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Comparison of sodium dodecyl sulfate depletion techniques for proteome analysis by mass spectrometry

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ABSTRACT

In proteomics, sodium dodecyl sulfate (SDS) is favored for protein solubilization and mass-based separation (*e.g.* GELFrEE or SDS PAGE). Numerous SDS depletion techniques are available to purify proteins ahead of mass spectrometry. The effectiveness of the purification has a controlling influence on the success of the analysis. Here we quantitatively assess eight approaches to SDS depletion: in-gel digestion; protein precipitation in acetone or with TCA; detergent precipitation with KCl; strong cation exchange; protein level and peptide level purification with Pierce detergent removal cartridges; and FASP II. Considering protein purity, FASP II showed the highest degree of SDS removal, matching that of in-gel digestion (over 99.99% depleted). Other methods (acetone, strong cation exchange, Pierce cartridges) also deplete SDS to levels amenable to LC–MS (>99%). Accounting for protein recovery, FASP II revealed significant sample loss (<40% yield); other approaches show even greater protein loss. We further assessed acetone precipitation, having the highest protein recovery relative to FASP II, to process GELFrEE fractionated *Escherichia coli* ahead of bottom-up mass spectrometry. Acetone precipitation yielded a 17% average increase in identified proteins, and 40% increase in peptides, indicating this approach as a favored strategy for SDS depletion in a proteomics workflow.

1. Introduction

Sodium dodecyl sulfate (SDS) has considerable utility in proteomics, facilitating cell lysis and protein solubilization [1], imparting mass-based protein separation (*e.g.* SDS PAGE or GELFrEE fractionation) [2], or enhancing enzyme digestions, particularly for membrane proteins [3]. For effective solubilization, Speers and Wu recommend a buffer with greater than 1% SDS [3], while Wisniewski et al. have suggested 4% SDS for maximal protein extraction [4]. Unfortunately, downstream processes for proteome analysis are severely compromised by such high levels of SDS. Trypsin activity is reduced in 0.1% SDS [5], while levels above only 0.01% SDS can be detrimental to chromatographic separation and cause severe suppression in electrospray MS [6]. SDS alternatives, including organic solvents, chaotropic agents, nonionic, acid-cleavable or phase transfer surfactants introduce concerns of reduced solubilization potential, LC–MS or enzyme incompatibility [7], or simply higher costs [8]. Effective protocols to deplete SDS are therefore an integral aspect of many proteomics workflows.

Numerous formats are available to separate SDS from proteins ahead of LC–MS [8], among which are column-based methods, membrane filtration, precipitation, and electrophoretic approaches. A popular approach is the FASP II protocol (filter aided sample preparation), which describes the use of urea or other suitable reagent to weaken SDS–protein interactions, allowing capture of proteins on a molecular weight cutoff (MWCO) as the surfactant washes through [4]. Following enzyme digestion, the retained proteins are recovered from the filter as peptides. In this sense, the approach parallels that of in-gel digestion, which also traps intact proteins (within a gel matrix), and finishes with the release of digested peptides. Beyond FASP, other solution-based depletion protocols are more amenable to top-down analysis of intact proteins; protein precipitation in organic solvents, or detergent precipitation with KCl are examples of protein level depletion. For a given depletion method, analyte recovery is optimized according to the type of sample (protein or peptide), though some methods (*e.g.* Pierce detergent removal spin cartridges) claim to be amenable to either protein or peptide level depletion. The efficiency of an SDS depletion method is often described through the number of protein/peptide identifications by MS, wherein strong MS signals are indicative of sufficient SDS being removed [9–18]. However,

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considering MS signal intensity, this value will be influenced by both the purity (level of SDS removal) and concentration (level of analyte recovery) of proteins or peptides in the sample. Focusing exclusively on MS counts for proteins or peptides, it is realized that this value is only minimally influenced by analyte concentration beyond a certain loading, reported previously as 1 μg total protein [16]. The number of identifications is also highly influenced by the MS detection platform (scan speed, sensitivity), as well as sample characteristics (concentration, complexity, *etc.*), and therefore cannot be used to accurately compare the effectiveness of various depletion strategies.

SDS depletion efficiency is best described by quantifying the residual SDS, relative to the starting concentration. Liu et al. [12] described a tube gel electrophoresis strategy to deplete SDS, reported to remove 85% of the initial SDS. Such efficiency is surprisingly low, considering the approach makes use of the proven strategy of in-gel digestion. Placed into context, a starting concentration of 1% SDS generally requires a minimum 99% of the SDS to be removed to be compatible with LC–MS. Botelho et al. [6] have previously reported that protein precipitation in acetone will deplete 99.9% of the SDS. Sun et al. [19] describes a strong cation exchange approach that is 99.99% efficient at SDS depletion. Again, it is difficult to directly compare such values, given the influence of sample composition, or possibly also the methods used to quantify residual SDS. The methylene blue active substances assay (MBAS) [20] is the most widely reported approach to quantify SDS, but is highly susceptible to interferences from anionic components which also complex with the cationic dye and extract into chloroform. Other colorimetric assays exist (Stains-All) [21]. GC–MS has also been used following pyrolysis of the detergent [22], as has LC–MS, though not in the context of quantifying trace levels of detergent from protein-containing solutions.

