 4 min with this capillary-based setup. Moreover, this instrumentation simplifies the introduction of individual, intact cells without the use of a microscope.

Characterizing heterogeneous populations of cells is of considerable interest in areas such as biomedicine.[1] However, owing to the low detection sensitivity of conventional biochemical methods, intracellular chemistry is often determined by sampling an aliquot that is representative of a large population (e.g., millions) of cells, and an average value is obtained for the measurement that is then extrapolated to the level of one cell. An example of this type of approach in a clinical setting is in the measurement of hemoglobin using blood from a finger stick.[2] The drawback with such an approach lies in the assumption that this average analyte measurement is indicative of each individual entity in the population. For situations in which all cells of a population have their chemistry dramatically altered by a given mechanism, this approach is acceptable. However, in situations where only a few cells of the population exhibit chemical differences, an approach that measures analyte composition directly from individual cells is desired.

To obtain distinct information from high numbers of individual cells, the technique of flow cytometry was developed.[3] In flow cytometry, classes of intracellular compounds are reacted with fluorescent labels, and the cells are sent single file through a tube and focused hydrodynamically using a sheath-flow orientation.[1,4−6] The corresponding fluorescence from thousands of cells is collected and quantitated, with sophisticated flow cytometers able to monitor four colors of fluorescence simultaneously (using four lasers).[4] In addition, some of these instruments can indicate size information by light scattering (and correlate size with analyte fluorescence) and others can serve as cell sorters.[4,5] However, despite the throughput capabilities of flow cytometry, this technique is restricted to compounds that must be labeled with a fluorophore, and subsequently, differentiation must result from labeling each member of a class of compounds (e.g., proteins) with reagents that have different spectral characteristics (i.e., different colors), as there is no other basis for distinction besides spectroscopy. To distinguish multiple analytes without labeling in continuously introduced cells, Fung and Yeung developed a mass spectrometric method in which intact cells were sent through a capillary by hydrodynamic flow and into a mass spectrometer for the determination of histamine and serotonin in single mast cells.[7] Our objective was to utilize the power of a microscale separation method (i.e., capillary electrophoresis, CE) while increasing the throughput to analyze a greater number of samples without manual cell injection.

Capillary electrophoresis coupled with various detectors has demonstrated superior detection limits and quantitative capabilities for the analysis of single cells, including non-nucleated cells (i.e., red blood cells) as well as nucleated cells. Representative analyses of nucleated cells by CE include mast cells,[8] neurons,[9,10] chroma-

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ffin cells,[11,12] pancreatic β-cells,[13] and cancerous cell lines.[14−16] Electrophoresis of hemoglobin from single, intact red blood cells was actually first demonstrated in 1965 with a microelectrophoresis device.[17] In the past decade, progress on the analysis of single erythrocytes has been achieved using CE. These determinations include Na^+ and K^+ with indirect fluorescence,[18,19] proteins with native fluorescence[20−22] or off-column derivatization,[23] glutathione with derivatization by monobromobimane (mBBr),[18,24,25] and enzyme-based assays of lactate dehydrogenase[26] and glutamate.[27] In addition, on-line coupling of CE with electrospray ionization/mass spectrometric detection has been used successfully for single-cell analysis.[28,29] Hofstadler et al.[28] used mass spectrometry coupled with CE to determine hemoglobin in individual erythrocytes, and Cao and Moini resolved the α- and β-chains of the hemoglobin tetramer originating from intact individual cells.[29]

One distinct advantage of using a microseparation method, such as CE, for separating intracellular analytes from individual cells is the capability to manipulate and analyze ultrasmall sample volumes (i.e., ≤pL). Capillaries with inner diameters of ≤25 μm work very well to inject a cell with a diameter of 5−15 μm. Another distinct advantage of sampling from single cells is that the chemical information from one cell is not averaged with the other cells in the population. Such an advantage becomes significant in the area of monitoring populations of cells for chemical damage or abnormalities. Only a technique capable of single-cell measurements can obtain such information.

A severe limitation to existing CE methodology for single-cell analysis is the injection of intact, individual cells into the separation capillary, although both methods that are used to inject analyte solutions into capillaries (i.e., electrokinetic and hydrodynamic) have been successful for injecting individual cells. Hydrodynamic injection, induced by creating suction at the outlet end of the capillary, is straightforward and works well for suspended cells.[30] Electrokinetic injection is favorable when the cells of interest are adhered to a surface (e.g., coverslip), rather than suspended in solution,[11] or when the outlet end of the capillary is inaccessible, such as in mass spectrometric detection.[28] For the analysis of individual cells, both of the conventional injection methods—hydrodynamic and electrokinetic—are tedious, lack control, and only allow for the injection, verification, and separation run of one cell at a time. Another injection method, optical trapping, has been used to inject individual secretory vesicles into a separation capillary for CE.[31] This laser-based method provides exceptional control of the sample during injection; however, it is also limited to the injection of one biological entity (e.g., cell or organelle) at a time, resulting in maximum analysis rates of only a few cells per hour. To enhance single-cell CE for the practical analysis of individual cells, there is a need to develop high-throughput techniques that include continuous cell introduction.

