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Preparation of 20-μm-i.d. Silica-Based Monolithic Columns and Their Performance for Proteomics Analyses

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We describe the preparation and performance of high-efficiency 70 cm × 20 μm i.d. silica-based monolithic capillary LC columns. The monolithic columns at a mobile-phase pressure of 5000 psi provide flow rates of ~40 nL/min at a linear velocity of ~0.24 cm/s. The columns provide a separation peak capacity of ~420 in conjunction with both on-line coupling with microsolid-phase extraction and nanoelectrospray ionization-mass spectrometry. Performance was evaluated using a *Shewanella oneidensis* tryptic digest, and ~15-amol detection limits for peptides were obtained using a conventional ion trap and MS/MS for peptide identification. The sensitivity and separation efficiency enabled the identification of 2367 different peptides covering 855 distinct *S. oneidensis* proteins from a 2.5-μg tryptic digest sample in a single 10-h analysis. The number of identified peptides and proteins approximately doubled when the effective separation time was extended from 200 to 600 min. The number of identified peptides increased from 32 to 390 as the injection amount was increased from 0.5 to 100 ng. Both the run-to-run and column-to-column reproducibility for proteomic analyses were also evaluated.

Current proteome analyses typically aim at broad protein characterization, which inevitably involves highly complex samples.[1] Mass spectrometry (MS) plays a key role in these analyses; the combination of MS with two-dimensional polyacrylamide gel electrophoresis (2-D PAGE) separations has enabled the fractionation of thousands of proteins in less than 1 day.[2−7] Despite its outstanding separation power, 2-D PAGE has several limitations that include incompatibility with very small, very large, and hydrophobic proteins, in addition to difficulties associated with automation, reproducibility, and quantitation.[3,8] Recent advances based on capillary liquid chromatography (LC)-MS approaches for proteome-wide analysis of enzymatic digests (peptides) circumvent many of these limitations.[9−18] Capillary LC, using for example 15−150-μm-i.d. reversed-phase columns can provide high separation efficiencies and high sensitivity when combined with MS using electrospray ionization (ESI). However, LC-MS analysis of very small samples (e.g., cells from small tissue samples obtained using laser capture microdissected[19]) is an open-ended challenge, and more sensitive, higher throughput, and broader dynamic range proteomics measurements are still needed to facilitate many areas of biological research. The varying sensitivity of ESI-MS as a function of liquid flow rate is the basis for increasing sensitivity by using smaller i.d. columns.[11,17] The number of charged species produced by an electrospray increases only weakly with increased flow rate; thus, lower flow rates provide increased ionization efficiencies, extending to the limit at low-nanoliter per minute flow rates where ionization efficiencies have been predicted to approach 100%.[17] By using narrower diameter separation columns, the concentration of the eluted solutes increases for a given sample loading, and ESI-MS sensitivity

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improves.[11,20] For example, the use of high-efficiency 87 cm × 15 μm i.d. capillary columns packed with 3-μm C-18 particles with ion trap-MS/MS enabled identification and confirmation of six tryptic peptides from only 7 amol of a BSA tryptic digest sample.[15] However, packing <25-μm-i.d., long columns (>80 cm) can be difficult because of the very high pressure required to overcome the low column permeability.

An approach that potentially avoids the difficulties of packing narrow-bore columns is to prepare monolithic columns, having small-sized skeletons and relatively large through pores to provide both a low-pressure drop and high column efficiency. Polymer-based monolithic columns have been prepared in a single step by in situ polymerization.[21−31] The porous structure of polymer monoliths can be tailored through careful control of the polymerization kinetics.[32−34] However, most polymers swell or shrink in organic solvents, which leads to dramatic effects on the chromatographic performance of monolithic columns and a lack of mechanical stability. In addition, because polymer-based monolithic columns are prepared in a single step, it is difficult to simultaneously ensure that the pores are large enough to limit the back pressure and that the mesopores (which contribute substantially to the overall surface area) are distributed over the desired size range. For example, a PS−DVB monolithic column demonstrated high separation efficiency for DNA fragments and proteomic samples;[27,28,31] however, because the back pressure was very high (2600−2900 psi for a 6 cm × 200 μm i.d. column with a flow rate of 2.6 μL/min at 50 °C[27]), it would not be feasible to prepare long columns suited for the analysis of very complex samples. A 10 cm × 20 μm i.d. PS−DVB monolithic column was also reported to provide 10-amol sensitivity[23] demonstrated by the detection of three peptides from a bovine catalase tryptic digest. However, the peak capacity of this PS−DVB monolithic column was relatively low.

