research articles Journal of proteome research

# Evaluation of the Application of Sodium Deoxycholate to Proteomic Analysis of Rat Hippocampal Plasma Membrane

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**Received March 23, 2006**

Detergents have been widely used for the solubilization of membrane proteins and the improvement of their digestion. In this paper, we have evaluated the application of sodium deoxycholate (SDC) to the solubilization and digestion of rat hippocampal plasma membrane (PM) proteins. For in-solution digestion, rat hippocampal PM fraction from sucrose-density gradient centrifugation was solubilized by boiling in 1.0% SDC, and directly digested without dilution. During the in-gel digestion of the hippocampal PM proteins separated by SDS-PAGE, 0.1% SDC was added. Before analysis of peptide mixture by liquid chromatography and electrospray mass spectrometry, SDC in the tryptic digests was removed by centrifugation following acidification. Use of 1.0% SDC in solubilization and in-solution digestion of rat PM proteins had led to 77 PM or membrane-associated proteins identified, a more than 2-fold increase over that by use of SDS. The addition of 0.1% SDC to the in-gel digestion of SDS-PAGE-resolved membrane proteins remarkably enhanced the coverage of tryptic peptides and the number of hydrophobic membrane proteins identified. Being a cheaper and more tractable acid-insoluble detergent, SDC could be used at higher concentration in the solubilization and tryptic digestion of proteins including PM proteins with the purpose of enhancing the protein solubility and at the same time making no interference with trypsin activity and subsequent analyses.

**Keywords:** plasma membrane • proteomics • sodium deoxycholate • rat • hippocampus • detergent

## Introduction

The plasma membrane (PM) provides main sites for exchanges of information and substances. Proteins that are integral to or associated with lipid membranes often have complex functions and can thus provide key information that is of great interest to the researchers studying the functions of a biological system. However, the very amphiphilic nature that allows them to be localized in the membrane also makes them water-insoluble and notoriously difficult to study. In recent years, although PM proteomics technologies have made rapid progress, membrane protein analysis has lagged behind soluble proteins and still presented a great challenge. Hippocampus is a very biologically important tissue in the brain and plays a functional role in learning, cognition, and memory. Many neurological diseases are also closely related to the tissue. Hippocampal cells are the representatives of central neurons. A comprehensive profile of their PM proteome would provide an insight into the mechanisms of such as learning and memory and propose a therapeutic potential in neurological disease. However, although some of the proteins in the tissue were studied,[1−3] the proteomic research on its PM is still a subject to be further understood.

Because current proteomic strategies limit the direct analysis of intact proteins, solubilization of PM proteins and generation of detectable peptides are crucial to the identification of PM proteins. The efficiency of digestion is greatly dependent on the solubilization of PM proteins. Most refinements have been directed at the solubilization of membrane fractions and improvement of PM protein digestion in solution and in gel.

In shotgun digestion approaches, some researchers have used mild and strong detergents to extract and solubilize membrane proteins,[4−6] and then the proteins are digested with protease in an aqueous solution containing a certain concentration of detergent. Sodium dodecyl sulfate (SDS) was one of the most frequently used detergents. However, these detergents present some critical disadvantages that they significantly reduce the trypsin activity, interfere with high-performance liquid chromatography (HPLC) separation, and affect the subsequent peptide analysis by mass spectrometry.[7] Although most membrane proteins can be solubilized in a solution containing high concentrations of detergents, the solution must subsequently be diluted to reduce the concentration of detergents prior to trypsin digestion lest the detergents should reduce the activity of the enzyme. Nevertheless, the solubility of the PM proteins could not always be maintained during the course of proteolytic digestion due to the dilution. On the other hand, although certain measures have been made to remove interfering detergents from tryptic peptides prior to liquid chromatography and mass analysis, it was unclear how efficiently detergents such as SDS were eliminated.[7,8]

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10.1021/pr060112a CCC: $33.50 



Published on Web 09/19/2006

In-gel digestion is one of the critical steps for sensitive identification of proteins resolved by gel-based approaches. Efficient protein digestion is required to obtain peptide peaks necessary for protein identification by mass spectrometry. Once the protein bands or spots have been selected and excised from the gels, they are in-gel-digested into peptides, which are then extracted from the gel pieces for subsequent protein identification. A limitation of the in-gel digestion approaches for membrane proteins is the hydrophobicity of the proteins. It is usually difficult to obtain high-sequence coverage because the membrane-spanning segments are either not readily accessible to proteolytic enzymes or not easily extracted from the gel pieces after digestion. For improvement of in-gel digestion of membrane proteins, various in-gel digestion methods have been recently developed using some detergents such as sodium 3-[(2-methyl-2-undecyl-1,3-dioxolan-4-yl) methoxy]-1-propanesulfonate (ALS), *n*-octyl glucoside (OG), and 5-cyclohexyl-1-pentyl-b-D-maltoside (CYMAL-5). For example, pretreatment of gel pieces containing protein spots separated by 2-DE with a small amount of ALS, an acid-labile surfactant, prior to trypsin digestion led to increase in the digested peptides eluted from the gels.[9−17] However, these detergents are usually expensive, and many procedures still remain to be optimized for them.

Sodium deoxycholate (SDC), a cheaper and more readily available acid-insoluble detergent, is originally a native strong ionic detergent and is found in mammalian biles at high concentrations.[18] It is relatively safe to use as a common chemical reagent. Being a potentially membrane-damaging surfactant, SDC was also employed by some researchers to solubilize and extract certain PM proteins at low concentrations, alone or as a component of a complex sample lysis solution.[4,5,19] In this paper, we report the application of SDC alone to the solubilization and extraction of rat hippocampal plasma membrane proteins at concentration of 1.0% and to the in-gel digestion of the membrane proteins at concentration of 0.1% for the enhancement of the digestion efficiency and peptide recovery. Our data demonstrated that, compared with SDS, addition of SDC had significantly increased the coverage of tryptic peptides and the number of hydrophobic membrane protein identified.

