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Anal Chem. Author manuscript; available in PMC 2024 July 06.

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A Nonionic, Cleavable Surfactant for Top-down Proteomics

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Abstract

Nonionic surfactants are often used as general reagents for cell lysis enabling protein extraction, stabilization, and purification under non-denaturing conditions for downstream analysis in structural biology. However, the presence of surfactants in the sample matrix often has a deleterious effect on electrospray ionization (ESI)-mass spectrometry (MS) analysis of proteins and complexes. Here, we report a nonionic, cleavable surfactant, n-decyl-disulfide-β-D-maltoside (DSSM), for top-down proteomics. DSSM was designed to mimic the properties of one of the most common surfactants used in structural biology, n-dodecyl-β-D-maltoside (DDM) but contains a disulfide bond that allows for facile cleavage and surfactant removal before or during MS analysis. We have shown that DSSM is compatible with direct electrospray ionization (ESI)-MS analysis and reversed-phase liquid chromatography (RPLC)-MS analysis of proteins and protein complexes. We have demonstrated that DSSM can facilitate top-down proteomic characterization of membrane proteins such as a model ion channel protein and a G protein-coupled receptor as well as endogenous proteins from cell lysates for the determination of

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ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at. Summary of TopPIC results (Table S1). NMR of DSSM (Figure S1); ESI-MS for DSSM (Figure S2); Time-course of DSSM degradation (Figure S3); Picture of DSSM after degradation and centrifugation (Figure S4); ESI-MS of carbonic anhydrase in DDMS at 2x and 20x CMC (Figure S5); Removal of DSSM by CID for ESI-MS of proteins and protein complexes (Figure S6); ESI-MS carbonic anhydrase with and without DSSM (Figure S7); ESI-MS of alcohol dehydrogenase with and without DSSM (Figure S8); Comparison of DDM and DSSM for RPLC-MS (Figure S9); Base peak chromatogram of standard protein mixture in DSSM (Figure S10).

The University of Wisconsin-Madison has filed a provisional patent application (P220246US01) on the nonionic, cleavable surfactant DSSM. Y.G., S.J., M.K.G., and K.A.B. are named as inventors.

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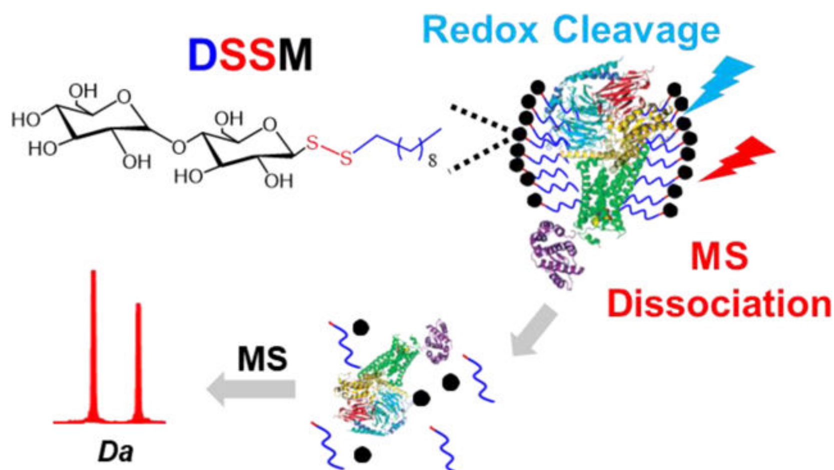
sequence variations and posttranslational modifications (PTMs). Conceivably, DSSM could serve as a general replacement for DDM in proteomics experiments and structural biology studies.

Graphical Abstract



Nonionic surfactants are versatile tools for the solubilization and purification of proteins from cells and are critical reagents used in structural biology.[1,2] One of the most popular nonionic surfactants for extracting proteins from their native environment and stabilizing them for downstream biophysical techniques such as crystallography and cryogenic electron microscopy is n-dodecyl-β-D-maltoside (DDM).[3]

However, the presence of surfactants, even mild ones like DDM, often has a deleterious effect on top-down proteomics for protein sequencing to identify posttranslational modifications (PTMs) and sequence variations.[4–6] Surfactant-related signal suppression is generally caused by the higher ionization efficiency and signal-to-noise ratio of the low molecular weight species. Moreover, the presence of surfactant can negatively impact common front-end protein separation techniques such as reversed-phase liquid chromatography (RPLC), which could cause potential problems in reproducibility and robustness.[5,6]