Porous monolithic inorganic materials have been developed to overcome some of the above-mentioned drawbacks.[35−37] Silica-based monolithic columns with bimodal pore structures (through pores and mesopores) have been prepared via a sol−gel process involving the hydrolysis and polycondensation of alkoxysilanes.[37−39] Such columns have demonstrated high efficiencies and low back pressure as a result of the ability to independently control the hydrolysis and polycondensation of alkoxysilane to form the macroporous structure, followed by heating of the whole monolith to form the mesopores in the presence of urea in the starting solution.[40,41] However, the separation efficiencies obtained with these monolithic columns were not impressive.[42]

In this study, we report on the preparation of high-efficiency 20-μm-i.d. silica-based monolithic columns and their performance when combined with an on-line solid-phase extraction (SPE) column and with ESI-MS detection. This system was demonstrated to enhance sample-processing capabilities and separation efficiency and to provide very high sensitivity for analysis of complex proteomic peptides samples.

EXPERIMENTAL SECTION

Materials. Fused-silica capillary tubing with polyimide outer coating was purchased from Polymicro Technologies (Phoenix, AZ). Poly(ethylene glycol) (MW = 10 000), urea, acetonitrile (HPLC grade), toluene (anhydrous), dichloromethane (anhydrous), trifluoroacetic acid, and hexamethyldisilazane were purchased from Aldrich (Milwaukee, WI). Tetramethoxysilane and *n*-octadecyltriethoxysilane were purchased from United Chemical Technologies (Bristol, PA). The two 10 000 psi positive-feedback six-port switching valves (C2XH-0906) were purchased from Valco Instruments (Houston, TX). PEEK tubing were purchased from Upchurch Scientific (Oak Harbor, WA). Deionized water (18.2 MΩ) was prepared using a Milli-Q system from Millipore (Bedford, MA).

Silica Monolithic Column Preparation. The fused-silica capillary tubing was first treated with 5 mol/L NaOH solution at 40 °C for 3 h, then washed with water and treated with 6 M HCl at 40 °C for 3 h, washed again with water, and flushed with He. Poly(ethylene glycol) (0.88 g) and urea (0.9 g) were dissolved in 10 mL of 0.01 mol/L acetic acid. Tetramethoxysilane was added to this solution (35/65, v/v), and the solution stirred at 0 °C for 45 min. The resultant homogeneous solution was pressured into a 1-m-long pretreated fused-silica capillary and allowed to react at 30 °C for 20 h. The silica monolithic column was then treated with ammonia generated by hydrolysis of the urea for 3 h at 120 °C to form the mesopores,[39] followed by a wash with water and a second wash with acetonitrile for 4 h at ~3000 psi. Heat treatment of the column was carried out at 330 °C for 25 h. Surface modification of the silica monolith was carried out on-column by continuously feeding a solution of *n*-octadecyltriethoxysilane in toluene (10% v/v) under 1500 psi at 110 °C overnight. The column

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Table 1. Parameters for NanoLC Monolithic Columns Operated at 5000 psi[a]

LC columns (cm × μm i.d.)	u (mm/s)	F (nL/min)	ϵ
50 × 46.4 (50)	1.6	135.1	0.83
70 × 20 (20)	2.4	38.8	0.86

[a] Linear velocity (u) and volume flow rate (F) were measured as described in the Experimental Section; column porosity (ϵ) was calculated according to $\epsilon = 4F/\pi u d_c^2$, where d_c are the capillary inner diameter measured by SEM and the values in parentheses are the manufacturer's specification.

was subsequently washed with toluene followed by acetonitrile and dried at 180 °C for 2 h by flowing He through the column. Hexamethyldisilazane solution (20% in dichloromethane) was poured into the column and reacted at 160 °C for 3 h to block unreacted silanol moieties. After washing with excessive toluene followed by acetonitrile, the column was ready for use.

The 20-μm-i.d. silica-based monolithic columns were evaluated at a pressure of 5000 psi using the method of Shen et al.[11] $NaNO_2$ was used as a marker to measure the linear velocity with a UV detector. The flow rate was measured by placing two UV detectors across a 15 cm × 50 μm i.d. open tubular capillary connected to the column outlet. The flow rate was calculated from the elution time of a marker through the two UV detectors. The column porosity (ϵ) was determined using the accurately measured capillary i.d., the mobile-phase linear velocity, and volume flow rates (see Table 1).

On-Line MicroSPE-NanoLC-ESI-MS and MS/MS Experiments. The nanoESI emitter tip was pulled from a 140-μm-o.d. and 10-μm-i.d. fused-silica capillary while the capillary was flushed with compressed carbon dioxide.[11] A stainless steel union was used to connect the nanoESI emitter and the nanoLC monolithic column. Before the monolithic column was connected, a stainless steel screen (2-μm pores) was placed in the union to prolong the lifetime of the nanoESI emitter. The union body was purchased from Valco and was modified to contain internal channels with diameters of 10 μm and a size variance of ±0.5 μm.[11]

The on-line microSPE-nanoLC-ESI-MS and MS/MS studies were carried out as described previously.[14] Briefly, a 4 cm × 50 μm i.d monolithic column was used as an on-line microSPE precolumn for sample trapping. The loaded sample was then switched on-line to the nanoLC monolithic column. Operation of the nanoLC involved two 10 000 psi positive-feedback six-port switching valves, and both microSPE and nanoLC were directly connected to the valves using PEEK tubing with a stainless steel screen (2-μm pores) in the valve adapter. Purified water with the addition of 0.2% acetic acid and 0.05% trifluoroacetic acid (TFA) was used as mobile phase A. An aqueous solution of HPLC-grade acetonitrile (ACN/H_2O/TFA, 90:10:0.1, v/v/v) was used as mobile phase B. The mobile phases were pumped through the column using two Isco LC pumps (model 100DM, Isco, Lincoln, NE) to maintain the gradient at constant pressure (5000 psi); separation was achieved using an exponential gradient.[10]