## Materials and Methods

**Materials.** Trypsin (proteomics sequencing grade), dithiothreitol (DTT), iodoacetamide (IAA), trifluoroacetic acid (TFA), 4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid (HEPES), α-cyano-4-hydroxycinnamic acid (HCCA), and sucrose were purchased from Sigma-Aldrich (St. Louis, MO). Acrylamide, bisacrylamide, thiourea, urea, glycine, tris, and sodium dodecyl sulfate (SDS) were from Amresco (Solon, OH). Sodium deoxycholate (SDC) was obtained from Sigma-Aldrich (St. Louis, MO). Bio-Rad DC protein assay kit was from Bio-Rad Laboratories (Hercules, CA). HPLC-grade acetonitrile and acetone were purchased from Shanghai Chemical Reagent Company of National Medicine Group of China (Shanghai, China). Water was obtained with a Milli-Q Plus purification system (Millipore, Bedford, MA). All other regents were domestic products of highest grade available. Sprague−Dawley rats (weighting 150−200 g) were from Hunan Academy of Traditional Chinese Medicine (Changsha, China).

**Effect of SDC on Trypsin Activity.** By the use of bovine serum albumin (BSA) as a model protein, tryptic digestion (1:50 w/w enzyme-to-protein ratio) was performed at 37 °C overnight in solutions of 25 mM $NH_4HCO_3$ (pH8.0) containing 0.0%, 1.0%, 2.0% SDC, respectively. For quenching the enzymatic reactions and removing SDC prior to mass spectrometric analysis, the solutions were acidified by adding 0.1% TFA (final concentration) and centrifuged at 15 000*g* for 15 min. The resultant supernatants were analyzed by matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry (MALDI-TOF MS) for detection of digestion efficiency.

**Preparation of Rat Hippocampal PM.** Rats were killed, and their hippocampal tissues were excised and then washed with 0.9% NaCl. All fresh tissues were grinded into powder in liquid nitrogen using a pestle and subsequently lysed in an ice-cold solution containing 50 mM HEPES (pH7.4), 1 mM $CaCl_2$, and 0.1 mM phenylmethylsulfonyl fluoride (PMSF). The mixture was centrifuged at 600*g* for 10 min at 4 °C to remove nuclei and unbroken cells. The supernatant was collected and then centrifuged at 24 000*g* (Beckman; SW28 rotor) at 4 °C for 30 min. The yielding membrane pellets were transferred to SW-28 tubes and subjected to linear sucrose-density gradient centrifugation. Linear gradients of 10−50% sucrose (w/w) were prepared. A 1-mL volume of the sample fraction was layered on the top, and then centrifuged at 100 000*g* (Beckman; SW28 rotor) at 4 °C for 8 h. The floating PM band was collected from the centrifugation tubes, washed twice with ice-cold solution containing 50 mM HEPES buffer (pH7.4) and 0.1 mM PMSF, and then stored at −70 °C until use.

**Solubilization and In-Solution Digestion of PM Proteins.** Rat hippocampal PM fraction from linear sucrose-density gradients was solubilized by boiling in 20 μL of 1.0% SDC/25 mM $NH_4HCO_3$ (pH8.0). PM proteins were quantified by Bio-Rad DC Protein Assay kit with BSA as a standard. Membrane proteins were reduced by 10 mM DTT at 50 °C for 1 h and alkylated by 55 mM IAA at room temperature in the dark for 1 h. Finally, trypsin (1:50 w/w enzyme-to-protein ratio) was added, and the mixture was incubated at 37 °C overnight. The final SDC concentration in the digestion mixture was 1.0% in a total volume of 100 μL. For comparison, referring to the reports in the literatures,[8,20−22] another aliquot of rat hippocampal PM fraction containing the same amount of PM proteins was solubilized by boiling in 20 μL of 0.5% SDS in 25 mM $NH_4HCO_3$. After conventional reduction and alkylation, the protein mixture was diluted to 0.1% SDS in a total volume of 100 μL. Subsequently, the same amount of trypsin was added, and the mixture was incubated at 37 °C overnight.

**Fractionation of In-Solution Digestive Peptide Mixture.** To decrease the complexity of sample, the peptide mixture of in-solution enzymatic digestion, after acidification and centrifugation to remove SDC, was fractionated on a WATERS Alliance2690 HPLC system using a $C_{18}$ column (3 mm × 250 mm, 5 μm, Phenomenex). The solvents were 0.1% TFA (buffer A) and ACN containing 0.1% TFA (buffer B). After column equilibration in solvent A, 100 μL of the peptide mixture (equivalent to 100 μg of proteins) was injected. Detection wavelength was set at 215 nm. Peptides were eluted from the column by a 9-step protocol. Step 1 consisted of a 20-min 0% B elution. The following steps were 10-min of *x*% solvent B elutions. The 10-min solvent B percentages (*x*) were 15%, 20%, 25%, 30%, 40%, 50%, 60%, respectively. The final step was a 10-min 100% solvent B elution. All the flow rates were 0.5 mL/min. Fractions eluted by various gradients were collected and dried in a Speed Vac, respectively. The peptide mixture of in-solution enzymatic digestion in the presence of SDS was also fractionated on the same HPLC system and under the same conditions.

