

Ammonium Dodecyl Sulfate as an Alternative to Sodium Dodecyl Sulfate for Protein Sample Preparation with Improved Performance in MALDI Mass Spectrometry

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Sodium dodecyl sulfate (SDS) is a strong surfactant that is widely used in protein sample preparation. While protein and peptide samples containing up to ~1% SDS can be analyzed by matrix-assisted laser desorption/ionization mass spectrometry (MALDI MS) using a two-layer matrix/sample deposition method, the presence of SDS in a protein sample generally degrades mass resolution and mass measurement accuracy. This degradation in performance is found to be related to the formation of sodium–protein adducts in the MALDI process. If the instrument resolving power is insufficient to separate these adduct peaks from the protonated molecular ion peak, peak broadening is observed in the protein molecular ion region, and as a result, the peak centroid shifts to a higher mass. In this work, we present a method using ammonium dodecyl sulfate as a viable alternative to SDS for protein sample preparation with much improved MALDI MS performance. Three non-sodium-based dodecyl sulfate surfactants, ammonium dodecyl sulfate (ADS), hydrogen dodecyl sulfate, and tris(hydroxymethyl)aminomethane dodecyl sulfate were investigated. Of the three surfactants tested, it is found that ADS gives the best performance in MALDI. For proteins with moderate molecular masses (i.e., up to ~25 kDa), the presence of ADS in a protein sample does not result in significant degradation in mass resolution and accuracy, and the protonated molecular ion peak is the dominant peak in the MALDI spectrum. The ammonium adduct ions dominate the MALDI spectra when the protein mass exceeds ~25 kDa; however, ADS still gives better results than SDS. The behavior of ADS in gel electrophoresis was also investigated. It is shown that cell extracts dissolved in ADS can be separated by normal SDS–polyacrylamide gel electrophoresis by simply mixing them with the SDS sample buffer. The application of ADS as the surfactant for protein solubilization with improved performance in MALDI analysis is demonstrated in the study of a detergent insoluble fraction from a Raji/CD9 B-cell lymphocyte lysate.

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) is widely used for protein analysis. Compared to other methods such as electrospray ionization, MALDI can tolerate a relatively high level of contaminants present in a sample.¹ The MALDI spectrum consists of peaks from mainly singly and doubly charged protein molecular ions. These attributes make the MALDI technique particularly useful for direct analysis of complex protein mixtures, such as those obtained from cell extracts or affinity purification. Samples obtained from these preparation methods may contain surfactants, which are commonly used reagents in protein sample workup. Surfactants help to solubilize hydrophobic proteins and reduce protein aggregation and protein adsorption on the container walls.^{2,3} Because of the possible adverse effect on MALDI performance caused by various types of surfactants, special care must be exercised in dealing with protein samples containing surfactants.

Sodium dodecyl sulfate (SDS) is a strong solubilization reagent that is commonly used for extracting proteins remaining in cells or tissues after they have been subjected to sequential extractions by nonionic detergents.^{4–6} SDS is also used as a denaturing reagent for SDS–polyacrylamide gel electrophoresis (PAGE). Early reports showed that SDS was detrimental to MALDI signals and should be avoided or removed from the samples prior to MS analysis.^{7–10} Electroelution or electroblotting of proteins, solvent extraction, and protein precipitation are several techniques that

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have been developed to remove the SDS interference.^{11–15} These techniques obviously add an undesirable step for MALDI analysis. The sample-cleaning step can result in protein loss, especially for hydrophobic or membrane proteins.^{16,17} An alternative approach is to develop new surfactants that have properties similar to those of SDS but will be MS-compatible. For example, an acid-labile surfactant was developed to replace SDS that was found to be compatible with MS analysis.^{18,19} However, this surfactant at present has only found limited use and its overall performance as a viable alternative to SDS in protein chemistry remains to be determined.

The effect of SDS on MALDI MS has been recently studied in more detail. Recent studies have indicated that the negative effect by SDS on protein analysis was strongly dependent on sample preparation conditions.^{20–25} For example, Roepstorff and co-workers reported that useful MALDI signals could be obtained for protein samples containing 0.2% SDS when the pH of the sample solution was kept under 2.²⁰ They also indicated that SDS affected the mass resolution. Limbach and co-workers recently illustrated that SDS could assist in analyzing membrane proteins and improve ionization of hydrophobic peptides.^{21–23} Amado and co-workers showed that the MALDI signal was improved if the SDS concentration was above 0.23%.²⁴ In our recent study, we presented a practical and robust technique based on a two-layer matrix/sample deposition method for the analysis of protein and peptide samples containing SDS by MALDI MS.²⁵ The two-layer method involves the deposition of a mixture of sample and matrix on top of a thin layer of matrix crystals. We found that, for SDS-containing samples, the intensity of the MALDI signals could be affected by the conditions of sample preparation, such as on-probe washing,

choice of matrix, deposition method, solvent system, and protein-to-SDS ratio. However, we found that, under appropriate conditions, the two-layer method gave consistent MALDI signals for samples with levels of SDS up to ~1%.