An ion trap mass spectrometer (Finnigan model LCQ XP, ThermoQuest Corp., San Jose, CA) with heated capillary temperature of 140 °C and ESI voltage of 1.4 kV was used for analyses. For MS experiments, a scan time of ~1.4 s (three microscans with a maximum ion injection time of 300 ms) with an m/z range of 400−2000 was used, and the five most abundant peaks from each MS spectrum were selected for MS/MS analysis. A "collision energy" setting of 45% was applied for ion fragmentation, and dynamic exclusion was used to discriminate against previously analyzed ions (data-dependent analysis).

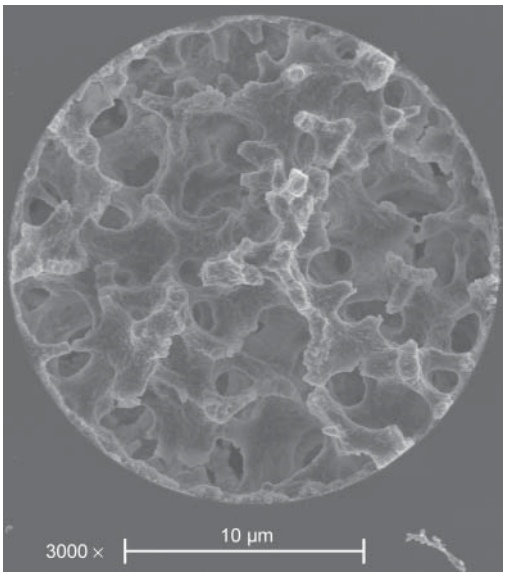

Figure 1. Scanning electron micrographs of a silica-based monolithic column (cross-section view).

Data Analysis. Peptide assignments and protein identifications were obtained by using the SEQUEST algorithm (ThermoQuest Corp.) to search against the translated predicted proteome databases for *Shewanella oneidensis*, according to previously established peptide assignment rules.[12]

Sample Preparations. A *S. oneidensis* tryptic digest was used to evaluate the performance of nanoLC-ESI-MS. The sample was prepared by using a procedure similar to that was previously reported.[43] A bovine serum albumin (BSA, Sigma, St. Louis, MO) tryptic digest was also used for the sensitivity evaluation.[15]

RESULTS AND DISCCUSION

Preparation and Characterization of the Monolithic Columns. Silica-based monolithic columns have also been recently used for proteomic analysis.[42,44] This type of column is prepared by doing the following: (1) pretreating a fused-silica capillary to attach the silica monolith to the inner wall, (2) hydrolyzing the sol−gel precursor [the most widely used are alkoxysilanes, e.g., tetramethoxysilane (TMOS) and tetraethoxysilane], (3) forming a silicate network by polycondensation in aqueous acetic acid, (4) thermal treating the silica monolith with ammonia to form the mesopore, (5) modifying the surface of the silica monolith with octadecyl reagent, and (6) end-capping to block the unreacted silanol group. Unfortunately, the peak capacities for these columns were relatively low.[44−46] Thus, improving the efficiency of silica monolithic columns will likely require careful optimization of the