**SDS-PAGE and In-Gel Digestion.** Rat hippocampal PM fraction from linear sucrose-density gradient centrifugation was

dissolved in sample lysis solution containing 8 M urea, 2 M thiourea, 4% (w/v) CHAPS, 1% (w/v) NP-40, 65 mM DTT, and 0.5 mM PMSF. The PM proteins were precipitated by acetone, and the pellets were used for SDS-PAGE. Approximately 200 $\mu$g of PM proteins, quantified by Bradford method, were dissolved in loading sample buffer containing 10 mM Tris-HCl, pH 6.8, 20% glycerol, 4% SDS, 100 mM DTT, and 0.005% bromophenol blue and boiled for 3 min. After centrifugation at 10 000*g* for 15 min, the proteins in the supernatants were resolved in parallel lanes (100 $\mu$g each) via SDS-PAGE using 4.8% stacking gel and 11.5% separation gel and then stained with Coomassie blue R-350. The Middle Molecular Weight Calibration Kit (Generay, China) was used as standard molecular weight marker proteins and also loaded in parallel lanes. The bands containing proteins were excised from the gels, destained with 50% ACN in 50 mM $NH_4HCO_3$, and dried in a Speed Vac. The proteins were reduced with 10 mM DTT in 25 mM $NH_4HCO_3$ at 56 °C for 1 h, and alkylated by 55 mM IAA in 25 mM $NH_4HCO_3$ in the dark at room temperature for 45 min. After washes with 50% and 100% ACN in 25 mM $NH_4HCO_3$ sequentially, the gels were dried in a Speed Vac.

For detecting the effects of SDC on in-gel digestion of proteins, the dried PM protein-containing gel pieces from one lane were re-swollen in 7 $\mu$L of 12.5 ng/$\mu$L trypsin in 25 mM $NH_4HCO_3$ containing 0.1% SDC (final concentration), and those from the other lane were used as controls and were re-swollen in 7 $\mu$L of 12.5 ng/$\mu$L trypsin in 25 mM $NH_4HCO_3$ containing no SDC. Some standard protein bands from another two lanes were also used for the detection. After an incubation of 16 h at 37 °C, the released peptides were extracted twice with a 50% ACN solution in 15-min sonication. The pooled extracts were acidified and centrifuged to remove SDC, and the supernatants were concentrated in a Speed Vac.

**Analysis Using MALDI-TOF MS.** Partial tryptic digests were analyzed by mass spectrometry for evaluation of the efficiency of digestion. The analyses were performed on a Voyager-DE-STR mass spectrometer (Applied Biosystems) in a positive ion reflector mode with accelerating voltage of 20 kV and delayed extraction of 250 ns. HCCA was used as the matrix. An external calibration was done prior to the analysis of samples.

**CapLC−MS/MS Analysis of Tryptic Digests.** Fractionated tryptic digests from in-solution digestion and peptide mixtures from in-gel digestion were analyzed by tandem mass spectrometry coupled with high-performance liquid chromatography using a capillary column (75 $\mu$m × 150 mm, Waters) for protein identification (CapLC−MS/MS). Peptides were reconstituted in an aqueous solution of 5% ACN before injection. The LC solvents were 0.1% formic acid/4.9% ACN/95% $H_2O$ (v/v/v) (buffer A) and 0.1% formic acid/4.9% $H_2O$/90% ACN (v/v/v) (buffer B). The on-line LC separation used a gradient from 5% to 50% B in 65 min and then 65% to 95% B in 10 min, followed by 95% B for 10 min. The flow rate was 3 $\mu$L/min. Peptides eluted from CapLC were directly injected into the coupled Q-TOF mass spectrometry (Micromass, Manchester, U.K.) with a nanoelectrospray. Peptides were analyzed using the data-dependent MS/MS mode over the $m/z$ range of 400−2000. The three most abundant ions detected in each MS scan were selected for collision-induced dissociation (MS/MS) analysis. MS and MS/MS data were acquired and processed automatically using the software MassLynx (Micromass, Manchester, U.K.).

**Data Analysis and Bioinformatics.** The initial MS and MS/MS data were analyzed using a Perl script which was written in-house to parse significant hits from Mascot output files (html files) into tab delimited data files suitable for subsequent data analysis. The Bioperl libraries were used to generate FASTA formatted protein sequence from the international protein index (IPI) rat databases for proteins identified by each MS experiment, and an automated sequence retrieval script was written in Perl. The theoretical molecular weights, isoelectric point (p*I*), and percentage of protein sequence covered by the matched peptides were retrieved from Mascot output files. The grand average hydropathy (GRAVY) values for identified proteins and peptides were analyzed using the ProtParam program (available at http://cn.expasy.org). The proteins exhibiting positive GRAVY values were recognized as hydrophobic.[23] Mapping of transmembrane (TM) regions for the identified proteins was conducted using the transmembrane hidden Markov model (TMHMM) algorithm (available at http://www.cbs.dtu.dk/services/TMHMM) by submitting the FASTA files.[21,24] Further information on the subcellular location and function of identified proteins were predicted by gene ontology (GO) component and function terms, respectively, text-based annotation files which were available for download from GO database ftp site at ftp://ftp.geneontology.org/pub/go.[25]

**Table 1.** Comparison of Statistical Parameters of PM Proteins or Membrane-Associated Proteins Identified Using SDC or SDS In-Solution Tryptic Digestion

| parameter | method | |
|---|---|---|
| | using SDC | using SDS |
| Total membrane proteins | 77 | 31 |
| Total matched peptides | 88 | 48 |
| No. of proteins with one or more trans-membrane domains | 50 | 22 |
| No. of membrane proteins with positive GRAVY values | 23 | 16 |
| Average p*I* | 7.07 | 7.10 |
| Average MW (kDa) | 78 | 68 |
| Average coverage (%) | >12 | 10 |

## Results and Discussion

**Effect of SDC on Trypsin Activity.** In this experiment, BSA tryptic digests in 0.0%, 1.0%, and 2.0% SDC were detected by MALDI-TOF. All the three spectra (spectra not shown) showed very close similarity, and there were no obvious differences in the number and signal intensity of main peptides between the spectra. This suggested that SDC did not significantly affect the activity of trypsin at the concentrations up to 2.0%, and a high concentration of SDC needed for enhancing solubility of PM proteins was compatible with tryptic digestion. In contrast, many other detergents, such as SDS,[7,10,26] severely decreased the tryptic activity even at low levels and must be diluted prior to tryptic digestion.