One approach to overcome the incompatibility of the surfactants for downstream proteomic analysis is to insert a cleavable bond (e.g., acid[7–9] or light-labile[10,11]) that allows for controlled degradation of the molecule into innocuous byproducts before MS analysis. Cleavable surfactants commonly used for proteomics contain denaturing, anionic head groups, such as sulfate, that preclude their use for applications where non-denaturing conditions are desirable.[12,13] Thus, there is an urgent need for cleavable surfactants that can aid in traditional biochemical preparation methods under non-denaturing conditions yet are still amenable for downstream proteomic applications. Here, we demonstrate for the first time the use of n-decyl-disulfide-β-D-maltoside (DSSM), a nonionic, cleavable surfactant, for top-down proteomics (Figure 1).

DSSM was originally developed to mimic the properties of DDM while providing a platform for high-throughput detergent exchange for biophysical assays.[14] The nonionic maltose head

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group resembles DDM, but the addition of the disulfide bond between the sugar and the hydrophobic decyl tail imparts cleavable properties. After synthesis and characterization of DSSM (Figure S1-2), we evaluated its compatibility with electrospray-ionization (ESI)- MS analysis for intact proteins.

The compatibility of DSSM with direct ESI-MS analysis was evaluated using carbonic anhydrase (29.1 kDa) in denaturing conditions. The surfactant was degraded with 5 equivalence of TCEP (tris(2-carboxyethyl)phosphine) at 4 °C for 2 h (Figure S3). Insoluble degradation products, which commonly pose an issue for acid-cleavable surfactants like RapiGest,[8] were not observed after DSSM degradation and centrifugation (Figure S4). We observe no difference in signal between the control sample and that with DSSM after degradation (Figure 2).

In contrast, even at the relatively low concentration of 2× CMC (0.02%), DDM is the dominant species suppressing intact-mass analysis of carbonic anhydrase (Figure 2 middle panel). When a large excess of DSSM was used (20× CMC), a species corresponding to the maltose head group was observed as the dominant peak (Figure S5). No deleterious effects were observed from the inclusion of TCEP. Nonetheless, this is a critical consideration for experiments using a redox reaction to degrade the surfactant.

Next, we tested if ion activation (i.e. collision-induced dissociation [CID]) could be used to dissociate the surfactant from the proteins and protein complexes for ESI-MS analysis under non-denaturing conditions[13] (Figure S6). MS analysis of carbonic anhydrase (29.1 kDa) ammonium acetate with and without DSSM at a concentration of 2× CMC (critical micelle concentration) yielded spectra with significant signal suppression from DSSM monomers at lower collisional activation (2–10 V) (Figure S6). When higher collisional activation (20–30 V) was applied, quality MS spectra were observed with a similar charge state distribution to the sample in ammonium acetate alone (Figure S6). Similar results were observed for carbonic anhydrase in DDM at 2× CMC (Figure S7).

For the tetramer forming complex, alcohol dehydrogenase (147.5 kDa), quality spectra could be obtained at a low collisional voltage (10 V) with and without DSSM at 2× CMC (Figure S8). Therefore, we further tested DSSM at a higher concentration (5× CMC) using collisional activation energies of 20–30 V to achieve direct ESI-MS analysis (Figure S6). Alternative activation methods, such as surface-induced dissociation (SID),[15] ultraviolet photodissociation (UVPD), [16,17] infrared laser activation (IRMPD),[18] may be implemented to remove DSSM in the gas phase for direct ESI-MS analysis.[13,18,19]

To evaluate the surfactant's compatibility with RPLC-MS, we analyzed a mixture of standard proteins (ribonuclease A, myoglobin, and carbonic anhydrase) with and without DSSM or DDM. DSSM did not influence the separation or the signal intensity of the standard proteins even at 20x CMC (Figure S9). The improved compatibility with RPLC-MS compared to direct ESI-MS analysis results from the fact that the maltose head group after the degradation of DSSM elutes in the void volume before the proteins during LC separation (Figure S10). Similarly, the addition of TCEP did not appear to have a deleterious effect. DDM, on the other hand, led to significant signal suppression in the chromatogram

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and mass spectra (Figure S9). This demonstrates the promise of DSSM as a general replacement for nonionic surfactants like DDM for RPLC-MS applications.