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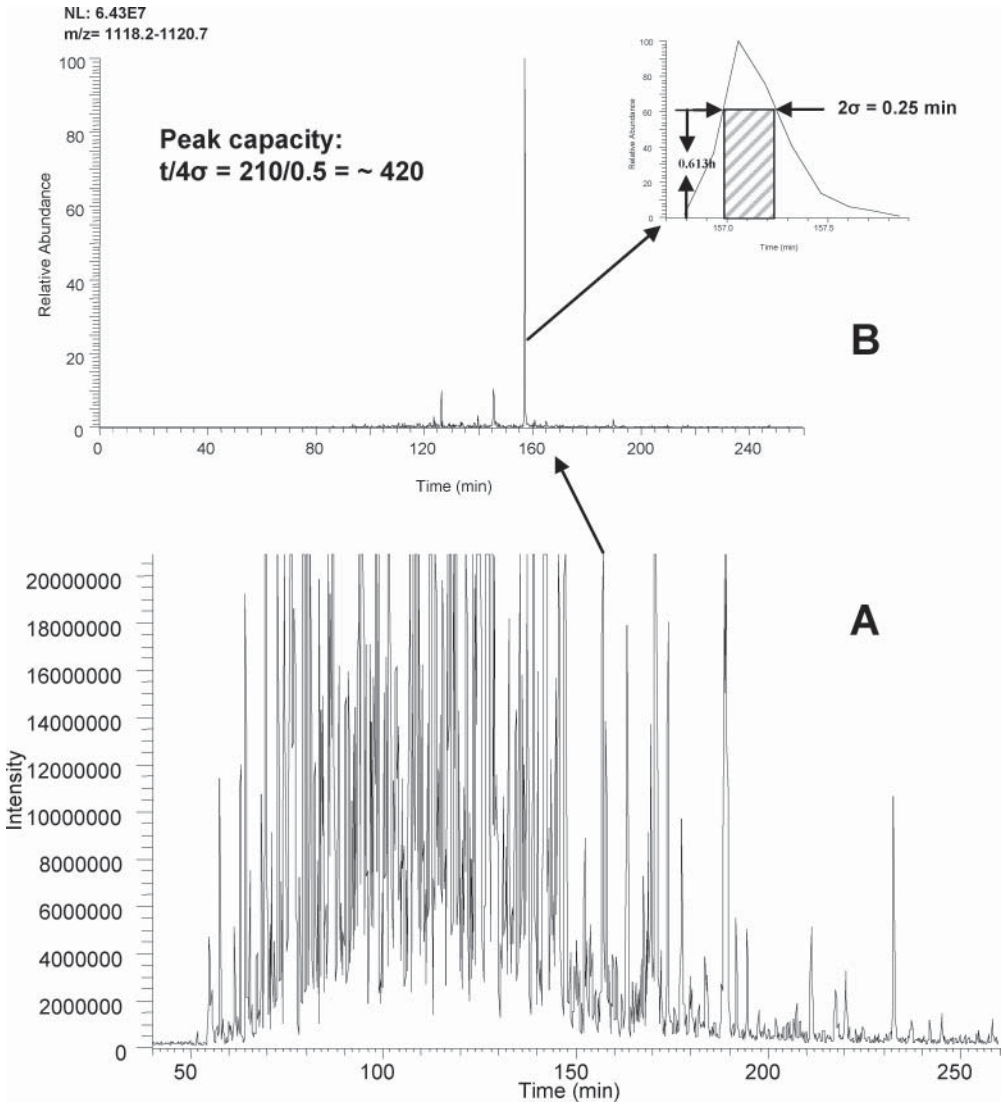

Figure 2. Base peak chromatogram from the high-efficiency on-line microSPE-nanoLC-ESI-MS/MS analysis of a 2.5-μg tryptic digest *S. oneidensis* protein sample (A) and peak capacity of the separation depicted in (A), using extracted ion chromatograms (B). Conditions: 70 cm $\times$ 20 μm i.d. silica-based monolithic column; 5000 psi; mobile phase A (H_2O, 0.2% acetic acid, and 0.05 TFA) to 75% B (90% ACN, 10% H_2O, 0.1% TFA, v/v) in 260 min and data collection at the start of the gradient. A 4 cm $\times$ 50 μm i.d. monolithic column was used as the on-line microSPE precolumn. A six-port positive feedback switching valve with a sample loop of 10 μL was used for sample introduction.

sol−gel process. Factors such as pH, temperature, reagent concentration, reaction time, and nature of the catalyst are used to control the porous structure of the sol−gel matrix.[47] The sol−gel skeleton shrinks dramatically during preparation. This shrinkage is associated with polymerization and can be avoided by attaching the sol−gel skeleton to the capillary wall. However, very large through pores can form, which will lead to an increase in the mass-transfer resistance from the mobile phase to the stationary phase.

For the present studies, we used a much higher concentration of TMOS in the starting solution (35%). An SEM micrograph of the internal structure of such a silica monolith is shown in Figure 1. The monolith appears as porous network with ~3-μm through pores. This denser sol−gel skeleton not only decreases the mass-transfer resistance from the mobile phase to the stationary phase but also increases the surface area of the silica skeleton, which determines the sample loading capacity of the column, as well as analyte retention. Chromatographic peaks become broadened and asymmetrical if the column loading capacity is too low, and low retention limits the use of gradient LC. Blocking unreacted silanol moieties greatly improves separations of biomacromolecules. To improve the end-cap efficiency, the monolithic column was immersed in an ultrasonic water bath for 5 min while the hexamethyldisilazane solution was pumped through.

Column Efficiency Performance. Gradient elution is the most powerful LC mode for the separation of complex samples. Determining the peak capacity is a very practical way of evaluating the LC separation quality and its potential for applications involving such samples.[48,49] By coupling an SPE column on-line to a high-

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efficiency nanoscale monolithic column, the sample-processing capabilities and extraction/elution characteristics can be enhanced to accommodate more complex samples. A shorter SPE column with a relatively large i.d. benefits rapid sample extraction. The shortest practical length for connecting the SPE column to the switching valve port is ~4 cm for an arrangement described previously by Shen et al.[14] The optimal i.d. of the SPE column based on previous studies in our group should be 2−3-fold larger than that of the nanoscale separation column. Therefore, in this study, we used a 4 cm × 50 μm i.d. monolithic column as the on-line microSPE column. The sample solution flow rate was ~2 μL/min through the microSPE column at 5000 psi. These optimized conditions increased the SPE-LC capability for processing solution volumes by ~50-fold compared to a single-dimension LC column with the same dimensions and enabled high-efficiency peptide separations with the 20-μm-i.d. monolithic column.

