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Electro-ultrafiltration to remove sodium dodecyl sulfate in proteins extracted for proteomics

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Sodium dodecyl sulfate (SDS) is commonly used to extract membrane proteins in proteomics studies; however, it can reduce the efficiency of tryptic digestion and interfere with the results of liquid chromatography-mass spectrometry (LC-MS) analysis. Available methods for removing surfactants, such as ultrafiltration, acetone precipitation, and gel electrophoresis, are not completely satisfactory. Therefore, in this study, a new method for the depletion of SDS was established, named electro-ultrafiltration, and its performance was compared with other conventional pretreatment methods. Electro-ultrafiltration combines electrophoresis and ultrafiltration to remove SDS from protein samples. This method uses an electric field as the driving force and an ultrafiltration membrane as the separation medium. The performance of the electro-ultrafiltration method in terms of both the signals of LC-MS and the number of proteins identified was superior to that of simple ultrafiltration, but was slightly worse than that of acetone precipitation. These results demonstrate that the electro-ultrafiltration method could help to reduce the influence of SDS on protein digestion and identification, demonstrating its feasibility for application in proteomics.

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Introduction

As a powerful technique, shot-gun proteomics has been extensively used across broad applications for the identification of proteins.^{1,2} It is based on liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis of the peptides digested from protein samples.^{3,4} One of the main goals of proteomics research is analysis of membrane proteins that are bound strongly to the hydrophobic portion of cell membranes, which requires the use of a surfactant for extraction, such as the most frequently used sodium dodecyl sulfate (SDS). However, this pretreatment has a negative influence on the subsequent trypsin digestion and LC-MS analysis.^{5,6} Even if only 0.1% SDS remains in the sample, the trypsin digestion efficiency will be substantially suppressed.⁷ SDS has a strong capacity for absorption in a reversed-phase liquid chromatography (RPLC) column, and therefore can largely interfere with the separation of peptides by RPLC because of their binding with SDS.⁸ Furthermore, SDS can reduce a peptide's ionization efficiency in MS analysis. Therefore, it is necessary to remove the surfactants after using.

The available methods for removing surfactants from proteins include ultrafiltration, acetone precipitation, gel electrophoresis,

and use of an ion exchange cartridge.^{5,9–13} However, these methods are not entirely satisfactory, indicating the need to explore further improvements or develop new methods. Ultrafiltration removes detergents according to a size exclusion principle, and requires relatively expensive centrifugal ultrafiltration tube with a low protein recovery and moderate removal rate of SDS. Acetone precipitation is a convenient method that yields a high removal rate of SDS, but the protein precipitate is difficult to redissolve, leading to a low protein recovery rate.^{14,15} Gel electrophoresis-based methods result in a high removal rate of SDS approaching 100%, but also show low protein recovery in terms of the harvested peptides from in-gel digestion, and the procedure is time-consuming and laborious.

To overcome these limitations, the aim of our study was to develop a new method and device for the removal of SDS from a protein sample. The principle of the method is based on a combination of electrophoresis and ultrafiltration, and is so-named electro-ultrafiltration. Proteins and SDS molecules move in a conductive solution by exposure to an electric field, and are then separated on an ultrafiltration membrane according to their different molecular sizes. To our knowledge, there has been no similar report to remove detergents from protein samples for use in proteomics research. In this study, the effects of electro-ultrafiltration for the depletion of SDS on the subsequent LC-MS analysis and protein identification were investigated and compared with those of other conventional methods to test its feasibility in application.

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Experimental

Material

Sequencing-grade modified trypsin (Promega, Madison, WI, USA), proteinase inhibitor and iodoacetamide (Roche, Basel, Switzerland), HPLC-grade acetonitrile and formic acid (Fisher Scientific, Waltham, MA, USA), ammonium bicarbonate (Beijing Chemical Company, Beijing, China), and all other chemicals (Sigma, St. Louis, MO, USA). Water was prepared by a Milli-Q system (Millipore, Billerica, MA, USA).