**Comparison of SDC and SDS Used in Proteomic Analysis of PM Based on In-Solution Digestion.** As shown in Tables 1 and 2 and Figure 1, compared with using SDS, solubilization of rat hippocampal PM proteins with 1.0% SDC and direct digestion in the same solution had obtained much better proteomic analysis results, mainly reflecting in the higher numbers of the total PM proteins or membrane-associated proteins, the total matched peptides, the proteins with one or more transmembrane domains, and the membrane protein with positive GRAVY value. In addition, average peptide coverage and average molecular weight of proteins identified using

**Table 2.** PM Proteins or Membrane-Associated Proteins Identified Based on In-Solution Tryptic Digestion in the Presence of SDC or SDS

| accesssion no. | protein name | MW(Da) | p*I* | GRAVY[a] | TM[b] | GO annonation[c] | method[d] |
|---|---|---|---|---|---|---|---|
| IPI00231885 | Splice Isoform Glt-1A of Excitatory amino acid transporter 2 | 62320 | 6.44 | 0.428 | 9 | PM | A, B |
| IPI00213663 | Splice Isoform Glt1 of Excitatory amino acid transporter 2 | 62579 | 6.29 | 0.413 | 9 | PM | A, B |
| IPI00564673 | Glutamate transporter GLT1b | 61390 | 6.15 | 0.483 | 9 | PM | A, B |
| IPI00454545 | Retina glutamate transporter GLT1c | 15911 | 4.74 | 0.143 | 1 | PM | A, B |
| IPI00195326 | 29 kDa protein | 29239 | 9.01 | 0.51 | 4 | PM | A, B |
| IPI00231102 | Myelin proteolipid protein | 26940 | 8.22 | 0.781 | 4 | PM | A, B |
| IPI00230956 | Splice Isoform GLAST-1A of Excitatory amino acid transporter 1 | 54527 | 6.93 | 0.35 | 8 | PM | A |
| IPI00324377 | Splice Isoform GLAST-1 of Excitatory amino acid transporter 1 | 59830 | 8.51 | 0.379 | 10 | PM | A |
| IPI00206170 | Synaptotagmin-1 | 47765 | 8.44 | −0.417 | 1 | PM | B |
| IPI00339124 | Sodium/potassium-transporting ATPase beta-1 chain | 35762 | 8.83 | −0.524 | 1 | PM | A, B |
| IPI00204447 | Vesicle associated membrane protein 2B | 14557 | 5.44 | −0.113 | 1 | PM | A |
| IPI00205372 | Splice Isoform 1 of Syntaxin binding protein 1 | 67925 | 6.49 | −0.41 | 0 | PM | A |
| IPI00230938 | Splice Isoform 2 of Syntaxin binding protein 1 | 69091 | 6.32 | −0.414 | 0 | PM | A |
| IPI00194875 | Splice Isoform WB of Plasma membrane calcium-transporting ATPase 2 | 137922 | 5.7 | −0.166 | 8 | PM | A |
| IPI00231309 | Splice Isoform WA of Plasma membrane calcium-transporting ATPase 2 | 132666 | 5.98 | −0.126 | 8 | PM | A |
| IPI00231310 | Splice Isoform XA of Plasma membrane calcium-transporting ATPase 2 | 129880 | 5.98 | −0.13 | 8 | PM | A |
| IPI00231311 | Splice Isoform YA of Plasma membrane calcium-transporting ATPase 2 | 131260 | 5.98 | −0.109 | 8 | PM | A |
| IPI00231312 | Splice Isoform ZA of Plasma membrane calcium-transporting ATPase 2 | 128473 | 5.98 | −0.112 | 8 | PM | A |
| IPI00231313 | Splice Isoform XB of Plasma membrane calcium-transporting ATPase 2 | 135135 | 5.7 | −0.171 | 8 | PM | A |
| IPI00231314 | Splice Isoform YB of Plasma membrane calcium-transporting ATPase 2 | 136515 | 5.7 | −0.15 | 8 | PM | A |
| IPI00231315 | Splice Isoform ZB of Plasma membrane calcium-transporting ATPase 2 | 133729 | 5.7 | −0.154 | 8 | PM | A |
| IPI00231316 | Splice Isoform WC of Plasma membrane calcium-transporting ATPase 2 | 133410 | 6.08 | −0.135 | 8 | PM | A |
| IPI00339139 | Splice Isoform XC of Plasma membrane calcium-transporting ATPase 2 | 130623 | 6.08 | −0.139 | 8 | PM | A |
| IPI00339140 | Splice Isoform YC of Plasma membrane calcium-transporting ATPase 2 | 132003 | 6.08 | −0.118 | 8 | PM | A |
| IPI00339141 | Splice Isoform ZC of Plasma membrane calcium-transporting ATPase 2 | 129217 | 6.08 | −0.121 | 8 | PM | A |
| IPI00557164 | Tax_Id=10116 Protein | 129684 | 6.49 | −0.134 | 8 | PM | A |
| IPI00558864 | Tax_Id=10116 Protein | 133452 | 7.92 | −0.186 | 8 | PM | A |
| IPI00561115 | Tax_Id=10116 Protein | 131091 | 6.49 | −0.152 | 8 | PM | A |
| IPI00568107 | 64 kDa protein | 64117 | 6.11 | −0.214 | 4 | PM | A |
| IPI00390542 | 38 kDa protein | 38542 | 9.08 | −0.086 | 0 | PM | A |
| IPI00568168 | 112 kDa protein | 113618 | 5.36 | −0.024 | 8 | PM | A, B |
| IPI00231451 | Sodium/potassium-transporting ATPase alpha-3 chain | 113045 | 5.26 | −0.007 | 8 | PM | A, B |
| IPI00563682 | 55 kDa protein | 55621 | 6.26 | −0.039 | 4 | PM | A, B |
| IPI00326305 | Sodium/potassium-transporting ATPase alpha-1 chain precursor | 114293 | 5.3 | 0.002 | 10 | PM | A, B |
| IPI00204644 | Splice Isoform SNAP-25b of Synaptosomal-associated protein 25 | 23528 | 4.66 | −0.865 | 0 | PM | A |
| IPI00390795 | Na+/K+-ATPase alpha 4 subunit | 115260 | 5.55 | 0.034 | 10 | PM | A, B |
| IPI00188119 | Splice Isoform Long of Potassium-transporting ATPase alpha chain 2 | 115643 | 6.16 | 0.034 | 8 | PM | A, B |
| IPI00231462 | Splice Isoform Short of Potassium-transporting ATPase alpha chain 2 | 103238 | 5.88 | 0.181 | 8 | PM | A, B |
| IPI00365705 | Potassium-transporting ATPase alpha chain 1 | 115544 | 5.58 | 0.061 | 8 | PM | A, B |
| IPI00211360 | Synaptophysin | 33574 | 4.91 | −0.09 | 4 | PM | B |
| IPI00558343 | Na,K-ATPase alpha-1 subunit | 27339 | 6.99 | −0.159 | 0 | PM | A |
| IPI00421874 | Voltage-dependent anion-selective channel protein 1 | 32513 | 8.62 | −0.357 | 0 | PM | A, B |
| IPI00231420 | Splice Isoform SNAP-25a of Synaptosomal-associated protein 25 | 23549 | 4.74 | −0.877 | 0 | PM | A |