Figure 2A shows a chromatogram from an on-line microSPE-nanoLC-ESI-MS/MS separation of a *S. oneidensis* proteins tryptic digest obtained using the 70 cm × 20 μm i.d. monolithic column. The mobile phase flow was ~40 nL/min at an operating pressure of 5000 psi. Peak elution began ~50 min after the gradient start and was completed in ~260 min. Symmetric peaks were observed throughout the entire separation. As a result of operating at constant pressure with static mobile-phase mixing, the mobile-phase gradient was smooth and reproducible. The separation quality was estimated by examining the elution profile of the sample individual components on extracted ion chromatograms. The peaks have 2σ values of between 0.15 and 0.25 min for retention times from 50 to 80 min, and 0.25−0.35 min for retention times from 200 to 250 min. Figure 2B shows an example of elution profile for a high-intensity peak (157 min) from the separation shown in Figure 2A. During the overall effective separation time period of 210 min (i.e., from 50 to 260 min; see Figure 2A), the separation yielded a peak capacity of ~420. The silica-based monolithic column efficiency was somewhat lower than that for a porous 3-μm silica particle-packed column. According to the relationship recently described,[43] the peak capacity provided by a 70 cm × 20 μm i.d. silica-based monolithic column should be similar to that of a 15-cm, 3-μm silica particle-packed column. However, in practice, it is very difficult to pack long narrow-bore columns because of the very high pressure required to overcome the low column permeability. The present monolithic columns are particularly attractive for the preparation of long (>50 cm) and small i.d. column (<25 μm) columns, and where they can be effectively utilized with only moderate pressures (<5000 psi).

Sensitivity of NanoLC Ion Trap-MS/MS Analyses Using 20-μm-i.d. Silica Monolithic Columns. The sensitivity of the monolithic columns for protein identification was evaluated using a BSA tryptic digest. A sample containing 15 amol of BSA tryptic digest in 10-μL solution was loaded onto the on-line SPE 50-μm-i.d. monolithic column, separated by the 20-μm-i.d. monolithic column, and analyzed by ESI ion trap-MS/MS (Figure 3) with peptide identification using the SEQUEST algorithm and assignment rules.[12] As an example of the sensivitity achieved, three doubly charged tryptic peptides were confidently identified with high SEQUEST scores from 15 amol of tryptic-digested BSA.

Proteomic Analysis Using a 20-μm-i.d. Silica Monolithic Column and nanoLC Ion Trap-MS/MS. A tryptic digest of *S. oneidensis* proteins was used to evaluate the performance of the silica monolithic column in the nanoLC-ESI-MS/MS system. Using data-dependent MS/MS scanning, a full MS scan between 400 and 2000 *m/z* was obtained and followed by five full MS/MS scans for the five most intense ions from the MS scan. Figure 4 shows