While the two-layer method can readily produce MALDI signals from protein samples containing SDS, the protein peaks observed are generally broad and the peak centroids are shifted to higher masses. In this work, we address these issues and present a method using ammonium dodecyl sulfate as a viable alternative to SDS for protein sample preparation with much improved MALDI MS performance.

EXPERIMENTAL SECTION

Chemicals and Reagents. Bovine insulin, equine cytochrome c, equine myoglobin, bovine trypsinogen, bovine carbonic anhydrase II, bovine serum albumin (BSA), lactoferrin, α -cyano-4-hydroxycinnamic acid (HCCA), diammonium hydrogen citrate (DAHC), sulfuric acid, and SDS were purchased from Sigma Aldrich Canada (Oakville, ON, Canada). Ammonium dodecyl sulfate (ADS) and dodecyl sulfate tris(hydroxymethyl)amino-methane salt (TDS) were purchased from Fluka, Canada (Oakville, ON, Canada). Tris(hydroxymethyl)aminomethane (Tris) base was purchased from Bio-Rad (Mississauga, ON, Canada). Dodecyl sulfuric acid (HDS) was generated in our laboratory by exchanging Na^+ of SDS for H^+ cations using AG 50W-X8 ion-exchange resin purchased from Bio-Rad. Approximately 450 g of resin was packed into a column. The hydrogen form of the resin was generated by washing the column with 300 mL of water, followed by 400 mL of 3 M HCl, and flushing with water until the effluent from the column was neutral.²⁶ About 50 mL of 1% SDS (i.e., ~35 mM) was then loaded onto the column, and the effluent was monitored for acidity. The first 20 mL of acidic effluent was discarded, and the following 10 mL was collected for MALDI sample preparation. The molar concentration of HDS collected was ~35 mM. HCCA was purified by recrystallization from ethanol prior to use. Analytical grade acetone and methanol were purchased from Caledon Laboratories (Edmonton, AB, Canada). Water used in all experiments was from a Nanopure water system (Barnstead/Thermolyne).

Sample Preparation. Standard protein samples containing different types of dodecyl sulfate (DS) compound were prepared by mixing the protein solution with the DS solution, followed by vortexing. The concentration of the DS compound (w/v) indicated in this work is for the protein samples prior to mixing with the MALDI matrix.

The two-layer method was employed for MALDI sample deposition.^{27,28} Briefly, 1 μL of a 12 mg/mL solution of HCCA in 80% acetone/methanol was deposited onto the target and was allowed to dry, forming a very thin layer of fine crystals. A 1- μL aliquot of the protein sample was mixed with HCCA second-layer solution (saturated in 40% methanol/water), and a 0.5- μL aliquot of this mixture was deposited on top of the first layer and allowed to air-dry. On-probe washing is always performed. The washing step involves adding 2 μL of water to the sample spot and then blowing away the water with air. This procedure was performed twice on each sample spot.

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SDS-PAGE. SDS-PAGE was carried out in a Bio-Rad Mini-Protein III system using 12% polyacrylamide minigels. The SDS sample buffer contained 2% mercaptoethanol (v/v), 1% SDS, 12% glycerol, 50 mM Tris-HCl, and 0.01% bromophenol blue. Prior to electrophoresis, the protein sample was mixed in the sample buffer and heated at $\sim 95^\circ\text{C}$ for 4 min. Visualization of protein bands was done by using Bio-Rad's Biosafe Coomassie blue stain reagent or silver stain kit.

Cell Lysis. Lymphoblastoid cell lines were grown in RPMI-1640 culture, buffered with 25 mM *N*-2-hydroxyethylpiperazine-*N*-2-ethanesulfonic acid (HEPES) and supplemented with 10% phosphate-buffered saline (PBS), 2 mM L-glutamine, 100 units of penicillin, and 100 $\mu\text{g}/\text{mL}$ streptomycin. Cell concentration was kept in logarithmic growth phase at $(2-5) \times 10^5$ cells/mL medium. Approximately 3.5×10^8 cells were spun down in a Beckman CS-6KR swing-bucket centrifuge, rinsed twice in ice-cold PBS, and lysed in 5 mL of ice-cold lysing buffer (1% Triton X-100 RipA buffer). The composition of the RipA buffer is as follows: 50 mM Tris-HCl, pH 7.4, 1% Triton X-100, 0.25% sodium deoxycholate, 150 mM NaCl, 1 mM EDTA plus aprotinin, leupeptin, and pepstatin at 10 $\mu\text{g}/\text{mL}$ and 1 mM phenylmethanesulfonyl fluoride (PMSF). The detergent-insoluble fraction (DIF) is the detergent-insoluble material after the cell lysates were extracted with RipA buffer. The pelleted DIF was finally rinsed in distilled water to reduce the salt concentration.