Microfabricated devices have been shown to have the capability to manipulate individual cells using electroosmotic flow, as shown by Harrison's group for the transport and selective lysis of single erythrocytes (without separation of intracellular components).[32] More recently, Dovichi's group optimized capillary-based instrumentation for single-cell analysis (termed chemical cytometry), which permits a more automated approach to certain requisite steps, such as cell injection and reagent introduction for on-column derivatization reactions.[33] We have developed a capillary flow injection interface that demonstrates, for the first time, continuous cell introduction, transport of intact cells through the introduction capillary into a cell lysis junction, and migration and separation of the resulting single-cell lysate through a second capillary. Optimized separation conditions show that the two major proteins in human erythrocytes, hemoglobin and carbonic anhydrase, can be baseline-resolved with excellent detection limits.

EXPERIMENTAL SECTION

Reagents and Solutions. Carbonic anhydrase II (CA; human), hemoglobin A_0 (Hb; human), sodium tetraborate (Borax), sodium dodecyl sulfate (SDS), Triton X-100, polyoxyethylene 10 lauryl ether, and other detergents were purchased from Sigma (St. Louis, MO). Sodium hydroxide (10 M solution), sodium chloride, sodium phosphate dibasic, sodium phosphate monobasic, and glucose were purchased from Fisher Scientific (Fair Lawn, NJ). Deionized water (resistance ≥18 MW) was prepared from a Milli-Q system (Bedford, MA) and was used in all experiments. Borate buffer solution (50 mM) was prepared by dissolving sodium tetraborate in deionized water, unadjusted pH ∼9.1. NaOH (0.2 M) was used to rinse columns at the beginning of each experiment; rinses between electrophoretic runs were not necessary. Phosphate-buffered saline (PBS) consisted of 154 mM NaCl, 3 mM Na_2HPO_4, 1 mM NaH_2PO_4, and 5 mM glucose adjusted to pH 7.3−7.4 with 10 mM NaOH. Borate and PBS solutions were filtered using a 0.2-μm filter (Corning, Corning, NY) after preparation. PBS was refrigerated for storage, but brought to room temperature before use. Hemoglobin A_0 (30 μM) and carbonic anhydrase II (1 μM) solutions were prepared in 50 mM borate, stored in the freezer, and used for experiments within 2 weeks of preparation.

Cell Isolation and Lysate Preparation. Red blood cells were from a presumably healthy male adult donor. Five microliters of

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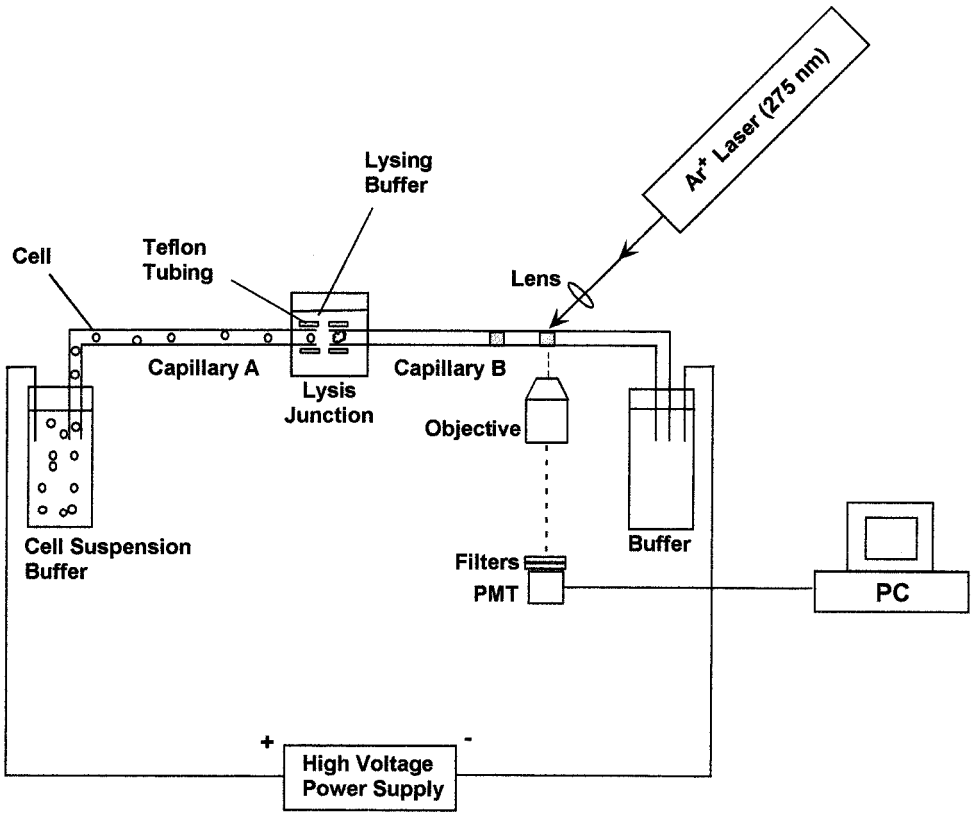