**Table 2** (Continued)

| accesssion no. | protein name | MW(Da) | p*I* | GRAVY[a] | TM[b] | GO annonation[c] | method[d] |
|---|---|---|---|---|---|---|---|
| IPI00205693 | Sodium/potassium-transporting ATPase alpha-2 chain precursor | 113457 | 5.39 | −0.007 | 8 | PM | A, B |
| IPI00210971 | Vesicle-associated membrane protein 3 | 11587 | 8.68 | −0.033 | 1 | integral to M | A |
| IPI00200466 | ADP,ATP carrier protein 2 | 32977 | 9.74 | 0.014 | 2 | integral to M | A |
| IPI00567131 | 31 kDa protein | 31591 | 8.82 | −0.413 | 0 | integral to M | A, B |
| IPI00209908 | Cytochrome *c* oxidase subunit 2 | 26096 | 4.6 | 0.269 | 2 | integral to M | A |
| IPI00551812 | ATP synthase beta chain, mitochondrial precursor | 56318 | 5.19 | 0.034 | 0 | integral to M | A, B |
| IPI00560581 | 43 kDa protein | 43621 | 7.48 | −0.288 | 0 | integral to M | A, B |
| IPI00231927 | ADP,ATP carrier protein 1 | 33065 | 9.81 | 0.029 | 4 | integral to M | A |
| IPI00209115 | Slc25a3 protein | 40052 | 9.38 | 0.045 | 2 | integral to M | B |
| IPI00562259 | Phosphate carrier protein, mitochondrial precursor | 39876 | 9.41 | 0.062 | 2 | integral to M | B |
| IPI00213516 | PREDICTED: camello-like 3 | 52355 | 9.35 | 0.238 | 4 | integral to M | B |
| IPI00188924 | Ubiquinol-cytochrome-c reductase complex core protein 2, mitochondrial precursor | 48400 | 9.16 | −0.068 | 0 | IM | A, B |
| IPI00368355 | PREDICTED: similar to dachsous 2 isoform 1 | 391380 | 4.67 | −0.182 | 1 | M | A |
| IPI00388687 | PREDICTED: similar to solute carrier family 25 (mitochondrial carrier, Aralar), member 12 | 51130 | 7.21 | −0.01 | 0 | M | A |
| IPI00564348 | 74 kDa protein | 74526 | 7.13 | −0.017 | 0 | M | A |
| IPI00567060 | 76 kDa protein | 77445 | 8.55 | −0.098 | 0 | M | A |
| IPI00230866 | Guanine nucleotide-binding protein, alpha-12 subunit | 43934 | 9.84 | −0.362 | 0 | M | A |
| IPI00199394 | 2′,3′-cyclic-nucleotide 3′-phosphodiesterase | 47638 | 9.03 | −0.443 | 0 | M | A, B |
| IPI00569825 | 35 kDa protein | 34876 | 9.42 | 0.012 | 2 | M | A |
| IPI00557819 | 26 kDa protein | 26454 | 5.85 | 0.253 | 2 | M | A |
| IPI00208315 | PREDICTED: similar to myosin-VIIb | 307188 | 8.65 | −0.299 | 0 | M | A, B |
| IPI00568360 | PREDICTED: similar to solute carrier family 25, member 5 | 29168 | 9.51 | 0.137 | 2 | M | A |
| IPI00363182 | PREDICTED: similar to solute carrier family 25, member 5 | 33326 | 9.85 | −0.001 | 2 | M | A |
| IPI00366141 | 34 kDa protein | 34329 | 9.13 | −0.013 | 2 | M | A |
| IPI00558425 | 28 kDa protein | 28337 | 9.32 | 0.01 | 2 | M | A |
| IPI0052085 | PREDICTED: similar to solute carrier family 25, member 5 | 21241 | 9.84 | 0.213 | 1 | M | A |
| IPI00564294 | 32 kDa protein | 31877 | 9.06 | −0.067 | 2 | M | A |
| IPI00569831 | 34 kDa protein | 35046 | 9.1 | 0.078 | 2 | M | A |
| IPI00373193 | PREDICTED: similar to PDZ domain containing 6 | 188971 | 8.94 | −0.358 | 1 | M | A |
| IPI00196627 | PREDICTED: similar to Synaptogyrin 3 | 24725 | 7.63 | 0.292 | 4 | M | B |
| IPI00365813 | PREDICTED: similar to voltage-dependent anion channel 1 | 25941 | 8.91 | −0.355 | 0 | OM | A |
| IPI00563424 | 31 kDa protein | 31649 | 6.16 | −0.191 | 0 | OM | A |
| IPI00566850 | 32 kDa protein | 32582 | 8.55 | −0.329 | 0 | OM | A, B |
| IPI00199872 | Guanine nucleotide-binding protein G(s), alpha subunit | 46091 | 5.69 | −0.597 | 0 | M fraction | A |
| IPI00204843 | Guanine nucleotide-binding protein G(o), alpha subunit 2 | 40437 | 5.69 | −0.353 | 0 | M fraction | A |
| IPI00231505 | Guanine nucleotide-binding protein G(o), alpha subunit 1 | 40482 | 5.34 | −0.343 | 0 | M fraction | A |
| IPI00210319 | 98 kDa protein | 98527 | 7.02 | −0.459 | 0 | endocytic vesicle M | A |
| IPI00389749 | Splice Isoform IIAA of Dynamin-2 | 98383 | 7.02 | −0.457 | 0 | endocytic vesicle M | A |
| IPI00563085 | 56 kDa protein | 56131 | 8.49 | −0.415 | 0 | endocytic vesicle M | A |
| IPI00389750 | Splice Isoform IIBA of Dynamin-2 | 98127 | 7.02 | −0.454 | 0 | endocytic vesicle M | A |
| IPI00198620 | ATP synthase delta chain, mitochondrial precursor | 17584 | 5.16 | 0.197 | 0 | organelle M | B |