Instrumentation. MALDI experiments were carried out on a Bruker Reflex III time-of-flight mass spectrometer (Bremen/Leipzig, Germany) using the linear mode of operation. Ionization was performed with a 337-nm pulsed nitrogen laser. The laser power was adjusted slightly above the threshold of the desorption/ionization process and was kept constant for a given set of protein samples (i.e., one protein at a given concentration with different DS salts). All data were processed using the Igor Pro Software package (WaveMetrics, Lake Oswego, OR).

RESULTS AND DISCUSSION

Effect of SDS on Mass Resolution. In our previous work, we described the two-layer sample preparation method and discussed how SDS affected the MALDI signal intensity.²⁵ With this method, good signal-to-noise ratios in MALDI spectra can be obtained from low-micromolar concentrations of protein samples containing up to 1% SDS. However, the molecular ion peak is usually quite broad, compared to that obtained from a sample containing no SDS. Analysis of low-mass proteins by this MALDI method reveals that peak broadening is mainly due to the formation of multiple sodium adduct ions with the proteins during the MALDI process. Figure 1 shows the MALDI mass spectrum obtained from a 2 μM bovine insulin solution containing 2% SDS. Several partially resolved peaks are observed in the molecular ion region of the protein. They are from the protonated molecular ions $[M + H]^+$ and sodium adduct ions $[M - (n - 1)H + nNa]^+$, where $n = 1, 2, 3$. In this case, the instrument resolving power is sufficiently high to resolve these adduct peaks. The protonated molecular ion can be readily identified, even though the peaks are not fully baseline-resolved. However, for higher mass proteins, sodium adduct peaks are not well resolved and can become the dominant peaks in the molecular ion region, resulting in poor mass resolution and a mass shift of the peak centroid to a higher mass. Since the extent of adduct formation

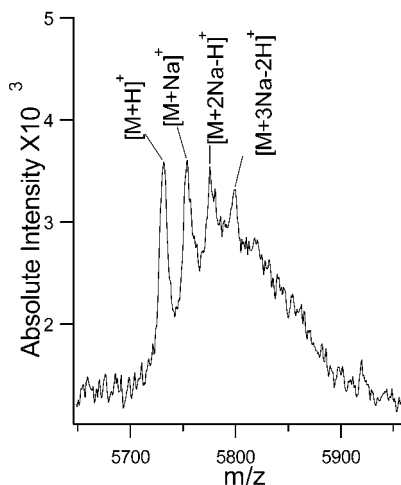


Figure 1. MALDI spectrum of 2 μM bovine insulin with 2% SDS. Analyte-to-matrix, HCCA (saturated in 40% MeOH/H₂O), ratio is 1:1.

cannot be predicted in the protein MALDI spectrum, the degree of mass shift is unknown. As a consequence, the protein mass cannot be accurately defined.

Effects of ADS, TDS, and HDS on Resolution. SDS is commonly used to extract hydrophobic or membrane proteins from cells.^{5,6} Most biopolymer samples such as cell lysates contain a lot of salts that can be the potential source of sodium ions in subsequent MALDI analysis. However, prior to the use of SDS for protein extraction, the water-soluble proteins are commonly first extracted by using a buffer system that may or may not contain a mild surfactant or detergent such as Triton. The detergent-insoluble component can be washed by using an aqueous solution to remove the salts. But, when SDS is used as a strong surfactant to solubilize any hydrophobic proteins remaining in the detergent-insoluble component, a large quantity of sodium ions from SDS is also introduced to the sample. Thus, the major source of sodium ions in the SDS-containing sample is from the SDS reagent. Since the surfactant property of SDS is mainly related to the dodecyl sulfate structure, other types of dodecyl sulfate salts still function like SDS for protein solubilization.²⁹ We have chosen two dodecyl sulfate salts, ADS and TDS, for this study. Ammonium and Tris were expected to interact less strongly with proteins in the gas phase and to form fewer adducts compared to the sodium ion. In addition, these two compounds are commercially available and relatively inexpensive—an important consideration for the wide use of these reagents in protein biochemistry and in mass spectrometry laboratories. For comparison, we have also examined the effect of HDS in MALDI analysis.