Figure 1. Schematic of instrumental setup for high-throughput CE analysis of single cells.

blood was dispensed in 1 mL of PBS, mixed gently, and then centrifuged at 126*g* for 6 min. The supernatant was discarded into a waste bottle containing 10% bleach. Then, 1 mL of PBS was added and mixed gently with the remaining cells. This washing procedure was repeated four times to remove all serum proteins. Finally, red blood cells were suspended in 1 mL of PBS and used within the next 8 h. The cell lysate was prepared by centrifuging 0.5 mL of cell suspension solution (126*g*, 6 min), discarding the supernatant, and adding 0.5 mL of deionized water to lyse the remaining cells. To prepare cell samples for single-cell experiments, 2 μL of suspension solution was diluted in 1 mL of 50 mM borate. The cells were counted with a hemacytometer (Hausser Scientific, Horsham, PA), and the cell count was determined to be $\sim 10^4$/mL. This very dilute cell suspension was necessary to minimize the possibility of multiple cells eluting very close together through capillary A (Figure 1).

High-Throughput Instrumentation. *Instrument Design.* A schematic of the experimental setup is shown in Figure 1. In this apparatus, two capillaries with lengths of 15- (capillary A) and 45 cm (capillary B) and the same inner and outer diameters (21 μm i.d. × 365 μm o.d.; Polymicro Technologies, Phoenix, AZ) were aligned together with a 5-mm length of Teflon tubing ($^1/_{16}$ in. o.d. × 0.010 in. i.d.; Alltech, Deerfield, IL) to create a gap between the two capillaries of $\sim$5 μm. The ends of the capillaries must be flat and without rough edges in order to reduce dead volume and improve alignment at the junction, which is located at the beginning of the separation capillary. To achieve clean cuts, a capillary cleaving tool (Supelco, Bellefonte, PA) was used. In addition, the capillaries must be aligned well so that the entire cell lysate can easily enter the second column. Each column was rinsed with 0.2 M NaOH followed by the running buffer (30 min each) prior to assembly of the junction. A 1-mL polypropylene vial (Eppendorf) was used to contain the lysis junction. Holes were made in the sides of the vial by inserting a 27-gauge needle in the horizontal direction. The end of one capillary was inserted through one of the holes so the end was inside the vial, and the 5-mm piece of Teflon tubing was slid over the capillary using tweezers. The second capillary was inserted first through the hole on the other side of the vial, then through the tubing, and positioned so that the inner channels were aligned with a $\sim$5-μm space between the two capillaries. Running buffer was placed into the vial to immerse the capillary junction. After construction of the junction region, the capillary inlet (capillary A) was dipped in dilute cell suspension (in borate) and the outlet (capillary B) was dipped in borate. A high-voltage power supply (Glassman High Voltage Inc., Whitehouse Station, NJ) was used to apply +14 kV to the inlet side; the outlet side was grounded. Because there is an electrolyte solution at the junction (in which the ends of the capillaries are immersed), applied voltage results in conductivity and a complete circuit (hence, electroosmotic flow, EOF) that is maintained across both capillaries. Cells were continuously pumped into the first capillary with electroosmotic flow and lysed on-column at the junction. After cells were lysed, cellular proteins were separated by CE and were detected by laser-induced native fluorescence.

Detection. An argon ion laser (Coherent, Innova Sabre TSM; Santa Clara, CA) was used as an excitation source, in constant-power mode (20 mW). A fused-silica prism (Edmund Scientific, Barrington, NJ) was used to isolate the 275-nm line from the laser plasma, and the beam (5.4 mW after reflective losses) was focused

onto the detection window with a fused-silica lens (1-cm focal length). The outer polyimide coating was burned with a lighter to create a ~1-cm detection window, and the incident angle of the excitation beam on the window was ~45°. The fluorescence was collected at 90° with a 10× microscope objective (Edmund), passed through two UG-1 band-pass filters (280−420 nm; $\lambda_{max} \approx$ 360 nm; Schott Scientific Glass, Inc., Parkersburg, WV) and one spatial filter to reject stray light, and imaged onto a photomultiplier tube (931B; Hamamatsu, Middlesex, NJ) operated at 1000 V. A low-pass electronic filter was used to reduce high-frequency noise before data acquisition. A 24-bit ChromPerfect data acquisition board was used to digitize the signal and were acquired at 10 Hz using ChromPerfect software (Justice Innovation, Palo Alto, CA). The original data files were converted to ASCII and the electropherograms were replotted using Excel.