The device made for electro-ultrafiltration

The device for electro-ultrafiltration was made up of two 0.5 mL 10 K centrifugal ultrafiltration tubes (Millipore). The schematic of the electro-ultrafiltration device is shown in Fig. 1. Both centrifugal ultrafiltration tubes were conglutinated into a whole in a mouth-to-mouth orientation, and three parts were separated by the two ultrafiltration membranes. Samples were added to the middle part, while the electrophoresis buffer (0.05 M Tris-HCl, pH 8.0) was added to the other two parts. Two platinum electrodes were inserted into both ends of the electro-ultrafiltration device, with the power source of an electrophoresis instrument (Liuyi, Beijing, China) as a stabilized voltage power supply.

Protein sample preparation

A protein mixture was extracted from cultured SH-SY5Y cells (iCell Bioscience Inc, Shanghai, China) by ultrasonic disruption with 50 mM Tris-HCl buffer (pH 7.5) containing protease inhibitors. SDS (0.5%) was added to the mixture, followed by different pretreatment methods for its removal, including centrifugal ultrafiltration, acetone precipitation, and electro-ultrafiltration. Furthermore, two different control samples were prepared based on the protein mixture extracted from the SH-SY5Y cells. One was tested without the addition of SDS, and the other was tested with the addition of SDS but with no subsequent pretreatment for its removal.

Pretreatment methods

Ultrafiltration. When conducting removal methods by ultrafiltration, the concentration of the detergent must be lower

than its critical micelle concentration, because only detergent monomers can be removed by size exclusion. Therefore, a 40 μ L protein sample containing 0.5% SDS was diluted by 400 μ L of 50 mM Tris-HCl buffer, which resulted in an SDS concentration below its critical micelle concentration (in the range of 5–600 g L⁻¹ (ref. 16)). The diluted solution was added to the 0.5 mL 10 K centrifugal ultrafiltration tube, and then centrifuged at 8000g for 15 min. The supernatant was sucked out and lyophilized by vacuum freeze-drying, followed by digestion and analysis.

Acetone precipitation. Cold acetone (400 μ L, -20 °C) was added to 80 μ L of the protein sample containing 0.5% SDS, left to stand for 10 min, and then centrifuged at 5000g for 10 min. The protein precipitate was dried under nitrogen to remove the acetone, followed by digestion and analysis.

Electro-ultrafiltration. A protein sample (80 μ L) containing 0.5% SDS was diluted by 800 μ L electrophoresis buffer (0.05 M Tris, 0.384 M glycine, pH 8.3) and then added into the middle part of the electro-ultrafiltration device. The electro-ultrafiltration was run for 30 min with the voltage stabilized at 100 V, and then the sample was sucked out and lyophilized by vacuum freeze-drying, followed by digestion and analysis.

Digestion, LC-MS/MS analysis, and protein identification

The dried samples were denatured and reduced in a solution containing 8 M urea, 10 mM dithiothreitol, and 50 mM NH₄HCO₃ at 37 °C for 4 h. Alkylation was performed in a 50 mM iodoacetamide solution at room temperature for 1 h in the dark, followed by dilution with 50 mM NH₄HCO₃ buffer to decrease the urea concentration to 1 M. Then, the samples were digested by trypsin at a concentration ratio of 50 : 1 (total protein : trypsin, w/w).

The digested samples were analyzed on an 1100 series HPLC system (Agilent, Santa Clara, CA, USA) coupled with an ion trap mass spectrometer (MSD Trap SL, Agilent) with a C₁₈ column (300 Å, 2.1 × 150 mm, Grace Vydac, USA) at a flow rate of 0.2 mL min⁻¹. The injection amount was 20 μ g. Gradient elution of the peptide samples was achieved with mobile phase A (0.1% (v/v) formic acid in H₂O) and mobile phase B (0.1% (v/v) formic acid in acetonitrile). The gradient program of mobile phase B consisted of 3% from 0–5 min, 3–40% from 5–55 min, 40–97% from 55–65 min, 97% from 65–68 min and 97–3% from 68–70 min, and 3% from 70–80 min. Electrospray ionization (ESI) trap MS was performed under dry gas at 10 L min⁻¹ at a temperature of 350 °C. The nebulizer pressure was 35 psi, and the capillary voltage was 3500 V. The MS mass window was 300–1800 amu.

