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Sodium dodecyl sulfate removal during electrospray ionization using cyclodextrins as simple sample solution additive for improved mass spectrometric detection of peptides

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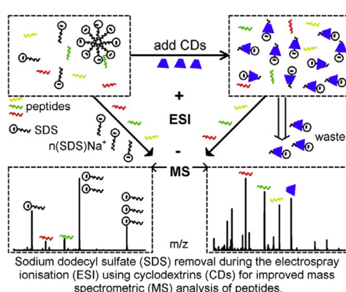
HIGHLIGHTS

- Uncomplicated solution chemistry based sample preparation for SDS containing peptide samples prior to ESI-MS.
- Sample preparation by addition of cyclodextrins (CDs) to the sample and no off-line extraction or use of solid supports.
- Up to 99% removal of SDS-related ESI-MS signals and instantly perceptible improvements in peptide to SDS signal ratio.
- Formation of stable CD-SDS inclusion complexes significantly decreased the formation of n(SDS)Na^+ multimers in the ESI.
- Improved %signal recoveries (up to 12x) for some peptides (e.g., bradykinin) by suppression of space charge effects by SDS.

GRAPHICAL ABSTRACT



Sodium dodecyl sulfate (SDS) removal during the electrospray ionisation (ESI) using cyclodextrins (CDs) for improved mass spectrometric (MS) analysis of peptides.

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ABSTRACT

Sodium dodecyl sulfate (SDS) removal is a vital procedure in SDS-assisted bottom-up proteomics because SDS affects the quality of the data in electrospray ionization mass spectrometry (ESI-MS). SDS removal methods provide efficient removal of SDS and improved peptide analysis, but would usually require time, specialised devices, and experienced analysts. Here, by simple addition of γ-cyclodextrin (γ-CD) to the solution at concentrations 1 to 2x the SDS in the sample, the SDS related signals in positive ionization ESI-MS can be significantly removed (70–99% reduction), without an additional sample manipulation step of extraction or purification. The mechanism for removal is based on the formation of tightly bound CD-SDS inclusion complexes, which hampered the generation of positively charged SDS multimers during ESI. For a sample with peptides (glu-val-phe, tyr-tyr-tyr, and bradykinin) and 3 mM SDS where 6 mM γ-CD was added, the %signal recoveries of peptides calculated by comparison with signals from standard samples without SDS were 49–59%. The space charge effect by SDS on bradykinin was also reduced, increasing the signal for bradykinin 12x in the presence of γ-CD. For a protein (bovine serum albumin, BSA) digest with 3 mM SDS, which is an expected concentration in trypsin treated samples, a noticeable 7-fold improvement in the peptide to SDS signal ratio and a 91% reduction of SDS signals were observed upon addition of 6 mM γ-CD. However, there were only small changes in the ESI-MS intensities

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for the BSA peptides (compared to without addition of γ-CD). This new approach to SDS signal removal using CDs in ESI-MS may find use in proteomic studies.

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1. Introduction

Sodium dodecyl sulfate (SDS) is a strong detergent that is widely used in proteomic studies for solubilization or extraction of hydrophobic or aggregated proteins and separation of proteins by SDS-polyacrylamide gel electrophoresis [1–5]. SDS changes the secondary and tertiary structures of proteins by ionic and hydrophobic interaction (with the head and tail of the surfactant, respectively) causing efficient solubilization [6]. In bottom-up proteomics, proteins are identified by in-solution protein digestion (typically with trypsin) followed by analysis of peptides by electrospray ionization-mass-spectrometry (ESI-MS) or liquid chromatography (LC)–ESI-MS. While 1–4% SDS has been recommended for protein solubilization, this concentration has to be reduced to <0.1% SDS (3.5 mM) in order to maintain the trypsin activity [7,8]. The SDS concentration has to be reduced further prior to ESI-MS. In positive ionization mode ESI-MS and at SDS concentrations ≥1 mM, strong SDS related signals (e.g., $n(SDS)Na^+$) will overwhelm the signals especially of low abundance peptides. Levels of >0.01% SDS (0.4 mM) can also cause ion suppression in ESI-MS and affect LC separation [9]. To achieve higher peptide or protein coverage in ESI-MS analysis, SDS removal is therefore required. Indeed, the development of SDS removal methods prior to ESI-MS analysis has become an important and active research area in proteomics [10]. These methods include solid phase extraction [11], filter aided sample preparation [4,8,12–15], electrofiltration [16,17], precipitation [18,19] and dialysis [10]. These sample preparation methods are all performed off-line and allow efficient removal of SDS, but are tedious, time consuming, and would often require experienced chemists.