We further assessed DSSM's compatibility with RPLC-MS to study membrane proteins, an important class of drug targets that are generally difficult to study using top-down proteomics owing to their inherent insolubility outside the plasma membrane and low abundance.[1,20,21] We performed DSSM-aided membrane proteomic analysis on a model ion channel protein, a pH-gated potassium channel (KcsA). After removing incompatible buffer components (salts, detergent, etc.) using a chloroform:methanol:water precipitation,[22] KcsA was solubilized in the DSSM (2× CMC). The surfactant was degraded with TCEP (in water or 50% isopropanol) and RPLC-MS/MS was performed using CID for fragmentation (Figure 3A-C). Using MASH Explorer[23] for peak assignment and validation, we observed good sequence coverage on an LC-MS time scale with 27b ions and 29y ions representing 36% residue cleavage. Many of the bond cleavages were found in the transmembrane domains (TMD), in line with previous studies that characterized the fragmentation trends of intact integral membrane proteins.[24,25] Furthermore, we were able to successfully map a mutation (E71A) that prevents channel inactivation (Figure 3).[26]

Furthermore, we demonstrated that DSSM could enable the top-down analysis of bacteriorhodopsin,[27,28] a commercially available GPCR. After bacteriorhodopsin was solubilized in DSSM and degraded using TCEP (in water or 50% isopropanol), RPLC-MS/MS yielded 37b ions and 21y ions corresponding to 23% residue cleavage (Figure 3D-F). A pyrrolidone carboxylic acid modification was localized to the N-terminus of the protein (Figure 3E)

Finally, DSSM was used to extract endogenous protein from mammalian cells using and directly analyzed using RPLC-MS/MS after surfactant degradation. Following TopPIC data analysis,[29] we identified a total of 276 proteoforms[30] from 206 protein groups over four LC-MS/MS experiments (Figure 4, Table S1). Additionally, PTMs such as phosphorylation, methylation, and trimethylation were successfully localized using CID (Table S1). Overall, we demonstrated DSSM is a valuable surfactant for cell lysis and enables proteoform identification using RPLC-MS/MS analysis.

In summary, we presented the first demonstration of n-Decyl-disulfide-β-D-maltoside (DSSM), a cleavable DDM mimic, for direct ESI-MS analysis of intact proteins and top-down proteomics. DSSM was generally compatible with ESI-MS as well as RPLC-MS analysis circumventing the characteristic signal suppression typically observed for surfactants. We demonstrated that DSSM enables the top-down proteomic characterization of a model ion channel (KcsA), GPCR (bacteriorhodopsin), and endogenous proteins extracted from cell lysates. DSSM represents an important and versatile surfactant that can facilitate protein sample preparation under non-denaturing conditions for a myriad of proteomic and structural biology applications and acts as a general replacement for DDM.

Supplementary Material

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ACKNOWLEDGMENT

This research was supported by National Institutes of Health (NIH) R01 GM117058 (to both S.J. and Y. G.). We would like to acknowledge funding from the HL096971, HL109810, GM125085, and S10 OD018475 (to Y.G.). K.A.B. would like to acknowledge the Vascular Surgery Research Training Program Grant T32 HL110853.

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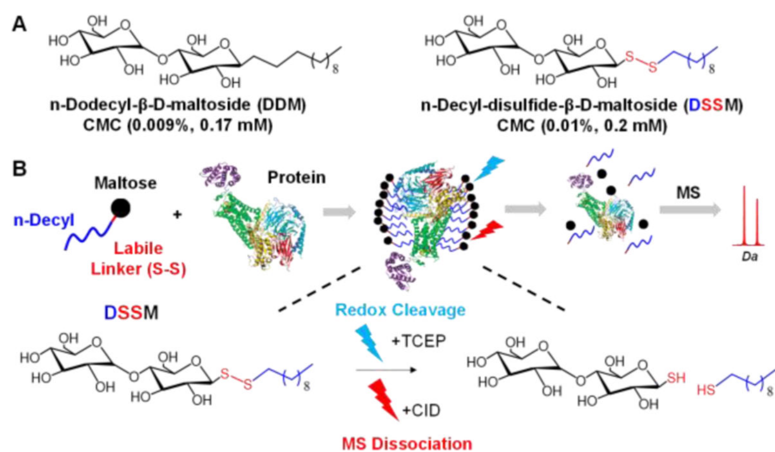

Figure 1.