The data produced by the RPLC/ESI-Trap MS/MS analysis were then searched against the Swissprot protein database by the MASCOT server (version 2.1; Matrix Science, Boston, MA, USA). The identification conditions were as follows: species option, human; protease, trypsin; missed cleavages, no more than 1; fixed modification, cysteine carbamidomethylation.

Results and discussion

In electro-ultrafiltration, proteins and SDS molecules are driven by an electric field. Although there have been some reports

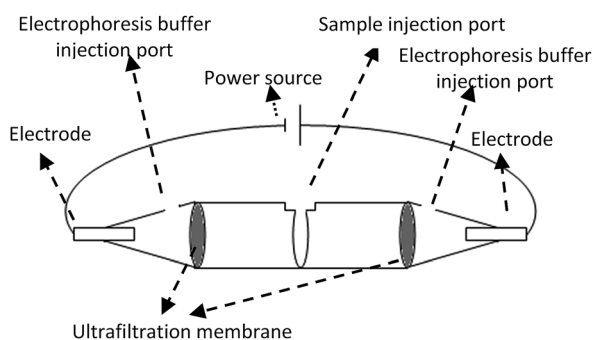


Fig. 1 Sketch map of electro-ultrafiltration device constructed by two centrifugal ultrafiltration tubes.



using electric field in the ultrafiltration for the separation of fermented or enzymatic products, the electric field was not used as a driving force for separation, but just an assistant means to reduce concentration polarization.^{17,18} In some sense, the proposed principle of electro-ultrafiltration is similar to that of gel electrophoresis. Both methods use an electric field as the driving force and size exclusion as a separation approach. The key difference is that gel electrophoresis requires a gel as the separation medium, which makes the procedure more time-consuming and laborious, whereas in electro-ultrafiltration, the separation is carried out in free solution without requiring the support of a solid medium.

The effects and efficiency of SDS removal based on different methods have often been compared and evaluated through assessment of the signals of LC-MS or the number of proteins identified.^{11,19–21} A stronger signal of LC-MS and a better separation effect would indicate that more SDS had been efficiently removed from the pretreatment. Fig. 2 shows the LC-MS total ion chromatograms (TICs) from the two different control samples with and without SDS. A larger and wider peak was observed at the retention time of 60–70 min in the TIC from the control sample with the addition of SDS than in that from the other control sample without the addition of SDS. This result clearly demonstrated that the large and wide peak was due to incomplete tryptic digestion by SDS, demonstrating the negative influence of SDS on RPLC separation, which is in accordance with the results of previous studies.^{6,22}

Fig. 3 compares the TICs obtained from the samples subjected to the various pretreatments for SDS removal. As shown in Fig. 3(a), the large and wide peak at the retention time of 60–70 min observed in the TIC from the control sample with SDS was diminished in the TIC from the sample pretreated by centrifugal ultrafiltration, suggesting that this treatment method improved the tryptic digestion and RPLC by effectively removing SDS to some extent. Fig. 3(b) shows that acetone precipitation induced further improvement in the tryptic digestion and RPLC compared to centrifugal ultrafiltration. Indeed, besides reduction in the peak at the retention time of 60–70 min, the peaks during the retention times before 60 min were obviously higher. As shown in Fig. 3(c), the newly developed electro-ultrafiltration could also diminish the peak at the retention time of 60–70 min, suggesting that electro-ultrafiltration could also improve tryptic digestion and RPLC by removing SDS to some degree.