Figure 2, from left to right, shows the MALDI spectra obtained from 2 μM insulin, cytochrome *c*, myoglobin, and trypsinogen, respectively, without the addition of any surfactant and with the addition of 0.5% various dodecyl sulfate surfactants. The molecular ion peaks for these four proteins are labeled in Figure 2A. In the MALDI spectra obtained from the samples containing 0.5% SDS (Figure 2B), the sodium adduct ion peaks are resolved from the protonated molecular ion peak for insulin and cytochrome *c*. For

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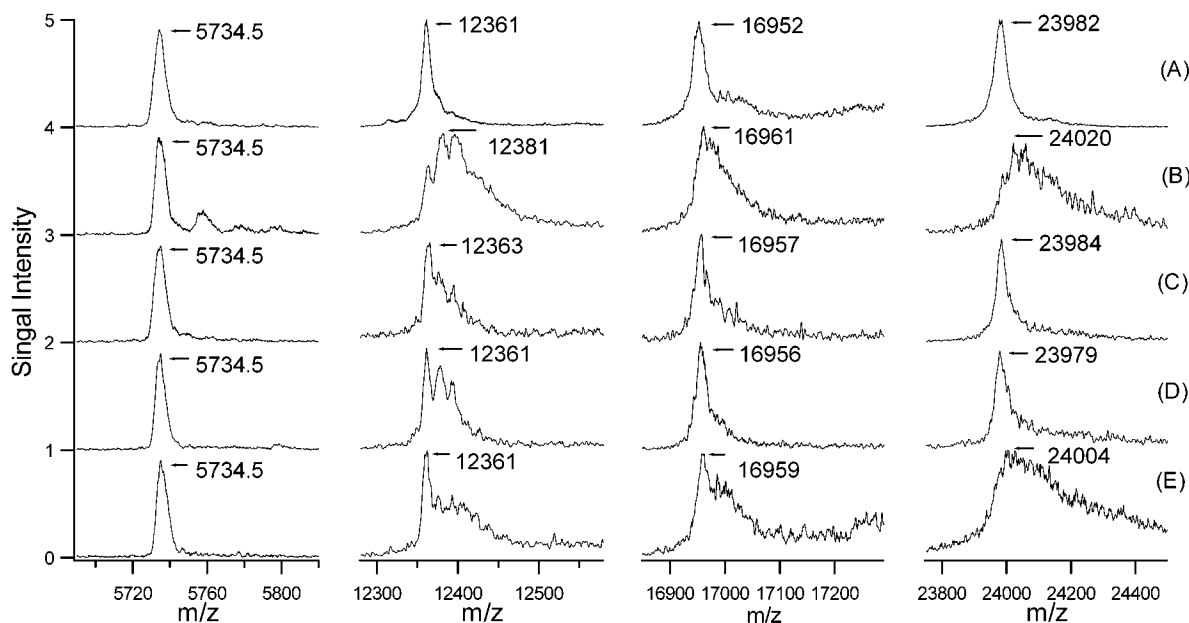


Figure 2. MALDI spectra of 2 μ M insulin, cytochrome *c*, myoglobin, and trypsinogen: (A) without surfactant, (B) with 0.5% SDS, (C) with 0.5% HDS, (D) with 0.5% ADS, and (E) with 0.5% TDS. Analyte-to-matrix ratio varies from 1:1 to 1:2.

insulin, the sodium adduct ion peaks are much less intense than the protonated molecular ion peak, whereas in Figure 1, the sodium adduct ion peaks are comparable to the protonated one (2 μ M insulin containing 2% SDS). It is generally the case that, as the concentration of SDS in a protein solution increases, the sodium adduct peaks in the MALDI spectrum become more intense. In the spectrum of cytochrome *c* with 0.5% SDS, the sodium adduct peaks become dominant. Even though the peaks are only partially resolved, the nominal molecular mass of cytochrome *c* still can be accurately determined. However, as the protein molecular mass further increases, the protonated and sodium adduct ion peaks are no longer well resolved, as illustrated in the spectra of myoglobin and trypsinogen containing 0.5% SDS. The peak centroids for these two proteins shift to higher masses than the molecular masses.

It is worth commenting on the differences in running protein samples containing common salts such as NaCl and those containing an equivalent amount of SDS by MALDI. In the case of protein samples containing NaCl, most salts will likely be excluded from incorporation into the cocrystals of proteins and matrix molecules during the sample/matrix preparation process. A significant fraction of salts present in the sample/matrix layer can be washed away by the on-probe washing procedure in the two-layer sample preparation method. As a result, the protonated protein molecular ions are dominant in the MALDI spectrum of protein samples containing NaCl. However, in the case of protein samples containing SDS, dodecyl sulfate interacts strongly with the protein molecule. Three species, namely, the protein, a protein–dodecyl sulfate complex, and dodecyl sulfate, can be incorporated into the matrix crystals. The presence of the latter two species in the matrix crystals would require counterions that would mainly be the sodium ions in a SDS-containing protein sample. Thus, matrix/analyte cocrystallization and on-probe washing can only reduce a certain amount of sodium ions. Many remaining sodium ions residing in the cocrystals will be ionized

in MALDI, which increases the possibility of forming adduct ions with the proteins in the gas phase.

The ZipTip sample cleaning technique is commonly used to reduce salt contaminants. We find it particularly effective for cleaning in-gel digests of proteins prior to MS analysis. However, we find that desalting the SDS-containing protein sample by using either ion exchange or C18 ZipTip does not result in any improvement in MALDI analysis. This is likely due to the difficulty of removing a large amount of sodium ions present in the SDS–protein sample with the ZipTip.