Single-Capillary CE. The experimental setup for high-throughput analysis, with a few modifications, was also used for single-capillary CE of standard proteins, cell lysate, and individual cells. For these experiments, the coupled capillaries and the unnecessary reservoir at the lysis junction were removed and replaced with one 21-μm fused-silica capillary (45-cm total length; 30-cm effective length) in the typical CE format. The running buffer and rinsing procedures were the same as with the high-throughput setup. Protein and lysate solutions were injected hydrodynamically by raising the injection end to a height of 5 cm relative to the outlet end for 10 s (injection volume ~52 pL). A voltage of +10.5 kV was applied to the inlet side and the outlet side was grounded. Triton X-100 (0.1% w/v in deionized water) was used as a neutral marker to measure electroosmotic flow of the single-capillary setup.

Injection of Single Cells. The procedure for injecting individual cells into the single-capillary setup was similar to that described previously.[14] Briefly, 10 μL of cell suspension was placed on a glass slide, in which the inlet end of the capillary was positioned at ~30°, using a mount attached to the microscope stage. A syringe containing running buffer was attached to the outlet end of the capillary with 10 cm of pump tubing (0.010 in. i.d.; Fisher Scientific, Pittsburgh, PA) and used to create suction to pull a cell into the column. Injection was monitored with a 10× microscope objective (100× total magnification). Injection of one cell was visually confirmed, both ends of the capillary were placed into buffer reservoirs, and electrophoresis was initiated. Because the running buffer contained detergent, an additional lysing step was not needed prior to electrophoresis.

RESULTS AND DISCUSSION

The separation of major proteins in red blood cells was chosen as a model system to demonstrate the application of our high-throughput instrumentation. Erythrocytes have several features that make this cell type well suited for optimizing high-throughput analysis, including ease of lysis and intracellular proteins that have been characterized. First, because rapid and efficient cell lysis is a critical step of the procedure, the non-nucleated structure of an erythrocyte permits lysis without complicated methods. It is well known that nucleated cells are more difficult to lyse in single-cell experiments than red blood cells.[11,12,33−35] Second, the major proteins, hemoglobin and carbonic anhydrase, have been characterized previously by CE-LIF,[20] and modification of an established system helped to minimize unnecessary development of the separation parameters. Thus, our efforts could be directed toward defining the high-throughput parameters. Finally, hemoglobin and carbonic anhydrase both exhibit native (or intrinsic) fluorescence, which is sensitive enough to permit single-cell detectability.[20−22,36] Intrinsic protein fluorescence relies on the fluorescence of constituent aromatic amino acids, primarily tryptophan and to a lesser extent tyrosine.[36] This property, permitting direct detection, was extremely important to simplify the instrumentation by not requiring a derivatization step for sensitive detection of intracellular analytes at the single-cell level.

Optimization of Protein Standards and Cell Lysate. A single-capillary orientation was used to assess the limit of detection (LOD) and separation efficiency of Hb and CA in standard solutions and in an erythrocyte lysate. Baseline separation of standard proteins is achieved in less than 6 min, as shown in Figure 2A. The LOD (S/N = 2; peak-to-peak noise) of Hb and CA are 37 and 1.6 amol, respectively, in 50 mM borate. Although these LODs are higher than previously reported in the literature,[20] they are sufficient to permit detection of Hb and CA in single cells, which are present in amounts of ~450 and 7 amol, respectively.[37] Figure 2A indicates that our separation efficiency is higher than previously reported[20]—the number of theoretical plates (N)[38] was determined to be 6.1×10^5 and 4.4×10^5 for CA and Hb, respectively, with a resolution of 9.3. Hemoglobin and carbonic anhydrase in a cell lysate were separated using 50 mM borate, as shown in Figure 2B. The resulting electropherogram corresponds approximately to the protein amounts in the equivalent of 1.3 cells (2.5×10^7 cells/mL × 52-pL injection volume). Comparing panels A and B of Figure 2 verifies that the detected cellular components in the lysate are the two major proteins.

Cell Lysis and Single-Cell Analysis. The LODs for Hb and CA indicate that our system should be able to measure these proteins in individual cells. However, one difference with injecting an intact cell compared to injecting solutions (i.e., protein or lysate) into the capillary is that the cell membrane must be lysed in order to gain access to the cytosol for separation of its components. This requires either having a running buffer that facilitates lysis or introducing a plug of lysing reagent after the intact cell has been injected into the capillary. The lysate sample for the electropherogram shown in Figure 2B was prepared by adding deionized water to a pellet of erythrocytes, and lysis was obtained by differences in osmotic pressure. In our experiment, our running buffer (50 mM borate) was insufficient to lyse human erythrocytes, although previous reports have found dilute running buffers without surfactants to be successful for lysing non-nucleated cells.[18−24] Repeated attempts to lyse red blood cells simply by suspension in 50 mM borate (pH ~9.1) were unsuccessful, and we found that the erythrocytes remained intact for at least 3 h. However, to analyze the contents of an individual cell, it must be first lysed so other methods were considered. Many ways to lyse