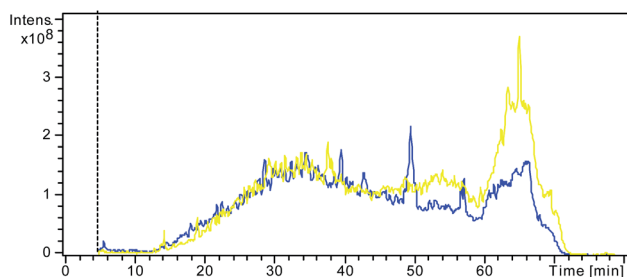


Fig. 2 Overlap of the LC/MS TIC from the control sample without addition of SDS (blue) and the other control sample with addition of SDS but no pretreatment to remove SDS (yellow).

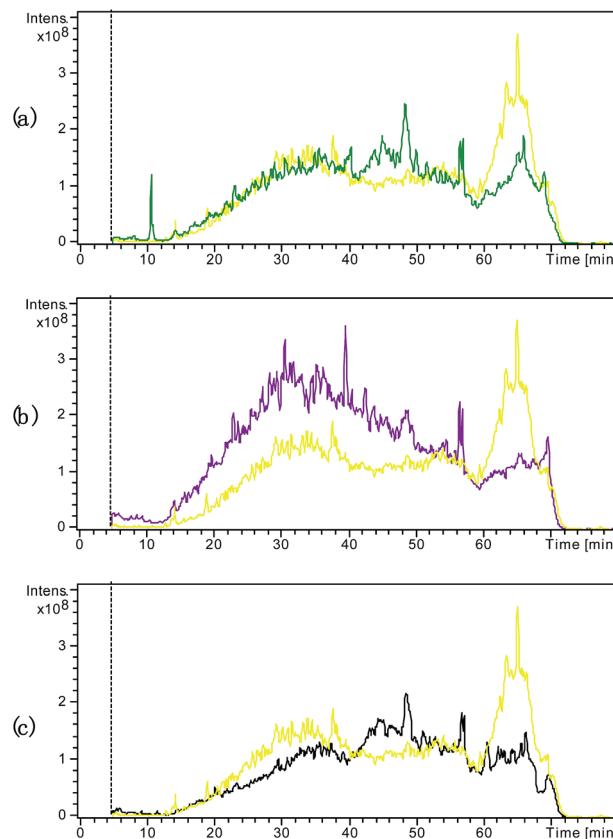


Fig. 3 Overlap of the LC/MS TIC from the control sample with addition of SDS but no pretreatment to remove SDS (yellow) and the different pretreated samples by centrifugal ultrafiltration (a), acetone precipitation (b) and electro-ultrafiltration (c).

Fig. 4 compares the extracted ion chromatograms (EICs) of a long retaining peptide with m/z 979.1 from different treated samples. The peptide was subsequently identified as VGAGAPVY-MAAVLEYLTAEILELAGNAAR from Histone H2A type. As can be seen, it is large and similar in size that the EIC peaks from the sample without SDS and the samples pretreated by acetone precipitation and electro-ultrafiltration, while the EIC peak from the sample pretreated by centrifugal ultrafiltration is smaller and no EIC peaks was observed from the sample with addition of SDS. The comparisons reflect the strong influence of SDS and the better effect of electro-ultrafiltration on removal of SDS than that of centrifugal ultrafiltration.

The data sets produced by the RPLC/ESI-Trap MS/MS analysis with the three types of samples were then searched against the Swissprot protein database by MASCOT. On average, 85 ± 9 proteins were identified from the analysis using the samples subjected to ultrafiltration, whereas 120 ± 7 and 108 ± 7 proteins were identified from samples subjected to acetone precipitation and electro-ultrafiltration, respectively. By contrast, only 54 ± 6 proteins were identified from the control samples without pretreatment for the removal of SDS, which suggested that ultrafiltration, acetone precipitation, and electro-ultrafiltration all improved the LC-MS/MS-based protein identification by removal of SDS to some degree. Although