When SDS is replaced with HDS, better mass resolution is obtained. This can be seen from Figure 2C. Protonated peaks are the dominant signals in the molecular ion region for all four proteins. However, for cytochrome *c* and myoglobin, HDS suppresses the MALDI signals to some extent. The reason for signal reduction is likely related to the strong acidity of HDS that may affect the protein incorporation and cocrystallization with the HCCA molecules. For example, the addition of 9 mM sulfuric acid to the cytochrome *c* sample, which results in the same acidity as that of 0.5% HDS, gave a signal reduction effect similar to what HDS did (spectra not shown). It should be noted that on-probe washing did improve signals for protein samples containing 0.5% dodecyl sulfate salts. However, washing must be limited for several seconds because a prolonged washing step would dissolve the matrix/analyte layer, resulting in poor signals. However, for samples containing sulfuric acid, a long washing period can be applied without dissolving the sample layer. In the case of cytochrome *c* containing sulfuric acid, if the sample spot was washed over 20 s, the protein signal can be recovered to the same level as that obtained from a sample with no strong acid added. But, for myoglobin, the additional washing step did not result in signal recovery. These results suggest that strong acids such as HDS and sulfuric acid can affect the MALDI sensitivity with the two-layer sample preparation method and on-probe washing may not always be effective to reduce this interference.

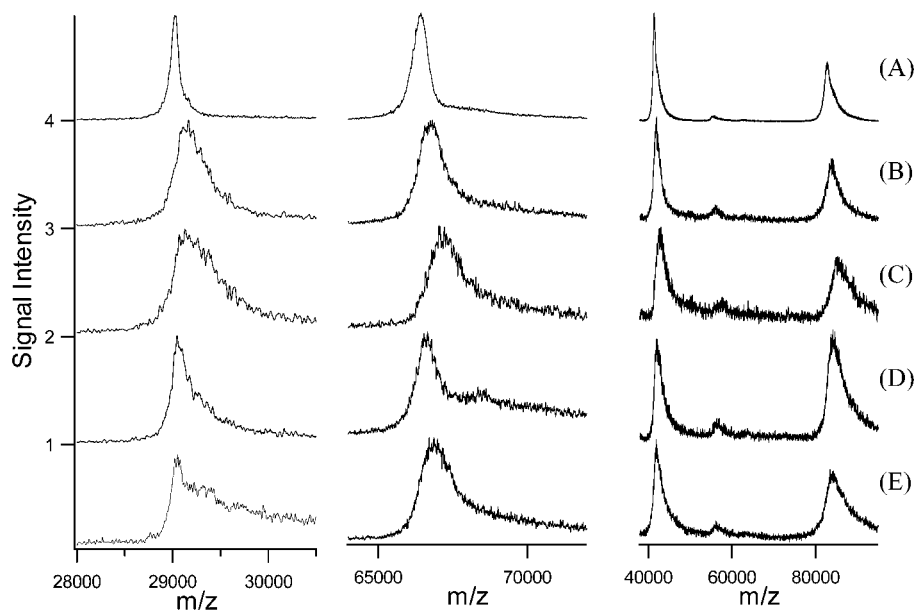


Figure 3. MALDI spectra of 5 μ M carbonic anhydrase, BSA, and lactoferrin: (A) without surfactant, (B) with 0.5% SDS, (C) with 0.5% HDS, (D) with 0.5% ADS, and (E) with 0.5% TDS. Analyte-to-matrix ratio varies from 1:1 to 1:2.

Figure 2D shows the MALDI spectra of the four protein samples containing 0.5% ADS. The spectral quality is comparable to that obtained from the protein sample with no surfactant added. Each spectrum displays a strong protonated molecular ion peak, from which accurate protein molecular mass can be determined. In the case of cytochrome *c*, ammonium adduct peaks are observed. The improvement of the MALDI spectral quality from the use of ADS over SDS is quite dramatic for the analysis of trypsinogen. With the presence of 0.5% SDS in the sample, the MALDI spectrum (Figure 2B) displays a broad peak which is clearly shifted to a higher mass. But, for the ADS-containing sample, a sharp protonated molecular ion peak is obtained.