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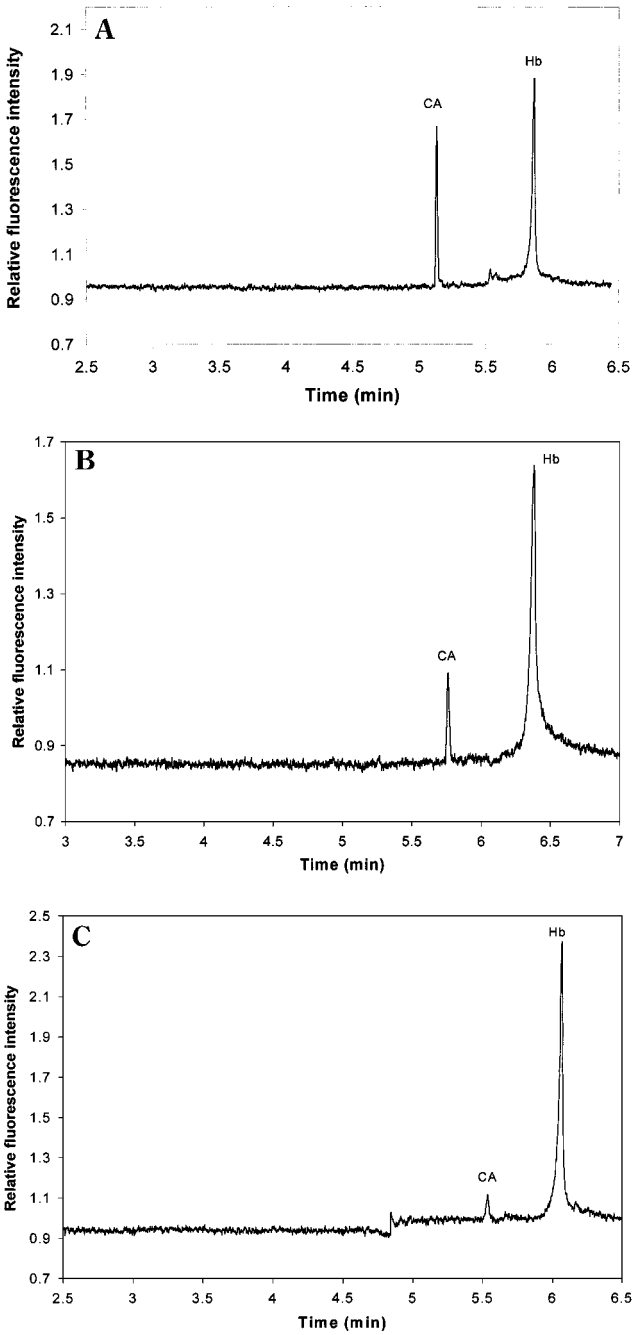

Figure 2. (A) Electropherogram of standard proteins using 50 mM borate running buffer. Conditions: 45 cm × 21 μm i.d. × 365 μm o.d. fused-silica capillary, 15 μM hemoglobin A_0 and 0.5 μM carbonic anhydrase II, 10 s hydrodynamic injection, +10.5 kV applied voltage. CA, carbonic anhydrase II; Hb, hemoglobin A_0. (B) Electropherogram of lysate of human erythrocytes using 50 mM borate running buffer. Approximately one cell was injected. Other conditions are the same as in (A). (C) Electropherogram of single human erythrocyte using 50 mM borate running buffer containing 0.05% polyoxyethylene 10 lauryl ether as buffer. The same column and applied voltage are used as in(A).

nucleated and non-nucleated cells have been described, with examples including the use of detergents,[8,11,14,33] a laser,[35] and even a strong electric field generated with a Tesla coil.[34] Owing to integration into our high-throughput instrumentation, lysing by detergents is the simplest method and was investigated initially. For our experiments, ideal detergents must not reduce electroosmotic flow (hence, lengthen analysis time), must have lysing capability, must not deteriorate the separation resolution, and must not adversely affect analyte signal, either by introducing background fluorescence or by quenching analyte fluorescence.

SDS is a commonly used detergent for lysing cells; thus, it was investigated for CE of standard proteins using 50 mM borate solution containing 0.1% SDS. However, the proteins coeluted, probably because SDS denatures proteins so that they have the same charge-to-mass ratio.[16,39−42] Hemoglobin and carbonic anhydrase could not be separated with SDS, so several nonionic surfactants[43−45] were investigated because of their neutrality and hopeful preservation of rapid analyte migration time. Of several detergents that we tested, we found that Triton X-100 and reduced Triton X-100 gave unacceptable background fluorescence, whereas octyl glucoside did not lyse the cell. Polyoxyethylene 10 lauryl ether (P10LE) was the optimum detergent of the ones we tested for CE of an individual human erythrocyte (single-capillary setup), and the electropherogram is shown in Figure 2C. Polyoxyethylene 10 lauryl ether and other alkylpolyoxyethylene glycol ethers meet almost all of our criteria for ideal lysing agents. A comparison of Figure 2C (single cell in borate + P10LE running buffer) with Figure 2A (protein standards in borate running buffer) reveals that the migration times of the proteins are the same. This indicates that the low concentration of neutral surfactant has no apparent effect on CE migration times and that this detergent appears to be appropriate for our system.