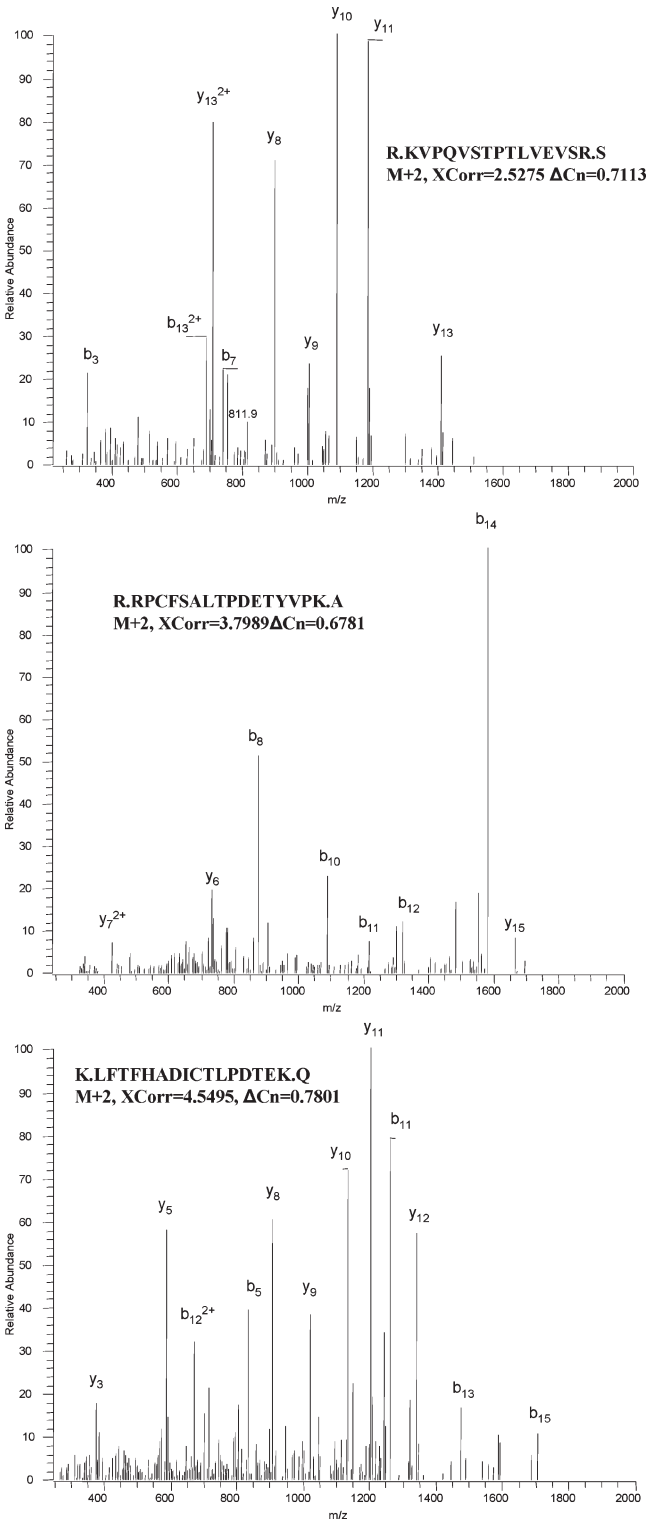

Figure 3. Results obtained from loading 15 amol of BSA tryptic digest onto the monolithic column for ion trap-MS/MS analysis. Conditions are the same as for Figure 2 except that a 1-h gradient was used. Peptide assignments were made using SEQUEST algorithm and Yates assignment rules.[12]

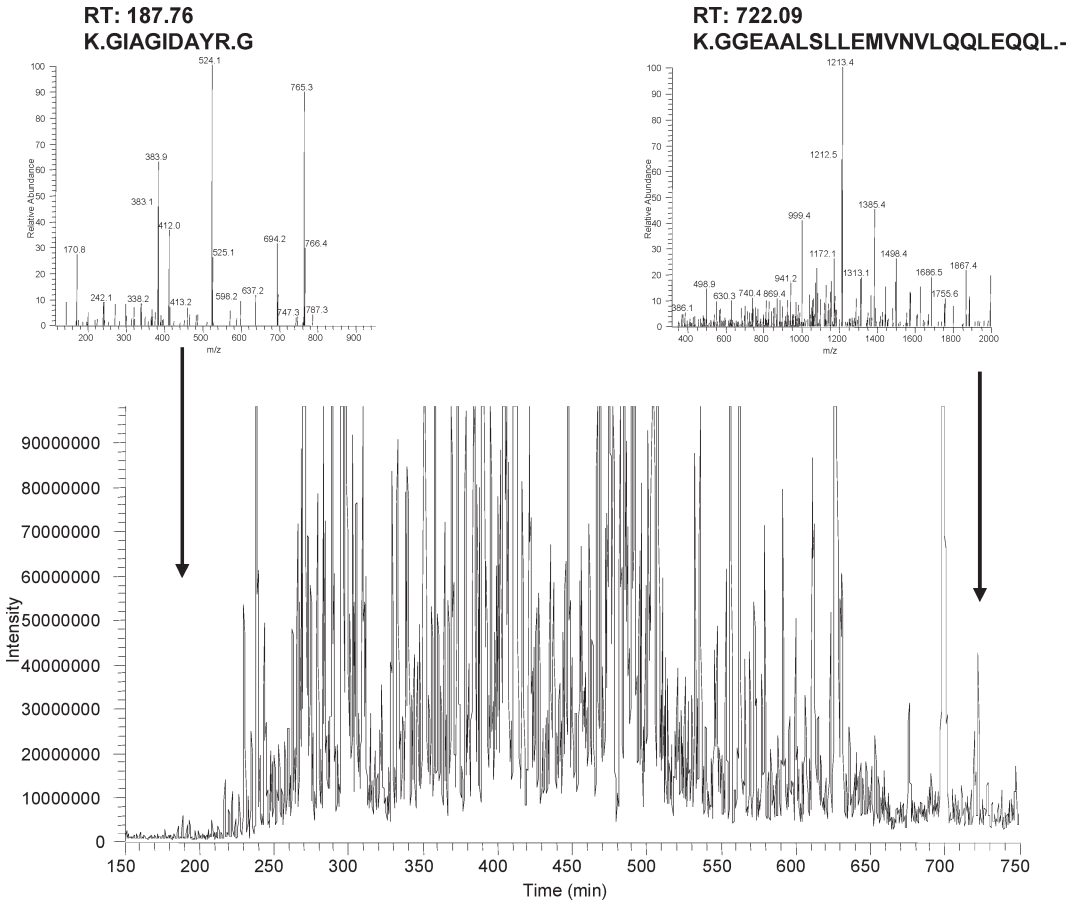

Figure 4. High-efficiency on-line microSPE-nanoLC-ESI-MS/MS analysis with extended LC gradient of for analysis of a 2.5-μg *S. oneidensis* tryptic digest sample. The experimental conditions are the same as for Figure 2, except that the gradient was extended to 750 min.

the base peak separation of a 2.5-μg tryptic digest of *S. oneidensis* proteins. Proteins were identified from peptides in the *S. oneidensis* database that had SEQUEST scores of ΔCn >0.08 and Xcorr >1.8, 2.5, and 3.5 for +1, +2, and +3 charge states, respectively. The first peptide that matched these criteria was eluted ~185 min after the gradient start. During the ~600-min effective separation time (150−750 min), 855 proteins were identified from 2367 different peptides (see Supporting Information_1).