(A) Comparison of the chemical structures of n-Dodecyl-β-D-maltoside (DDM) and n-Decyl-disulfide-β-D-maltoside (DSSM). (B) Overview of using a DSSM for proteomics

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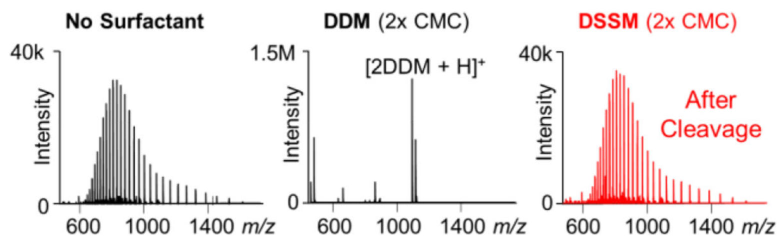

Figure 2. Compatibility of DSSM with ESI-MS of intact proteins.

MS spectra of carbonic anhydrase (left), carbonic anhydrase with DDM (2× CMC) (middle), and carbonic anhydrase in DSSM after degradation with TCEP (right).

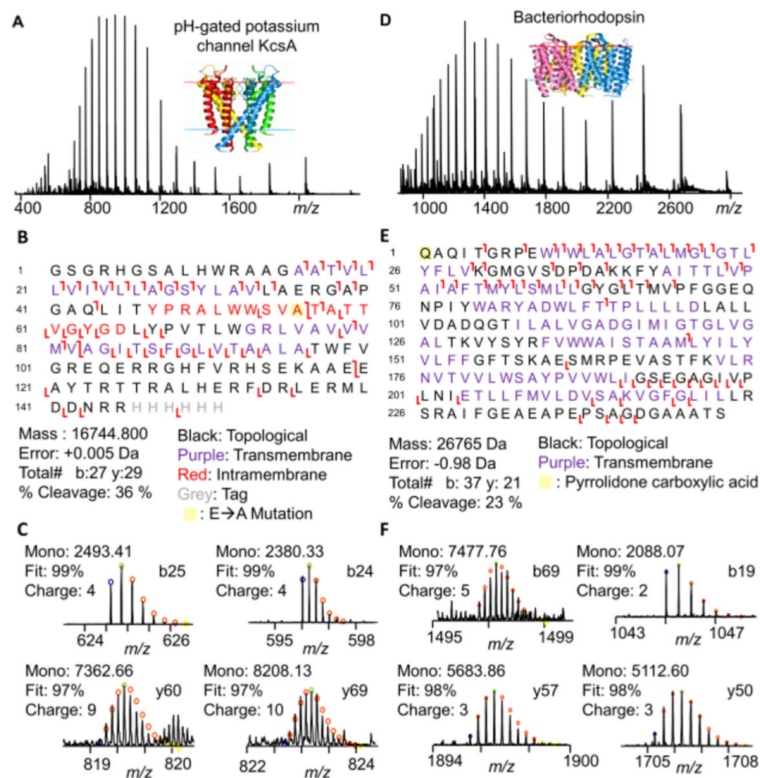

Figure 3. Top-down proteomics of DSSM solubilized membrane proteins.

Intact mass spectra, fragmentation map, and representative ions (with theoretical fits) for analysis of KcsA (A-C) and bacteriorhodopsin (D-F). Proteins were solubilized in DSSM and analyzed by LC-MS/MS after surfactant degradation.

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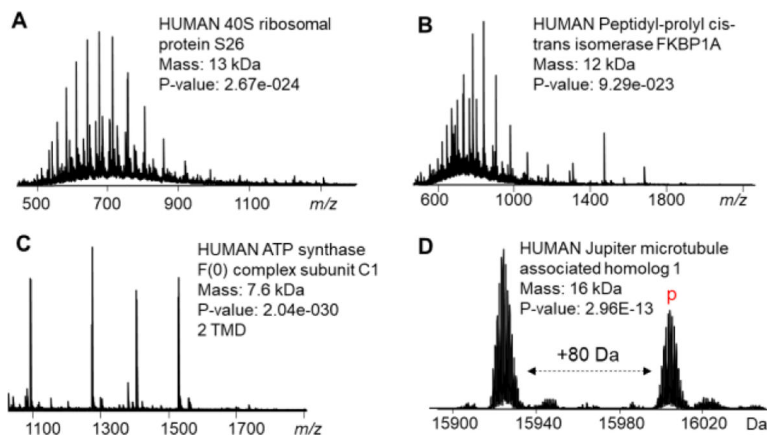

Figure 4. Top-down proteomics of endogenous proteins extracted from cell lysate using DSSM.
(A-D) Representative proteins were confidently identified from HEK whole cell or crude membrane lysate (A, B, and D from whole cell lysate and C from crude membrane lysate).

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