Continuous Injection of Cells. Cells are very sensitive to environmental parameters such as pH and ionic strength, and to prevent unwanted degradation or lysis, they are generally maintained at physiological conditions. After our washing procedures, human erythrocytes are suspended in PBS at pH 7.3. Although this buffer maintains intact cells, this buffer cannot be used as a separation buffer because it does not provide the desired resolution of the proteins of interest. In addition, if the cells can be suspended in the running buffer for direct introduction of cells and buffer into the capillary, then optimum separation efficiency is guaranteed. Furthermore, a CE running buffer, which is a conducting electrolyte solution, also permits EOF originating from the inlet sample vial (containing intact cells). As mentioned previously, we found (by viewing under a 100× microscope objective) that human erythrocytes could survive in 50 mM borate (pH ∼9.1) at least 3 h without any indication of cell damage or shrinkage. This time frame was more than sufficient for our studies, as one complete high-throughput experiment took only took 40 min, hence, was well within this 3-h window. Thus, we chose to suspend the erythrocytes in our borate running buffer.

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One of the essential steps in high-throughput CE of single cells is to introduce intact cells into the column continuously. In flow cytometry, cells are continuously pumped by hydrodynamic flow.[1,3−6]A liquid sheath is used to focus cells to form a single file. We considered using pressure-induced introduction of cells into capillary A; however, hydrodynamic flow induces a parabolic flow profile, resulting in low separation efficiency and resolution in CE. To maximize our separation efficiency in this instrumentation, we instead used electroosmotic flow to pump cells into the capillary. The pluglike flow profile of EOF ensures that separation efficiency is maintained. Furthermore, the use of EOF instead of pressure simplifies the instrumentation by not having to add additional components to regulate pressure flow. An inlet column with a small inner diameter (capillary A, Figure 1) was used to confine cells into a single file when cell suspension was diluted. This was verified with a microscope although the microscope was not used in the subsequent experiments. Cell movement in the capillary is a result of both electrophoretic movement of the cell and electroosmotic flow under an electric field. Apparent mobility of a cell can be described by the following equation:[46]

$$\mu = \mu_{eo} + \mu_{ep}$$

where μ is the apparent mobility of a cell, μ_{eo} is the electroosmotic mobility (i.e., EOF of the CE system), and μ_{ep} is the electrophoretic mobility of the cell. In this experiment, the measured $\mu_{eo} = +4.2 \times 10^{-4}$ cm^2/V·s. Identical electric field strengths in the single and coupled capillary setups ensure that the EOFs are the same. A cell is actually a charged particle, and the surfaces of most cells are negatively charged under physiological conditions. Sialic acid residues are thought to be primarily responsible for the negative charge of a cell, which is a property that can be used to separate cells from one another.[47] Cell electrophoresis is a separation technique for intact cells based on differences in their electrophoretic mobilities.[48−51] Under our experimental conditions, the electrophoretic mobility of human erythrocytes was found to be -2.0×10^{-4} cm^2/V·s. Compared with μ_{eo}, the cell electrophoretic mobility was small and had the opposite direction; therefore, under our conditions, the net movement of the cells was along the electroosmotic direction. When cells were pumped into a single capillary setup (i.e., without the lysis junction), only spikes from the light scattering of cells passing the laser beam were observed, as shown in Figure 3. The spikes correspond to individual cells and demonstrate that the buffer alone or in combination with the applied voltage across a single capillary is insufficient to lyse the cells.

Cell Lysis and Electrophoresis. As mentioned previously, the other important step in high-throughput CE of single cells is to lyse the individual cells. Lysing by a detergent was selected for the single-capillary experiment shown in Figure 2C because of its simplicity. On the basis of these results, our initial intent was to place a detergent solution in the lysis reservoir, such that the lysis agent would be delivered on-column into the junction by diffusing through the gap between the capillaries. Similar devices have been reported with CE for monitoring flow across a junction[52] and for post- and precolumn derivatization of amino acids.[53,54] However, we found that in the high-throughput setup single cells were lysed in the absence of lysing agent (i.e., with only 50 mM running buffer in the lysis reservoir), as shown in Figure 4A.

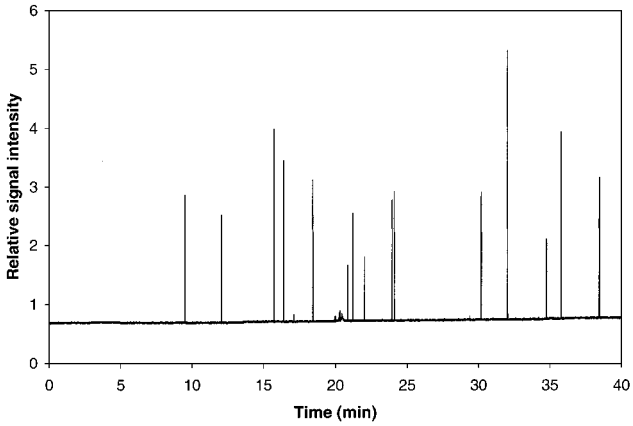

Figure 3. Electropherogram of intact human erythrocytes using 50 mM borate running buffer. The same column and applied voltage are used as in Figure 2A.