[a] Grand average hydrophathy values. [b] Predicted number of transmembrane helices retrieved by TMHMM. [c] Cellular location. Abbreviations: IM, inner membrane; M, membrane; OM, outer membrane; PM, plasma membrane. [d] A, in the presence of SDC; B, in the presence of SDS.

SDC were all higher than those using SDS. However, there was no significant difference in the average of p*I* between the two methods.

In total, 77 PM proteins or membrane-associated proteins were identified when SDC was used, many of which were ATPases, transporter proteins, binding proteins, or ion-channel proteins, such as $Na^+/K^+$-ATPase alpha 4 subunit (IPI00390795), Glutamate transporter GLT1b (IPI00564673), splice isoform 1 of Syntaxin binding protein 1 (IPI00205372), and voltage-dependent anion-selective channel protein 1(IPI00421874). The functions of these proteins fit well with the roles of PM membranes in a biological system. Of the identified PM proteins or membrane-associated proteins, 50 had been predicated to have one or more transmembrane domains, which was a more than 2-fold increase over that when using SDS. As seen in Figure 1, the difference resulted mainly from the markedly higher number of proteins with one, two, and eight transmembrane domains when using SDC method. Twenty-three membrane

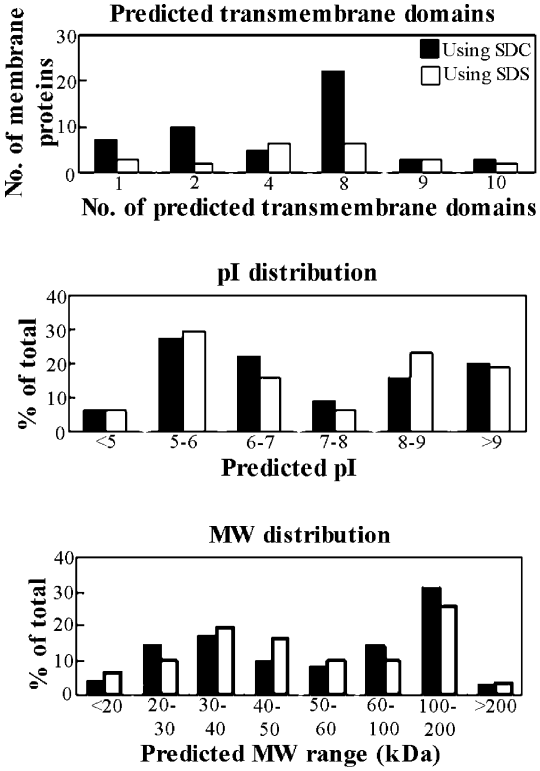


**Figure 1.** Physicochemical parameters (predicted transmembrane domains, p*I*, and MW) of membrane proteins identified by two different methods as showed in Table 1.

proteins identified using SDC had positive GRAVY value, which was recognized as a reliable hydrophobic marker of membrane proteins,[23] whereas only 16 membrane proteins were identified when SDS was used. These data strongly suggested that using SDC is more effective for improving the solubilization, digestion, and identification of hydrophobic membrane proteins than using SDS. As shown in Figure 2, SDC is facially amphipathic; that is, it contains both hydrophobic (lipid soluble) and hydrophilic (polar) faces, which is responsible for the disruption of plasma membrane and the solubilization of hydrophobic plasma membrane proteins. As SDC is itself amphipathic, it readily acted on the lipid bilayer and disrupted hydrophobic interaction. The hydrophobic region of the integral protein is coated with a layer of SDC molecules which enables the protein to remain in solution.[27] Helenius et al. demonstrated that the overall mechanism of SDC solubilization of membranes resembles that of SDS except that with SDC the various stages of