The gradient length of the LC separation plays an important role in that, the longer the gradient, the better the separation and the number of peptides and proteins that can be identified. We investigated the effect of gradient length of the 70 cm × 20 μm i.d. silica monolithic column with regard to the number of peptides and proteins identified using the same criteria (Figure 5). The number of unique peptides increased from 1131 to 2388, and the number of identified proteins, from 493 to 860 when the effective separation time was increased from 200 to 600 min. The peak capacity increased slightly (from ~420 to ~450) when the effective separation time increased from 200 to 600 min. The signal-to-noise ratio decreased by ~50% when the effective separation time was extended from 200 to 600 min, possibly due to the increased peak width. The number of proteins identified from various *S. oneidensis* sample sizes using ion trap MS/MS are summarized in Figure 6. The 2-h LC-ion trap MS/MS analysis resulted in identification of 217 proteins (from 390 unique peptides) from a 100-ng sample and 18 proteins (from 32 unique peptides) from a 500-pg sample based upon the same spectrum analysis criteria. These results

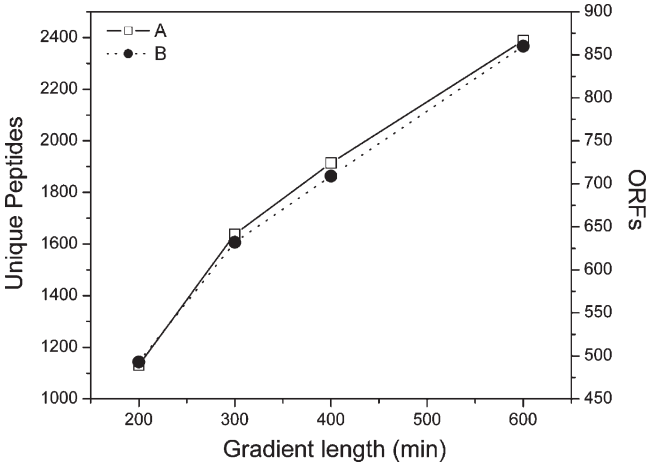

Figure 5. Effect of gradient length on the number of unique peptides (A) and proteins (B) identified from 2.5 μg of *S. oneidensis* protein tryptic digest using ion trap MS/MS.

show the potential of utilizing the small-bore silica monolithic column for analysis of samples composed of a limited number of cells, e.g., from laser capture microdissected cell samples.

Reproducibility of NanoLC-ESI-MS. We have investigated the retention reproducibility of the silica-based monolithic column on the single ion extracted chromatograms for *S. oneidensis* protein tryptic digest analyses. The run-to-run single-column and column-to-column reproducibility was determined by measuring

Table 2. Run-to-Run Reproducibility[a]

	relative retention time						relative retention time				
mass	run 1	run 2	run 3	av	RSD (%)	mass	run 1	run 2	run 3	av	RSD (%)
1309.46	0 (64.86)[b]	0 (59.45)[b]	0 (66.04)[b]	0 (63.45)[b]	0 (5.5)[c]	1622.82	0.029	0.028	0.029	0.029	2.1
2930.15	0.086	0.077	0.088	0.084	6.5	2026.28	0.144	0.131	0.136	0.137	4.9
2110.37	0.204	0.186	0.184	0.192	5.9	1716.04	0.264	0.248	0.238	0.250	5.2
1288.50	0.322	0.306	0.298	0.309	3.9	1699.94	0.381	0.370	0.361	0.371	2.8
2820.29	0.437	0.425	0.422	0.428	1.9	2470.93	0.491	0.480	0.476	0.482	1.6
1917.34	0.553	0.543	0.538	0.545	1.5	1940.16	0.592	0.582	0.578	0.584	1.3
2266.55	0.649	0.639	0.703	0.641	1.2	4304.89	0.713	0.707	0.703	0.708	0.7
2419.73	0.768	0.761	0.761	0.764	0.5	3545.94	0.806	0.798	0.801	0.801	0.5
3007.37	0.858	0.851	0.850	0.853	0.5	3771.35	0.909	0.901	0.900	0.903	0.6
3178.57	0.964	0.959	0.961	0.961	0.3	3958.59	1 (240.92)[b]	1 (241.19)[b]	1 (259.48)[b]	1 (247.20)[b]	0 (4.3)[c]

[a] Peptides were randomly selected across the elution time, other conditions are the same as for Figure 2. [b] Absolute retention time (min) of the reference peptides. [c] RSD of the absolute retention time of the reference peptides. For sequences and m/z of each peptides, see Supporting Information_2.