Kuhr et al. showed that fluid dynamics across a gap between two capillaries depend on the ionic strength of the gap buffer.[52] They found that in cases of high ionic strength gap buffer, there is greater radial diffusion and less siphoning into a second capillary, but when distilled water was placed at the gap, radial diffusion was nearly nonexistent and siphoning was pronounced.[52] In our orientation, however, the dimensions are much smaller than their 75-μm-i.d. capillaries and 50−100-μm gap; our setup has capillary inner diameters of 21 μm, a gap of ∼5 μm, and a cell diameter of ∼7 μm.[37] We believe that lysis is a result of mechanical disruption owing to deformation of the erythrocyte in the radial direction as it enters the gap, which is ≤7 μm, followed by siphoning into capillary B. As the electric field lines and flow patterns force the cell into the second capillary, the membrane is ruptured and the siphoning action sweeps the intracellular contents into the capillary.

Figure 4A shows a recursive two-peak pattern, with each peak pair corresponding to the major proteins from one cell. The difference between migration times of each pair ($\Delta t = 0.62 \pm 0.01$ min, $n = 6$) matches well with those for the lysate ($\Delta t = 0.61 \pm 0.01$ min, $n = 3$) shown in Figure 2B. It can be seen in Figure 4A that the peak areas of Hb and CA vary from cell to cell, which is expected to be from intercellular variation, as reported previously.[20] Furthermore, this variation does not appear to be owing to the performance of the interface, as there is clearly not a drift (i.e., gradually increasing or decreasing peak areas) from the first

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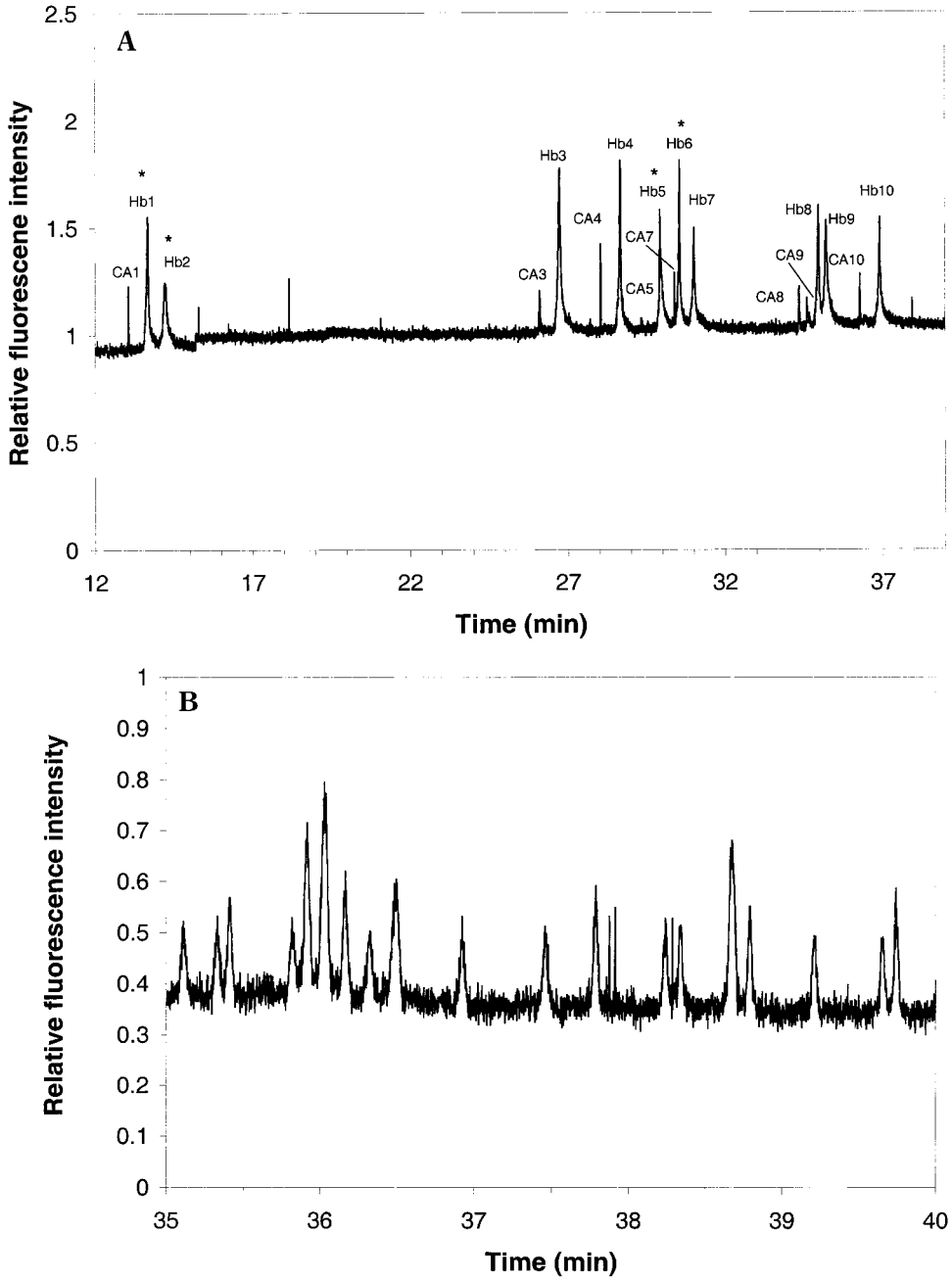