Cyclodextrins (CDs) belongs to a group of cyclic oligosaccharides with α(1–4) ether linkages of glucopyranose units. They have the shape of a truncated cone, with the internal cavity showing a hydrophobic character while the external surfaces are hydrophilic [20]. The great interest on CDs is primarily due to the ability of the CD's cavity to include a wide range of guest molecules, including surfactants. The cavities also impart enantioselectivity, which is the fundamental basis of the wide use of CDs in chiral separations by chromatography or capillary electrophoresis (CE) [21,22]. The hydrophobic tail of SDS is strongly included into the CDs' cavity, forming stable CD-SDS complexes [23]. CDs were even thought to act like "vacuum cleaners" that suck away surfactants in solution, causing the increase in the critical micelle concentration (cmc) of surfactants [24]. CD-SDS inclusion complexes have been observed in negative ionization but not in positive ionization mode ESI-MS [25]. This suggests that the CD-SDS inclusion complexes do not form positive gas phase ions from charged droplets, and thus were not introduced into the MS detector.

CDs have been immobilized into a solid support/resin for removal of SDS from a proteomic sample stream by extraction prior to ESI-MS [26]. Ref. [26] is a patent that described the use of a spin column for batch removal of SDS where separation is by gravity flow. Here, the potential of γ-CD as a simple solution phase additive for the removal or restraint of SDS related signals and improved detection of peptides in positive ionization ESI-MS was studied. The CDs were directly added and mixed into the sample solution at an appropriate concentration, and without an extraction or purification step, the resulting sample was directly infused to the ESI-MS. It is highlighted that in this work, the CDs were not immobilized into a solid support to perform off-line sample preparation. The proof-of-concept studies were conducted using three model peptides, different concentrations of SDS, and different SDS and CD concentration ratios. The extremely fast and simple approach was then tested using digested bovine serum albumin (BSA) with SDS at a relevant concentration for trypsin activity.

2. Experimental section

2.1. Reagents, stock solutions, and peptide samples

Hydrochloric acid (HCl), glacial acetic acid, formic acid, HPLC grade methanol and acetonitrile, SDS, dihydroxybenzoic acid (DHBA), tris-HCl, 1,4-dithiothreitol, iodoacetamide (IAA), ammonium bicarbonate (NH_4HCO_3), trypsin from bovine pancreas were from Sigma-Aldrich (St. Louis, MA) or Fluka (Buchs, Switzerland). γ-CD, α-CD, peptides (glu-val-phe (MW = 393.43), tyr-tyr-tyr (MW = 507.54), and bradykinin (MW = 1060.21)) and BSA were from Sigma-Aldrich. Purified water was from Milli-Q system (Millipore, Bedford, MA, USA). Stock solutions of the peptides were 1 mg/mL in purified water. Stock solutions of 50 mM SDS, 50 mM γ-CD, 50 mM α-CD, and 30 mM formic acid were prepared in purified water. Samples with the three peptides were prepared by dilution of aliquots of peptide, SDS, CD, and formic acid stock solutions with purified water.

2.2. SDS denaturation and tryptic digestion

100 μg of BSA was dissolved in 50 μL solution containing 100 mM tris-HCl, 10 mM DTT, and 1% by weight SDS. The solution was then incubated in a boiling water bath for 30 min. 10 μL of 100 mM IAA was added to the solution, and was stored in the dark for 1 h. Finally, the mixture was diluted with 10 mM NH_4HCO_3 (pH 7.8) to make 0.5 mg/mL or 0.1 mg/mL protein concentration. For the in-solution digestion, 10 μL of 0.5 mg/mL of trypsin prepared in 1 mM HCl was added and was incubated for 16 h at 37 °C. The digestion was quenched by addition of 10 μL of 25% acetic acid. The resulting concentration of SDS was 3 mM. The sample was diluted two-fold with 3 mM SDS prior to direct infusion ESI-MS.

2.3. Direct infusion ESI-MS and data analysis

The ESI-MS of model peptides and digested BSA samples were done on an Agilent 6320 Ion Trap system with an Agilent G1607A ESI interface. The sample was introduced using 75 μm i.d. capillary of 1 m total length at 1 bar. The capillary was cut using a diamond cutter and the polyimide coating at the tip was removed by heating. ESI voltage was +4 kV. Spray chamber parameters were as follows: nebulizer pressure 15.0 psi, dry gas flow-rate 5.0 L/min, drying gas temperature 325°C. Total ion scan was performed at 100–1200 *m*/*z*. Sample flow rates can affect the analysis of peptides by ESI-MS [27]. Different sample flow rates were then studied using the Agilent CE (50 mbar and 1 bar) and syringe pump (25–500 μL/h). Flow rate did not affect the signal of the peptides and SDS (with or without CD) and thus is irrelevant in this study.

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