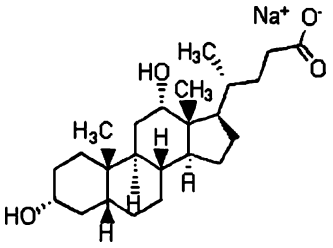

**Figure 2.** Structure of SDC ($M_r$: 414.6).

membrane disruption occur at about 10-fold higher equilibrium free detergent concentrations.[19] Although SDS was a most commonly used detergent in improving the solubilization of poor water-soluble proteins such as membrane proteins, the critical disadvantage of using SDS in in-solution digestion was that the high concentration of SDS severely decreased the enzyme activity, whereas diluting SDS to a low concentration (typically 0.1% or lower) would reduce the solubility of some hydrophobic proteins in digestion and, to some extent, cause a loss of hydrophobic proteins.[28]

In this paper, 1.0% SDC was used for dissolving the rat hippocampal PM and increased the solubility of PM proteins. Particularly, the sample was directly in-solution-digested without dilution to reduce the concentration of SDC, which made the PM proteins remain in a state of solubilization that was greatly in favor of the tryptic digestion. In addition, use of SDC did not significantly interfere with the mass spectrometric analysis because it was an acid-insoluble detergent and could be removed from the sample prior to the MS analysis by centrifugation following acidification. During the acidification, SDC and deoxycholic acid that was transformed from SDC formed gel-like precipitates.[29] In contrast, many other commonly used detergents including SDS were difficult to eliminate and, as a result, gave rise to strong MS background masking peptide signals.[26,30]

**Effect of SDC on Proteomic Analysis of PM Based on In-Gel Digestion.** For probing the probability of improving in-gel digestion of PM proteins with SDC, we had taken three SDS-PAGE-resolved standard proteins as model molecules and made preliminary experiments on in-gel tryptic digestion in the presence of 0.1% SDC, with the controls in the absence of SDC. Table 3 summarized the effects of SDC on the protein identification in terms of the Mascot score, the number of matched peptides per identified protein, and sequence coverage in percentage. As the whole table shows, most of the statistical parameters of the three standard proteins in the presence of SDC are higher than those in the absence of SDC.

**Table 3.** Effect of SDC on the Identification of In-Gel Digested Standard Proteins[a]

| | | 0 missing cleavages | | | 1 missing cleavage | | | 2 missing cleavages | | |
|---|---|---|---|---|---|---|---|---|---|---|
| protein name | treatment | SCR | PEP | COV | SCR | PEP | COV | SCR | PEP | COV |
| Trypsin inhibitor | + | 251 | 5 | 23% | 318 | 7 | 38% | 366 | 8 | 38% |
| | − | 152 | 4 | 18% | 209 | 5 | 21% | 209 | 5 | 21% |
| Rabbit phosphorylase b | + | 682 | 16 | 23% | 1285 | 29 | 41% | 1312 | 29 | 42% |
| | − | 598 | 16 | 25% | 1025 | 24 | 34% | 1105 | 27 | 40% |
| Ovalbumin | + | 680 | 14 | 50% | 769 | 17 | 55% | 766 | 18 | 55% |
| | − | 572 | 12 | 47% | 622 | 13 | 55% | 619 | 13 | 55% |
| *Average | + | 538 | 12 | 32% | 782 | 18 | 45% | 815 | 18 | 45% |
| | − | 441 | 11 | 30% | 619 | 14 | 37% | 644 | 15 | 39% |

[a] SCR, Mascot score; PEP, the number of peptides matched/protein identified; COV, sequence covered in percentage. +, with SDC treatment. −, without SDC treatment. *Average values of the corresponding parameters of standard proteins treated with or without SDC.

**Table 4.** Effect of SDC Treatment on the Identification of PM Proteins or Membrane-Associated Proteins Based on In-Gel Digestion

| parameter | with SDC treatment | without SDC treatment |
|---|---|---|
| Total membrane proteins | 26 | 19 |
| Total matched peptides | 49 | 42 |
| No. of proteins with one or more transmembrane domains | 10 | 5 |
| No. of membrane proteins with positive GRAVY values | 6 | 3 |

The evaluation based on these statistical parameters, particularly the average values, suggested that the addition of SDC could remarkably improve the protein identification based on in-gel digestion.

After the rat hippocampal PM proteins were separated by SDS-PAGE, we randomly selected and excised 10 protein bands from each of the two lanes in the gels. The statistical parameters on the PM proteins or membrane-associated proteins identified were summarized in Table 4. Totals of 26 membrane proteins were identified when 0.1% SDC was added to in-gel digestion as compared with 19 membrane proteins in the absence of SDC. Of the membrane proteins identified based on in-gel digestion in the presence of SDC, 10 proteins had one or more transmembrane domains, which were much higher than those in the absence of SDC. In addition, when SDC was used, the number of identified proteins with positive GRAVY values was 6, a 2-fold increase over that when no SDC was added. These statistical parameters on the identification of rat hippocampal PM proteins or membrane-associated proteins demonstrated that the addition of SDC to in-gel digestion solution had significantly improved the in-gel digestion, the extraction of released peptides, and hence, the reliability of protein identification.

## Conclusion

The results of our study demonstrated that SDC obviously assisted in the solubilization and the in-solution and in-gel digestion of proteins including poor water-soluble proteins such as PM proteins, whereby the reliability of protein identification was significantly enhanced. Compared with other detergents currently used in proteomic analysis, SDC is cheaper, easier to obtain, and more tractable due to its acid-insolubility. Most importantly, SDC can be used at higher concentrations both in the solubilization and tryptic digestion of proteins including membrane proteins, with the purpose of enhancing the protein solubility and at the same time making no interference with trypsin activity and subsequent analyses.

**Acknowledgment.** This work was supported by grants from National 973 Projects of China and the Special Program for Key Basic Research of the Ministry of Science and Technology, China (2001CB5102, 2004CCA00300), and National Natural Science Foundation of China (30430170, 90408017).