Figure 4. (A) Electropherogram showing high-throughput capillary electrophoresis of continuously injected single human erythrocytes with 50 mM borate running buffer in the lysis junction. Conditions: 15 cm × 21 μm i.d. × 365 μm o.d. fused-silica capillary A and 45 cm × 21 μm i.d. × 365 μm o.d. capillary B; +14 kV applied voltage. CA, carbonic anhydrase II; Hb, hemoglobin A_0. (B) Continuous electropherograms of high-throughput capillary electrophoresis of single human erythrocytes with 50 mM borate solution containing 0.3% SDS in the lysis junction. Other conditions are the same as in (A). The peaks are not labeled because each peak represents coelution of Hb and CA.

cell to the last cell in the electropherogram. For this application, in which there were two detectable analyte peaks, the separation pattern is uncomplicated and typically the peaks were resolved. However, more complex intracellular environments or greater numbers of analyzed cells will require balancing the parameters of cell dilution, EOF rate, and peak efficiency (within a narrow separation window) to distinguish each cell's electropherogram from those of nearby cells. Comparing migration times of Figure 2B with Figure 3, we know that the time for the cell to travel from the inlet to the junction was 4.5 min and the time for the components to travel from the junction to the detection window was 6 min. These travel times would give rise to a ~10.5-min lag time at the beginning of the high-throughput run; thus, the 14-min lag time in Figure 4A was slightly longer than the calculated total time of cell transfer by electroosmotic flow from the inlet to the junction and cell components traveling from the junction to the detection window. One possibility is because cells do not immediately enter capillary A. The absence of peaks observed in the period from 16 to 24 min, but the return of peaks after 24 min, supports that cells do not get into the column uniformly with time. In addition, we observed that fewer and fewer peaks come out with time. This is attributed to cell sedimentation, and as the cells sediment because of gravity, there are fewer and fewer cells available around the inlet of the capillary A.

Although the actual mechanism of lysis is likely physical disruption, the composition of the buffer in the lysis reservoir can still influence the intracellular components following lysis of the cell. We demonstrated this phenomenon by using the high-throughput setup in which the lysis reservoir was filled with 50 mM borate containing 0.3% SDS. Part of the electropherogram is shown in Figure 4B, in which many peaks are observed. Interestingly, the resultant peaks are as expected, in that only one peak is seen for each cell and no recursive two-peak pattern is observed. Each peak corresponds to one cell, with closely eluting cells probably giving rise to the closely migrating peaks. As discussed earlier, SDS interacts with the proteins resulting in similar charge-to-mass ratios and, hence, the same migration time. The results of this experiment demonstrate that the detergent is diffusing efficiently into the junction and also into capillary B (as a result of siphoning).

Quantitation. Amounts of carbonic anhydrase II and hemoglobin A_0 in 6 of the 10 human erythrocytes in Figure 4A were determined by using external calibration. The calibration curve was obtained using CE of standard proteins with a single capillary. The amounts in the other four cells could not determined because of peak overlap (these pairs are indicated by an *). The amounts of carbonic anhydrase per cell were in the range from 4.99 to 9.78 amol, with an average 7.1 ± 2.2 amol. This is very close to the literature value of 7 amol.[37] The amounts of hemoglobin per cell were in the range from 262 to 1025 amol, with an average of 535 ± 277 amol, which is a little higher than the literature value of 450 amol.[37] This discrepancy is probably owing to the individual donor and to the fact that the average of the limited sample may not represent the average of the population.

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CONCLUSIONS

The averaged time for the analysis of one cell in the high-throughput electropherogram was ∼4 min. However, the analysis time for one cell in single-capillary CE was 6 min (counting only the run time and not including the time for manual cell injection by suction). While the present investigation utilized continuously injected erythrocytes (from a single sample source), preliminary observations indicate that this instrumentation should be applicable to nucleated cells as well.[55] Challenges that remain in future optimization studies include overcoming sedimentation (to extend the number of analyzed cells and prevent injection bias toward less dense cells) and reducing the separation window to less than 1 min (in order to surpass the present theoretical limit of ∼60 cells/h). Nonetheless, our high-throughput instrumentation for CE of single cells demonstrates several features for faster analysis. Cell injection is automatic and does not require a microscope; total cell analysis can be finished in a much shorter time because cells can be injected continuously before cellular analyte zones pass by the detection window and the run is considered finished as in a single-capillary system. Furthermore, a shorter column can be used because there is not a restriction on the extra-capillary length in order to view cell injection with a microscope.

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