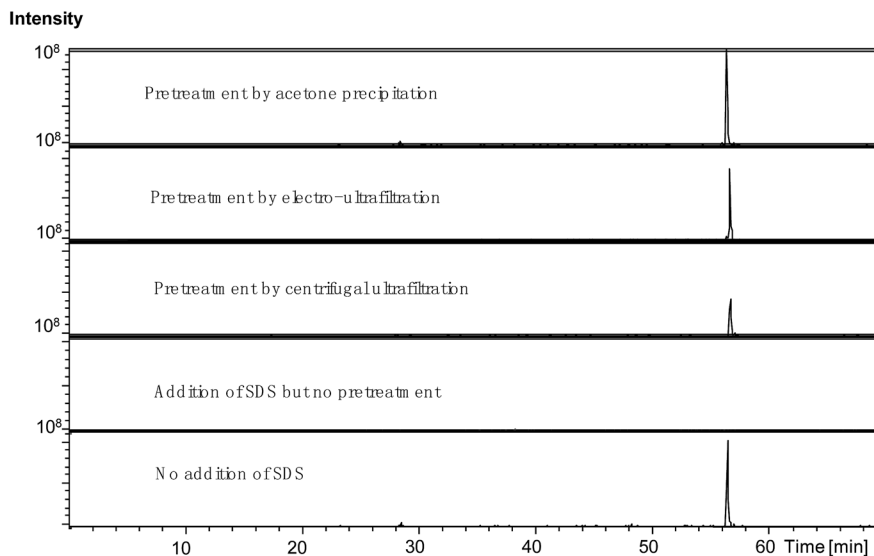


Fig. 4 The EICs of a long retaining peptide with m/z 979.1 from different pretreated samples. The peptide was identified as "VGAGAPVY-MAAVLEYLTAEILELAGN-AAR" belonging to Histone H2A type.

electro-ultrafiltration did not show the best identification result, its feasibility for improving LC-MS/MS-based proteomics research was nevertheless revealed. Moreover, the present work represents a preliminary study of electro-ultrafiltration, and therefore many improvements are expected to strengthen its SDS-removal function.

Ultrafiltration depends on the centrifugal force or other aspects of fluid pressure to drive solutions through the membrane and achieve the desired concentration and separation. However, a major operating problem for membrane filtration is concentration polarization, resulting in the buildup of solutes on the membrane surface, thereby reducing the separation efficiency.^{23–26} In electro-ultrafiltration, the power of filtration is derived from an electric field, which drives charged molecules to move through the solution. Moreover, the driving speed and directions differ for different molecules, depending on their charges, sizes, and masses. Therefore, SDS molecules would theoretically be expected to move faster toward the membrane surface than proteins in electro-ultrafiltration, and the variation in the rates of movement of different proteins would reduce the concentration polarization on the membrane surface. Furthermore, centrifugal ultrafiltration is highly dependent on the tenacity of the membrane due to the high fluid pressure, while electro-ultrafiltration is not. For electro-ultrafiltration, an ordinary dialysis membrane is sufficient, and further membrane support is not needed; thus, its manufacturing costs will be lower than those required for centrifugal ultrafiltration.

The electro-ultrafiltration method combines electrophoresis and ultrafiltration to remove SDS from a protein sample, but it cannot concentrate samples as well as ultrafiltration and acetone precipitation. However, some other convenient methods such as lyophilization can be used after electro-ultrafiltration to improve the concentration effect. Theoretically, electro-ultrafiltration should also be able to remove other

small charged ions and surfactants from protein samples besides SDS, thereby reducing their interferences on peptide signals in the subsequent MS analysis.

In summary, a new method termed electro-ultrafiltration was demonstrated to be able to improve protein digestion and LC-MS-based protein identification by the efficient removal of SDS, indicating its feasibility for application in proteomics research for membrane proteins. This study provides a foundation for the application of electro-ultrafiltration in proteomics in the future. However, as this is a preliminary analysis of electro-ultrafiltration, further improvements are needed to strengthen its function before it can be adopted widely in proteomics research.

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