## References

(1) Klein, J. B.; Gozal, D.; Pierce, W. M.; Thongboonkerd, V.; Scherzer, J. A.; Sachleben, L. R.; Guo, S. Z.; Cai, J.; Gozal, E. *Respir. Physiol. Neurobiol.* **2003**, *136*, 91−103.
(2) Mennerick, S.; Dhond, R. P.; Benz, A.; Xu, W.; Rothstein, J. D.; Danbolt, N. C.; Isenberg, K. E.; Zorumski, C. F. *J. Neurosci.* **1998**, *18*, 4490−4499.
(3) Agassandian, C.; Plantier, M.; Fattoum, A.; Represa, A.; der, Terrossian, E. *Brain Res.* **2000**, *887*, 444−449.
(4) Fountoulakis, M.; Gasser, R. *Amino Acids* **2003**, *24*, 19−41.
(5) Navarre, C.; Degand, H.; Bennett, K. L.; Crawford, J. S.; Mortz, E.; Boutry, M. *Proteomics* **2002**, *2*, 1706−1714.
(6) McCarthy, F. M.; Burgess, S. C.; van den Berg, B. H. J.; Koter, M. D.; Pharr, G. T. *J. Proteome Res.* **2005**, *4*, 316−324.
(7) Lu, X.; Zhu, H. *Mol. Cell. Proteomics* **2005**, *4*, 1948−1958.
(8) Wu, C. C.; Yates, J. R., III. *Nat. Biotechnol.* **2003**, *21*, 262−267.
(9) Katayama, H.; Nagasu, T.; Oda, Y. *Rapid Commun. Mass Spectrom.* **2001**, *15*, 1416−1421.
(10) Nomura, E.; Katsuta, K.; Ueda, T.; Toriyama, M.; Mori, T.; Inagaki, N. *J. Mass Spectrom.* **2004**, *39*, 202−207.
(11) Quach, T. T.; Li, N.; Richards, D. P.; Zheng, J.; Keller, B. O.; Li, L. *J. Proteome Res.* **2003**, *2*, 543−552.
(12) Katayama, H.; Tabata, T.; Ishihama, Y.; Sato, T.; Oda, Y.; Nagasu, T. *Rapid Commun. Mass Spectrom.* **2004**, *18*, 2388−2394.
(13) Meng, F.; Cargile, B. J.; Patrie, S. M.; Johnson, J. R.; McLoughlin, S. M.; Kelleher, N. L. *Anal. Chem.* **2002**, *74*, 2923−2929.
(14) Ross, A. R.; Lee, P. J.; Smith, D. L.; Langridge, J. I.; Whetton, A. D.; Gaskell, S. J. *Proteomics* **2002**, *2*, 928−936.
(15) Konig, S.; Schmidt, O.; Rose, K.; Thanos, S.; Besselmann, M.; Zeller, M. *Electrophoresis* **2003**, *24*, 751−756.
(16) Suder, P.; Bierczynska, A.; Konig, S.; Silberring, J. *Rapid Commun. Mass Spectrom.* **2004**, *18*, 822−824.
(17) Josic, D.; Brown, M. K.; Huang, F.; Callanan, H.; Rucevic, M.; Nicoletti, A.; Clifton, J.; Hixson, D. C. *Electrophoresis* **2005**, *26*, 2809−2822.
(18) Coleman, R.; Iqbal, S.; Godfrey, P. P.; Billington, D. *Biochem. J.* **1979**, *178*, 201−208.
(19) Helenius, A.; Fries, E.; Garoff, H.; Simons, K. *Biochim. Biophys. Acta* **1976**, *436*, 319−334.
(20) Stevens, S. M., Jr.; Zharikova, A. D.; Prokai, L. *Mol. Brain Res.* **2003**, *117*, 116−128.
(21) Cutillas, P. R.; Biber, J.; Marks, J.; Jacob, R.; Stieger, B.; Cramer, R.; Waterfield, M.; Burlingame, A. L.; Unwin, R. J. *Proteomics* **2005**, *5*, 101−112.
(22) Li, N.; Shaw, A. R. E.; Zhang, N.; Mak, A.; Li, L. *Proteomics* **2004**, *4*, 3156−3166.
(23) Kyte, J.; Doolittle, R. F. *J. Mol. Biol.* **1982**, *157*, 105−132.
(24) Krogh, A.; Larsson, B.; von Heijne, G.; Sonnhammer, E. L. *J. Mol. Biol.* **2001**, *305*, 567−580.
(25) Ashburner, M.; Ball, C. A.; Blake, J. A.; Botstein, D.; Butler, H.; Cherry, J. M.; Davis, A. P.; Dolinski, K.; Dwight, S. S.; Eppig, J. T.; et al. *Nat. Genet.* **2000**, *25*, 25−29.
(26) Zhang, N.; Li, L. *Rapid Commun. Mass Spectrom.* **2004**, *18*, 889−896.
(27) Mukhopadhyay, S.; Maitra, U. *Curr. Sci.* **2004**, *87*, 1666−1683.
(28) Blonder, J.; Goshe, M. B.; Moore, R. J.; Pasa-Tolic, L.; Masselon, C. D.; Lipton, M. S.; Smith, R. D. *J. Proteome Res.* **2002**, *1*, 351−360.
(29) Zhang, L.; Yang, Z. L.; Xiong, Y.; Peng, T. T.; Weng, S. F.; Wu, J. G. *Acta Phys.−Chim. Sin.* **2004**, *20*, 1196−1199.
(30) Nilsson, C. L.; Larsson, T.; Gustafsson, E.; Karlsson, K. A.; Davidsson, P. *Anal. Chem.* **2000**, *72*, 2148−2153.

PR060112A