A NOVEL SDS ANALOG COMPATIBLE WITH MS ANALYSIS OF PROTEINS AND PEPTIDES

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Overview

Protein solubilization and purification often necessitates the use of surfactants. However, surfactants cause ion suppression in mass spectrometric detection. In particular, ionic detergents such as sodium dodecyl sulfate (SDS) which are routinely used as denaturing reagents for proteins in SDS-polyacrylamide gel electrophoresis (PAGE), must be removed from the sample prior to the MS analysis. While a variety of techniques such as electroblotting, electroelution, on-line dialysis and on-line adsorption have been developed to remove deleterious interferences, execution of these steps on minute sample quantities inevitably results in sample loss. The most common approach to removing SDS is to electroblot a protein onto a membrane such as PVDF, wash away the residual surfactant, and recover the protein from the membrane using a suitable solvent. This technique is time-consuming and can result in significant protein loss due to strong non-specific interactions with the membrane, particularly for hydrophobic proteins and peptides.

In order to eliminate these problems, we have developed a novel family of acid-labile anionic surfactants that can be used as replacements for SDS in protein solubilization and Laemmli gels. These surfactants are compatible with electrospray and matrix assisted laser desorption ionization methods, and no electroblotting or electroelution is needed prior to the MS analysis. MS data of proteins which are purified by PAGE using both SDS and the novel acid-labile surfactants are compared, demonstrating an enhancement in detection when using the latter surfactants.
Introduction

SDS polyacrylamide gel electrophoresis (SDS-PAGE)\(^1\) is one of the most widely used tool for analysis and purification of proteins. SDS denatures proteins by forming a stable complex, and binds to them in a constant weight ratio of about 1.4:1. The resulting SDS-protein complexes have almost identical charge densities, and migrate in a polyacrylamide gel according to molecular weight. If the gel is of the correct porosity, a plot of log \(M_w\) vs. relative mobility, \(R_f\), results in a linear relationship. When combined with another electrophoretic technique, isoelectrofocusing, complex mixtures can be separated into several hundred discrete components.

Mass spectrometry has become an important tool in the characterization of proteins following their separation by gel electrophoresis. However, SDS can interfere with MS sensitivity. It can be difficult to adequately remove SDS due to strong surfactant-protein interactions. Several approaches have been tried to solve these problems: (1) using non-ionic surfactants such as octyl \(\beta\)-gluco-pyranoside\(^2\); (2) electroelution or electroblotting after PAGE onto a PVDF or nitrocellulose membrane; (3) protein precipitation with guanidium chloride\(^3\); (4) using ion-pair reagents\(^4\); (5) liquid-liquid extraction\(^5\); (6) reversed-phase HPLC\(^6\). However, all these techniques are time-consuming, and often result in significant sample loss.
Methods

Materials

Protein Standards and Myoglobin were obtained from Sigma (St. Louis, MO). 12% Tris-glycine gels were obtained from Novex (San Diego, CA). Sodium dodecyl sulfate (SDS) was purchased from Pierce (Rockford, IL). Acid-labile surfactants (ALS) were synthesized in-house. Zinc stain kit was purchased from Bio-Rad (Hercules, CA). Zinc sulfate, imidazole and Coomassie Blue were purchased from Sigma.

PAGE

Polyacrylamide gel electrophoresis was performed on protein standards using either 0.1% SDS or 0.1% ALS. The upper and lower buffer chambers were filled with buffer consisting of 0.025 M Tris, 0.192 M Glycine, and either 0.1% SDS or 0.1% ALS, pH 8.3.

Molecular weight standards were prepared as follows: Mark VI protein standards (containing 13.5 mg of lysozyme, β-lactogobulin, trypsinogen, pepsin, egg albumin, bovine albumin and bromphenol blue tracking dye) and Mark VII protein standards (containing 13.5 mg each of α-lactalbumin, trypsin inhibitor, trypsinogen, carbonic anhydrase, glyceraldehyde-3-phosphate dehydrogenase, egg albumin and bovine albumin) were reconstituted in 0.6 mL of buffer containing 0.125 M Tris-Cl, 20% (v/v) glycerol, 0.02% bromphenol blue pH 6.8. Reduction was performed by adding β-mercaptoethanol (1% v/v), and heating to 100 °C for 2 minutes.

Electrophoresis was performed at a constant current of 20 mA per gel.
Staining

SDS-PAGE gels were stained with either Coomassie Blue or zinc-imidazole stain according to the product instructions.