In addition to purity, protein recovery must also be considered in assessing the effectiveness of an SDS depletion protocol. With any sample manipulation, some loss will be expected, though numbers vary greatly in the literature. Considering only acetone precipitation, protein recovery values ranging from below 50% to nearly 100% have been reported [23–26]. Such variation in sample loss is typical of any SDS depletion protocol. Protein recovery is often a function of the sample composition and concentration, but may also simply depend on the user, as it is difficult to fully remove the supernatant without accidentally transferring a small portion of an already miniscule protein pellet [27].

Here, we employ a standardized system to test the effectiveness of SDS depletion, considering both the recovery and purity of protein and peptide samples. We evaluate solutions containing low and high concentrations of BSA, or of an *Escherichia coli* proteome extract to test the effectiveness of eight independent SDS depletion protocols. We reveal differences in the efficiency of the methods, most notably with respect to the recovery of protein or peptide following SDS depletion. We further assess the impact of protein recovery and purity resulting from two SDS cleanup approaches (FASP and acetone precipitation) on their capacity to identify proteins by LC–MS.

2. Materials and methods

2.1. Chemicals and reagents

Bovine serum albumin (BSA) and TPCK-treated trypsin (T8802) were purchased from Sigma (Oakville, Canada). Milli-Q water was purified to 18.2 MΩ cm. Organic solvents (acetone, methanol, chloroform, acetonitrile, isopropanol) were of HPLC grade and obtained from Thermo Fisher Scientific (Ottawa, Canada). Methylene blue was also from Fisher. Reagents for casting and staining SDS PAGE gels, as well as urea, DTT, iodoacetamide, and SDS were from Bio-Rad (Mississauga, Canada). Formic acid (98%) was from Fluka (Mississauga, Canada), while trichloroacetic acid (TCA), trifluoroacetic acid (TFA), Stains-All, and all remaining chemicals used were from Sigma.

2.2. E. coli *growth and protein extraction*

Escherichia coli (*E. coli*) was cultured according to established protocols (Qiagen Manual for Good Microbiological Practices). Cells were grown at 37 °C with shaking until an OD_{600} of 0.7, then isolated by centrifugation at 5000 × *g* (15 min). Proteins were extracted by suspending the cells in 2% SDS with heating (95 °C for 5 min). Cellular debris was pelleted by centrifugation (15,000 × *g*, 15 min) and discarded. The total protein content of the supernatant was determined using a BCA assay kit from Pierce (Rockford, IL), against a calibration curve of BSA in 2% SDS.

2.3. Sample preparation for SDS depletion

Stock solutions of BSA and of the extracted *E. coli* proteins were prepared at concentrations of 0.1 g/L and of 1.0 g/L, each including 1% SDS in water. Five 100 μL aliquots were prepared as replicates for each of the eight SDS-depletion protocols. For peptide level SDS depletion, the 100 μL protein aliquots were diluted to a final concentration of 0.025% SDS and digested with trypsin as described by Sun et al. [19]. Following digestion and acidification, samples were evaporated to dryness in a Speedvac, and reconstituted with sonication in 100 μL of the appropriate buffer system, restoring the SDS concentration to 1%.

2.4. SDS depletion methods

A brief description of methods is provided below, with full details available as supplementary files.

2.4.1. FASP II

As described by Wiśniewski et al. [4,28], a 30 kDa Micron YM-30 filter (Millipore, Cat. No. 42409) was selected, employing 8 M urea in 0.1 M Tris (pH 8.5) to facilitate removal of protein-bound SDS. 10 kDa YM-10 filters (Millipore, Cat. No. 42406) were also assessed, as were Amicon Ultra 2 10 kDa filters (Millipore, Cat UFC201024). The cleaned proteins were digested overnight on the filter with trypsin at a 1:100 ratio and the resulting peptides were released from the filter by centrifugation (14,000 × *g*, 40 min), followed by a wash of the filter in 0.5 M NaCl. Peptides were desalted as described by Wiśniewski et al. [4] using 10 mg Oasis HLB sample extraction columns (Waters, Milford), eluted with 1.25 mL of 70% ACN, dried and diluted to a final volume of 100 μL with water, with sonication.

2.4.2. Protein precipitation in 80% acetone

As described by Botelho et al. [6], proteins were precipitated through addition of 400 μL acetone, with overnight incubation at −20 °C and isolation of the pellet through centrifugation (15 min, 21,000 × *g*). The protein pellet was subject to two additional washing steps with 400 μL of acetone. The final protein pellet was resuspended in 100 μL water, with sonication. Sixty microliters were reserved for the SDS assays. The remaining 40 μL of sample was diluted with 40 μL of 2% SDS, sonicated to fully dissolve the suspended protein, and subjected to BCA protein assay.

2.4.3. Protein precipitation in TCA with acetone wash

Proteins were precipitated through addition of 1/10th volume of 100% (w/v) TCA [29]. The protein pellet was collected by centrifugation (15 min, 21,000 × *g*) and incubated overnight in 1 mL cold (−20 °C) acetone. The protein pellet was again collected by

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