The improved MALDI results from the use of ADS in place of SDS are not totally surprising. The use of ammonium to suppress the sodium adduct ions in MALDI has been practiced by many researchers in the area of oligonucleotide analysis.^{26,30} In electrospray ionization MS, the addition of ammonium salts to the protein or peptide solution can also enhance the protonated peaks over the sodiated peaks. During the ionization and ion transport processes, the protein–ammonium adduct ions can be dissociated to form the protonated ions with the loss of ammonia. Note that merely adding a large amount of ammonium salts such as NH_4Cl to a protein sample containing 0.5% SDS does not result in the reduction of sodium adduct ions. Sodium ions appear to interact with the protein more strongly than the ammonium ions do. This observation is similar to that found in oligonucleotide analysis by MALDI where desalting of the DNA sample prior to mixing it with the matrix and ammonium salts is critical to generate a high-resolution mass spectrum.^{26,30}

The results from the analysis of TDS-containing protein samples as shown in Figure 2E are somewhat surprising. In planning our experiments, we thought that the bulky structure of Tris would not readily form adduct ions with proteins. With the two-layer sample preparation method, MALDI signal intensity,

mass resolution, and mass measurement accuracy of proteins are unaffected by the presence of neutralized Tris base at levels up to 1% (spectra not shown). In analyzing protein samples containing Tris base, an acid was added to the second-layer solution to neutralize the Tris base. Otherwise, the first crystal layer would be dissolved by the basic second-layer solution. In the cases where TDS-containing samples were analyzed, for insulin and cytochrome *c*, the protonated peak is dominant, as shown in Figure 2E. However, extensive adduct ions are observed for myoglobin and trypsinogen. Interestingly, the patterns of the adduct ion peaks are similar to those from the SDS-containing protein samples. It is possible that the adduct ion peaks shown in Figure 2E are also from the sodiated species. The source of the sodium ions may come from the sample, the TDS reagent, and the containers used. During the matrix/analyte cocrystallization process, the bulky structure of Tris that should act as the counterion for dodecyl sulfate and the protein–dodecyl sulfate complex has to compete with other smaller positive ions such as sodium to be incorporated into the cocrystals. Even though the amount of sodium ions in the solution is small compared to Tris, its readiness to be incorporated in the cocrystals can result in an accumulation of a large quantity of sodium ions in the crystals. Once the sodium ions are trapped inside the crystals, they are not readily washed away by the on-probe washing process.

Comparative analysis is also carried out for relatively high mass proteins. Figure 3 shows the MALDI mass spectra obtained from 5 μ M carbonic anhydrase II (molecular mass 29 025 Da), BSA (molecular mass 66 430 Da), and lactoferrin with and without the dodecyl sulfate surfactants added. The spectrum shown in Figure 3A is from the protein samples with no surfactant added. With the presence of 0.5% SDS in the sample (Figure 3B), the molecular ion peaks become very broad. With HDS (Figure 3C), the peaks are still broad with their centroid masses shifted to high masses. When TDS is present in the samples (Figure 3E), the resulting spectra are similar to those obtained from samples containing SDS. However, when ADS is used (Figure 3D), good resolution is

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obtained. For example, in the carbonic anhydrase spectrum, a reasonably sharp peak is observed at m/z 29042, which seems to correspond to the ammonium–protein adduct ion, $[M + \text{NH}_4]^+$. Peak tailing to a higher mass suggests that some other adduct ions likely from the attachment of two and three ammonium ions are also formed.

The observation of the ammonium–protein adduct ion as the dominant peak in the protein mass spectrum shown in Figure 3D can be explained by considering the readiness of adduct ion formation and the energetics of adduct ion dissociation. As the protein mass increases, the increase in protein chain length provides more possible binding sites for ammonium ion attachment. During the MALDI process, protein molecules are gaining internal energies through collisions, reactions, and possibly direct absorption of the laser energy. However, the internal energy gained may not be sufficient to dissociate the ammonium adduct ions formed with a larger protein. As a consequence, protonated molecular ions are not extensively produced, as is the case for smaller proteins. A relatively sharp peak observed in the mass spectrum of the ADS-containing sample indicates that ions from the proteins with more than two ammonium ions attached are much less abundant than the adduct ion with one ammonium ion attached. Proteins with multiple ammonium ions attached likely dissociate into the $[M + \text{NH}_4]^+$ ions during the MALDI process. In the case of sodium adduct ions (see Figure 3B), since the binding between the sodium ion and the protein molecule is stronger, more energy is required to dissociate the multiple sodium adduct ions. An insufficient amount of internal energy placed on the protein–sodium adduct ions during MALDI would prevent them from dissociation, resulting in the observation of similar intensities of protein adduct ions with several sodium ions attached.

Negative Ion Mode Operation. The same protein samples were run in negative mode, to see whether these four dodecyl sulfate compounds have effects on mass resolution similar to those in positive mode. However, the sensitivity of negative ion detection of proteins is found to be much lower than that of positive ion detection. For example, for the $2\ \mu\text{M}$ cytochrome *c* solution, the molecular ion peak is barely detectable in the MALDI spectrum. For the $2\ \mu\text{M}$ insulin samples with and without the presence of any dodecyl sulfate surfactants (at the 0.5% level), similar mass resolution is observed (data not shown). However, for cytochrome *c* and other higher mass proteins, the signal intensity is weak even for a $5\ \mu\text{M}$ protein solution. Considering that the positive ion detection gives much better detection sensitivity and that good sensitivity is critical for biomolecular analysis, we did not attempt to make any further investigation on the effects of surfactants on mass resolution for the large proteins detected under the negative ion mode operation.