Gels run with ALS-1 were stained using a modification of the zinc-imidazole staining technique developed by Fernandez-Patron et al.\textsuperscript{7}. After electrophoresis, the gel was incubated for approximately 3 minutes in 200 mM zinc sulfate (Sigma, Z-0501), 100 mM imidazole, 0.1% ALS-1, pH 5.0. After the bands developed, the gel was rinsed in several changes of 50 mM Tris, pH 8.8.

Passive Elution

Gel slices containing protein bands were carefully excised from PAGE gels using a clean razor, transferred to a clean tube and washed with 1 mL of 2.5 mM Tris, 19.2 mM glycine. The slice was then washed twice in 1 mL deionized water. Subsequently, it was carefully diced to approximately 1 mm\textsuperscript{3} pieces with a clean razor, and then transferred to a microcentrifuge tube. 40 µL of elution solvent containing 80/20 (v/v) acetonitrile-water, 0.1% TFA was added to the tube. After vortexing and overnight mixing on a platform rocker, the tubes were spun at 12,000 rpm to pellet the polyacrylamide gel pieces. The eluate was collected using a gel loading pipette tip.
Acid-Labile Surfactant (ALS) Degradation

\[
\text{CH}_3(\text{CH}_2)_x \xrightarrow{\text{ALS}} \text{CH}_3(\text{CH}_2)_x + (\text{CH}_2)_y\text{SO}_3^-\text{Na}^+ \\
\text{O} \quad \text{O} \\
(\text{CH}_2)_y\text{SO}_3^-\text{Na}^+ \\
\text{OH} \quad \text{OH} \quad \text{A}
\]

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Kinetics of Surfactant Degradation

Degradation determined by $^1$H NMR in D$_2$O at 22°C. 10% TFA was used to acidify a 10% ALS-I solution. At pH 7, the 10% ALS-I solution is stable for months.
Comparison of SDS-PAGE and ALS-PAGE Gels

Coomassie Blue stained gels of Mark VI and Mark VII standards. Proteins were denatured by heating 2 minutes at 100 °C prior to gel loading.
Plot of log (MW) versus relative migration factor for reduced Mark VI and Mark VII proteins using SDS and ALS-I. Results show similar migration behavior.
Carbonic anhydrase behaves anomalously under native conditions and can be used to determine effectiveness of the acid-labile surfactant to denature proteins. It was determined that ALS-I may require heat (60°C for 10 min or 100°C for 2 min) to fully denature carbonic anhydrase. Heat treatment is often used in SDS applications as well, but is not required.
Direct ESI infusion of 5 µM myoglobin into a Waters Platform LC 50/50 (v/v) 20 mM ammonium acetate - acetonitrile with 1% acetic acid using: a) no surfactant, b) 0.1% ALS-II, c) 0.1% ALS-I, and d) 0.1% ALS-III. All surfactants degraded for 16 h prior to ESI-MS.
Direct ESI infusion of 5 µM myoglobin into a Waters Platform LC 50/50 (v/v) 20 mM ammonium acetate, pH 5.1 - acetonitrile with 1% acetic acid using: a) no surfactant, b) 0.1% SDS, and c) 0.1% ALS-I after 16 hours.
Direct infusion of a solution of 5 \( \mu \text{M} \) myoglobin in 50/50 (v/v) 20 mM ammonium acetate, pH 5.1 - acetonitrile was performed into a Waters Platform LC. Solutions contained different concentrations of SDS, as noted.
ESI-MS of Myoglobin: Effect of %ALS

Direct infusion of a solution of 5 µM myoglobin in 50/50 (v/v) 20 mM ammonium acetate, pH 5.1 - acetonitrile was performed into a Waters Platform LC. Solutions contained different concentrations of degraded ALS-II (16h), as noted.
Direct infusion electrospray mass spectra of myoglobin following PAGE. Myoglobin band excised and protein eluted following protocol described in Methods Section.
MALDI of Myoglobin following PAGE and Passive Elution

Data courtesy of Andy Whitehill, Micromass, Inc. MALDI performed on a Micromass TofSpec-2E. Myoglobin band excised followed by passive elution. Sample mixed with sinapinic acid and air-dried on MALDI target.
Conclusions

• SDS-PAGE vs. ALS-PAGE
  – Similar log MW vs. $R_f$
  – Denaturation capability slightly weaker with ALS, e.g., carbonic anhydrase, trypsinogen

• Rapid ALS degradation at acidic pH; very stable at neutral and basic pH

• Significantly enhanced ESI and MALDI MS sensitivity of proteins in presence of ALS vs. SDS

• ALS-I gives one myoglobin - ALS degradant adduct; ALS-II and ALS-III give two adducts

• Potential for other application areas:
  – HPLC
  – Bioprocessing
  – Protein preparation/solubilization
  – etc.
References