Table 3. Column-to-Column Reproducibility[a]

	relative retention time						relative retention time				
mass	column 1	column 2	column 3	av	RSD (%)	mass	column 1	column 2	column 3	av	RSD (%)
1261.46	0 (56.12)[b]	0 (61.01)[b]	0 (46.92)[b]	0 (54.68)[b]	0 (13.1)[c]	1236.41	0.055	0.060	0.055	0.057	5.5
1190.39	0.119	0.104	0.096	0.106	11.3	1400.69	0.161	0.155	0.153	0.156	2.8
2101.32	0.204	0.210	0.203	0.205	1.7	1824.98	0.244	0.238	0.225	0.235	4.2
2323.56	0.275	0.278	0.272	0.275	1.1	2370.62	0.316	0.322	0.303	0.313	3.1
2062.33	0.372	0.358	0.327	0.352	6.5	2074.40	0.410	0.394	0.358	0.387	6.9
1270.57	0.447	0.440	0.430	0.439	1.9	2389.62	0.492	0.498	0.461	0.484	4.0
1435.66	0.557	0.533	0.540	0.543	2.3	1756.02	0.604	0.597	0.589	0.597	1.3
1871.27	0.744	0.731	0.720	0.732	1.5	3325.71	0.773	0.749	0.728	0.750	3.0
2120.45	0.838	0.809	0.817	0.821	1.8	2325.66	0.897	0.822	0.873	0.884	1.4
2643.01	0.935	0.915	0.899	0.916	1.9	3683.08	1 (202.63)[b]	1 (195.40)[b]	1 (179.46)[b]	1 (192.5)[b]	0 (6.2)[c]

[a] Three 70 cm × 20 μm i.d. silica-based monolithic columns were used to test the column-to-column reproducibility; other conditions are the same as for Figure 2. [b] Absolute retention time (min) of the reference peptides. [c] RSD of the absolute retention time of the reference peptides. For sequences and m/z of each peptides, see Supporting Information_2.

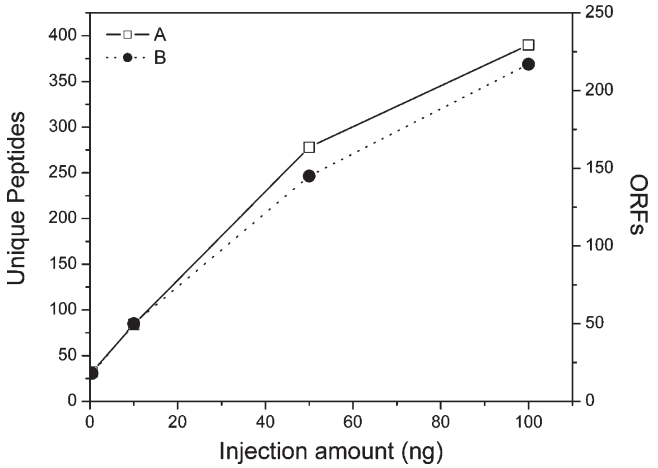

Figure 6. Number of unique peptides (A) and proteins (B) identified from various *S. oneidensis* sample sizes using ion trap MS/MS. The injection amounts were 0.5, 10, 50, and 100 ng, respectively.

the relative standard deviation (RSD) of the peptide relative retention times from three independent sets of measurements. The run-to-run reproducibility shown in Table 2 is better than 2.2% (average), and the column-to-column reproducibility shown in Table 3 for three columns was better than 3.5% (average). The normalization of elution times allows the effective correlation of analyses despite small variations in chromatographic conditions.[11] The average numbers of peptides and proteins identified in three runs with the same column were 1110 (RSD 9.3%) and 468 (RSD 1.4%), respectively, while the average numbers of peptides and proteins identified with three different columns were 1178 (RSD 16%) and 430 (RSD 8.4%), respectively.

CONCLUSIONS

Proteome analysis of small samples using a 20-μm-i.d. silica-based monolithic capillary columns with MS has been initially evaluated. The optimized connection of a 50-μm-i.d SPE column on-line to the nanoscale LC increased the sample volume loading speed by ~50-fold, enabling a 10-μL sample solution to be loaded in ~5 min. Under the reported optimized conditions, on-line SPE-nanoLC-ion trap MS provided peak capacities of ~420. High-sensitivity analysis of 15 amol of a BSA tryptic digest using on-line microSPE-nanoLC and ion trap MS was demonstrated to provide high-confidence MS/MS identifications of three different BSA peptides. Application of the high-sensitivity on-line microSPE-nanoLC-MS to the more complex *S. oneidensis* tryptic digest proteomic sample provided identification of 855 proteins from a

single 10-h nanoLC-MS/MS analysis of a 2.5-μg sample. Many potential proteomic applications that are presently impractical because of extremely small sample sizes stand to benefit from these nanoscale LC separations combined with ESI-MS. Further development of nanoLC monolithic columns using <20-μm-i.d. capillaries is in progress.

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SUPPORTING INFORMATION AVAILABLE

Additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

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