Effect of ADS on Gel Electrophoresis. ADS is a surfactant that possesses properties similar to those of SDS.³¹ Thus, it can be used to replace SDS for cell extraction and protein dissolution. However, for gel electrophoresis, the use of the ammonium ion instead of the sodium ion may cause interference. The effect of ADS on the performance of SDS–PAGE was investigated. In our

experiment, protein samples containing ADS was first mixed with the normal SDS sample buffer, followed by separation using a 12% SDS–PAGE gel. It was found that the presence of ADS in protein samples at a practical level up to 5% (and probably higher) does not affect the SDS–PAGE separation, which means that a protein sample can be prepared using ADS for MALDI analysis and the remaining used for gel electrophoresis.

We have also examined the use of ADS in place of SDS for the gel casting and running procedure (i.e., ADS–PAGE). Since the ammonium ion has the potential to dissociate to form ammonia and a proton during the electrophoretic process, the gels were run under constant current. The voltage needed for running the ADS gel was found to be slightly lower than that used for running the SDS gel. However, the major problem for running the ADS gel is associated with the staining process. When the ADS gel is stained by the Coomassie blue staining reagent, the background can be quite high, reducing the contrast between the protein bands and the gel background. Thus, the protein display sensitivity with Coomassie stain is lower in running ADS–PAGE. High background or reduced sensitivity was also observed to be true for silver-stained ADS gels. Therefore, in terms of detection sensitivity, SDS–PAGE is preferred to ADS–PAGE.

Application to Hydrophobic Protein Analysis. The DIF pellet from lymphoblastoid cells or Raji/CD9 B-cells was dissolved in 1% ADS or 1% SDS. This pellet should mainly consist of very hydrophobic membrane proteins. Without the addition of ADS or SDS, no MALDI signals could be obtained by simply mixing the DIF sample with the HCCA second-layer solution in the two-layer sample preparation. Figure 4 shows the MALDI spectra of DIF obtained with (A) SDS or (B) ADS. These spectra can be readily reproduced. Thus, the spectral differences observed in panels A and B of Figure 4 reflect the different effects of SDS and ADS on MALDI signals. From the spectra shown in Figure 4, we can see that low-mass proteins with masses up to ~ 20 kDa are detected in both cases. However, the spectrum obtained from ADS (Figure 4B) gives more peak information because the peaks are better resolved. For example, the broad peak labeled at m/z 7879 in Figure 4A actually consists of two major peaks as shown in Figure 4B at m/z 7816 and 7902. The peak shown in Figure 4B labeled as m/z 12 799 is not observed in Figure 4A. In addition, with ADS in the sample, a protein with molecular mass of ~ 33 kDa was detected while this protein was barely seen in the case of SDS (Figure 4A). As a control experiment, a portion of the DIF sample was dissolved in 1% SDS, mixed with gel loading sample buffer, and separated on a 17% SDS–PAGE gel (Figure 5). The spectrum obtained from ADS is in agreement with the gel control experiment (Figure 5), which indicates that there is a high amount of proteins in the low-mass range and another dark band in the mass range 30–35 kDa.

It should be noted that both spectra in Figure 4 were obtained by adding a small amount [$\sim 10\%$ (v/v)] of 0.2 M DAHC to the mixture of analyte and matrix (HCCA saturated in 40% MeOH/ H_2O).^{26,30,32} DAHC is a reagent that is sometimes used to assist analyte ionization by enhancing protonated molecular ion signals and suppressing the sodium adduct ions in DNA and protein samples. In the analysis of DIF, without the addition of DAHC,

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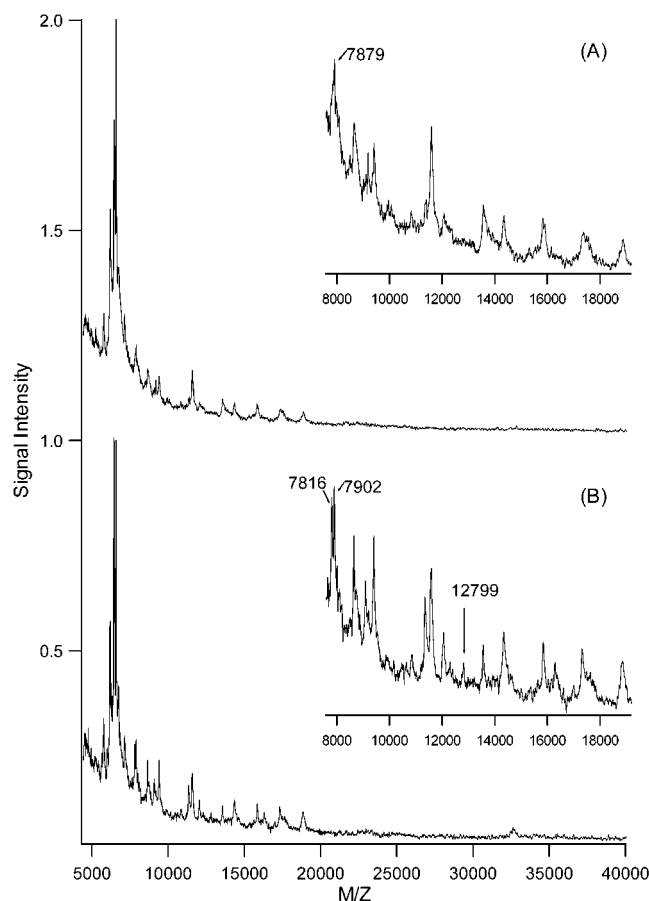


Figure 4. MALDI spectra of the CD9 cell lysate detergent-insoluble fraction pellet. The pellet was mixed with (A) 1% SDS and (B) 1% ADS.

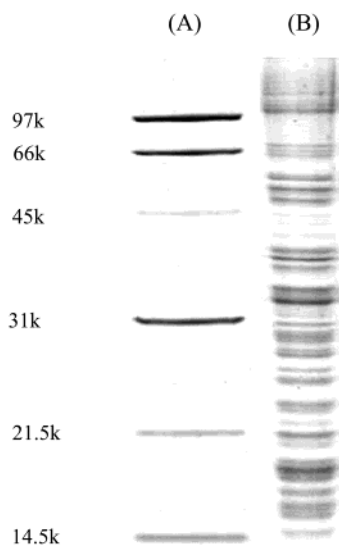


Figure 5. Gel image of the CD9 cell lysate detergent-insoluble fraction pellet. Lane A is from molecular weight markers, and lane B is from the DIF pellet.

the signals were low and only some of the low-mass proteins in the mass range up to 12 kDa could be obtained.

Although the gel image shown in Figure 5 detects a number of high-mass proteins, many of them are not detected in MALDI. This observation is similar to that obtained in analyzing water-

soluble proteins.³³ It appears that for both hydrophilic and hydrophobic proteins the low-mass proteins are more readily ionized and the high-mass protein signals are suppressed. However, MALDI analysis of very hydrophobic proteins is not as sensitive as for less hydrophobic proteins. For the analysis of DIF, which consists mainly of very hydrophobic proteins, the laser power required to produce protein signals was higher than that used for less hydrophobic proteins. The ionization efficiency for a hydrophobic protein via protonation is expected to be low because of the lack of many efficient protonation sites.

It is clear that the MALDI technique is compatible with the strong dodecyl sulfate surfactant, which should provide a new tool to tackle hydrophobic proteome analysis problems. For example, we are currently in the process of developing a protein digestion protocol to generate peptides directly from the DIF pellet of the lymphoblastoid cells. MALDI MS/MS will be used to generate peptide sequence information to identify the proteins present in the mixture. With the knowledge of accurate molecular masses obtained by MALDI-TOF, we are hoping to determine the origins of these low-mass proteins. These proteins could be the fragments of large membrane proteins or small gene products with or without posttranslational modifications. Our future work will also be directed to the improvement of ionization efficiency of MALDI for the analysis of very hydrophobic proteins that can only be soluble in dodecyl sulfate surfactant. To this end, the use of a transition metal ion such as silver salt will be attempted to examine whether cationization can be a better means of ionization for the very hydrophobic proteins, as one would expect from the polymer MALDI work. In that case, the analysis of less polar species such as polybutadiene is usually done by using cationization with a transition metal ion.³⁴

CONCLUSIONS

While protein samples containing up to ~1% SDS can be analyzed by the two-layer sample/matrix preparation method, the MALDI signal quality was found to be degraded because of the formation of protein-sodium adduct ions in the MALDI process. If the instrument resolving power is inadequate to resolve these adducts, peak broadening is observed and the broad peak centroid is shifted to higher mass. Ammonium dodecyl sulfate, hydrogen dodecyl sulfate, and tris(hydroxymethyl)aminomethane dodecyl sulfate were explored as alternatives to SDS. We found that the ADS was least detrimental to MALDI performance. The presence of ADS in the protein samples did not significantly degrade the signal resolution and accuracy, as was the case with SDS. With ADS in protein samples, the protonated peak was dominant in MALDI analysis from moderate protein mass up to ~25 kDa. As the protein molecular weight increases, the ammonium adducts become dominant in the MALDI spectrum, but ADS still yields the best results of the four surfactants. The performance of ADS-solubilized proteins for gel electrophoresis was also examined. We demonstrated that ADS-solubilized cell extracts could be separated by SDS-polyacrylamide gel electrophoresis by mixing the samples with normal SDS buffer. The improved MALDI performance of an ADS-solubilized detergent-

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insoluble fraction from Raji/CD9 B-cell extract was also demonstrated in this work. It can be concluded that ADS is a viable alternative to SDS in protein extraction and purification and should be used in MALDI analysis of very hydrophobic proteins.

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