Direct Matrix-Assisted Laser Desorption Ionization Mass Spectrometric Analysis of Proteins Immobilized on Nylon-Based Membranes

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Direct analysis of proteins adsorbed onto the surface of nylon membranes has been performed at the picomole level by matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS). Nylon-66 and positive charge-modified nylon (Zetabind) membranes fixed to MALDI probe tips were successfully employed to analyze picomole quantities of sample that were adsorbed onto these inert supports prior to adding a matrix-containing solution. Proteins and peptides are readily solubilized from these types of membrane with conventional matrix solvents and cocrystallize with the matrix on the membrane surface. Because solubilization of membrane-adsorbed protein is necessary for successful sample preparation, nylon membranes are more suitable for use with MALDI-MS than other protein transfer membranes such as polyvinylidene difluoride or nitrocellulose. When compared to samples prepared conventionally, no apparent loss of sensitivity or resolution is observed when analysis by MALDI-MS is performed from nylon-66 or positive charge-modified nylon membranes. Detection limits and resolution are not apparently affected by the membrane immobilization/washing procedure, and no change in the mass accuracy is observed when analysis is performed on the nylon surface. However, there is a time shift (increase) in ion flight time when analysis by MALDI-time-of-flight-MS is performed directly from the membrane fixed to the probe tip (about 200 ns for an ion of mass 379.3). To maintain mass accuracy, the use of internal standards or external calibration performed on a membrane support was necessary. The immobilization of proteins on nylon membranes can be used to facilitate removal of water-soluble contaminants because the sample is retained when the membrane is immersed in water prior to adding the matrix solution. The feasibility of performing both chemical and enzymatic modifications of proteins adsorbed onto inert nylon supports prior to analysis by MALDI-MS is also demonstrated. (*J Am Soc Mass Spectrom* 1994, 5, 230-237)

Matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS) has quickly developed into one of the principal means for the mass spectrometric characterization of biological macromolecules. Some notable features of this technique are a sensitivity into the low picomole range [1], an accessible mass range with an upper mass limit approaching 300,000 Da [1], the capability for analyzing mixtures [2], and a degree of tolerance for many buffers and salts commonly used in biological sample preparation. However, some sample contaminants, particularly surfactants, can preclude successful analysis by MALDI-MS [3].

Because the success of a MALDI-MS experiment is highly dependent on the crystallization process during sample preparation, the intolerance observed with cer-

tain contaminants and a particular matrix are likely due to interferences associated with either matrix crystal formation and/or the necessary interactions of the analyte with the growing matrix crystals. For example, samples containing more than 1 M guanidine, or a few percent of a relatively nonvolatile compound such as glycerol, often fail to crystallize and can be difficult to dry. The presence of surfactants may also modify analyte adsorption onto growing matrix crystals. This would likely reflect differences in analyte-matrix-solvent surface tension properties and result in a matrix-dependent response. Thus, whereas Mock et al. [3] report a threshold limit to the onset of spectral degradation from protein samples in the presence of > 0.01% sodium dodecyl sulfate (SDS) using sinapinic acid in acetonitrile/water/trifluoroacetic acid (TFA), Strupat et al. [4] have shown a MALDI mass spectrum of chicken egg albumin in the presence of 10% SDS using 2,5-dihydroxybenzoic acid in 70% formic acid.

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Other MALDI-MS contaminants commonly found in biological samples are high concentrations of alkali halide salts and/or buffers which may simultaneously crystallize and compete with matrix for the analyte in the crystallization process. This has been shown to suppress analyte signals to varying degrees, and also to generate a number of unresolved ions consisting of single or multiple cation adducts that skew the signal centroid toward higher mass, which can adversely affect accurate mass assignments [5].

The differential intolerance of MALDI-MS to contaminants such as surfactants, salts, or buffers manifests itself in the form of spectral degradation [3, 5]. This can be in the form of reduced signal intensity, peak broadening from multiple adduct formation, the need for higher laser irradiance, and/or nonpersistent signals from samples that have not completely dried. It would be advantageous then either to avoid or to minimize the use of additives during protein sample preparation, but this is often not possible. An alternative would be to remove the additives that are contaminants by a washing procedure prior to analysis by MALDI-MS.

Beavis and Chait [5] have described a washing procedure that exploits the relative insolubility of many MALDI matrices. In this procedure, the dried analyte-matrix deposit on the probe tip is briefly immersed in cold distilled water to remove water-soluble contaminants. Hefta et al. [6] were later successful in washing cytochrome P450 enzymes immobilized (before adding matrix) on either a copper stage or a piece of polyvinylidene difluoride (PVDF) membrane after the Beavis and Chait washing procedure proved ineffective for their analysis. Mock et al. [3] then described protocols for the immobilization of biological samples to roughened gold- or nitrocellulose-coated targets. In the latter case, the nitrocellulose had to be electro-sprayed onto the target because a membrane fixed directly onto the target became electrically charged and interfered with the MALDI-MS analysis.

There is considerable interest in the direct mass spectrometric analysis of biological samples that have been adsorbed onto a transfer membrane. Several types of these membranes are available for protein blotting from polyacrylamide gel electrophoresis (PAGE) gels. Characteristics of an ideal transfer membrane for use with MALDI-MS would include: (1) adsorption of protein and peptide samples during transfer from PAGE gels (by diffusion or electroelution), (2) retention of proteins/peptides during sample manipulation (i.e., multiple washings, enzymatic digestions, or chemical derivatization), (3) the ability to resolubilize the protein or peptides from the membrane into solvents that contain the MALDI matrix prior to introduction into the mass spectrometer, and (4) a surface that permits even crystallization and drying of the matrix-analyte solution (Figure 1).

In this report we describe the use of two nylon-based membranes for the immobilization of proteins prior to

Figure 1. Conceptual scheme for manipulation of low levels of proteins using immobilization membranes under conditions that permit direct analysis by MALDI-MS. Ancillary procedures allow for the removal of contaminants interfering with analysis by MALDI-MS and microscale derivatization or enzymatic digestions with minimal sample handling.

analysis by MALDI-MS. These types of membranes offer several notable features such as a high protein binding capacity, the capability for resolubilizing adsorbed protein with solvents typically used in MALDI-MS sample preparation, simple attachment to sample probes, and low cost. The results presented here indicate that the use of nylon-66 and positive charge-modified nylon (Zetabind) membranes are suitable for analyzing picomole levels of proteins that were immobilized by adsorption onto a nylon surface and washed to remove water-soluble contaminants prior to adding matrix. We also demonstrate that it is possible to perform enzymatic digestions and chemical modifications on proteins immobilized on Zetabind transfer membranes at the picomole level prior to the direct analysis by MALDI-MS.

Experimental Section

MALDI mass spectra were obtained on a VT-2000 (Vestec Corp., Houston, TX) linear time-of-flight (TOF) mass spectrometer equipped with a nitrogen laser (337 nm, 3 ns pulse). The accelerating voltage in the ion source was 30 kV. Data were acquired by using a

transient recorder with 5-ns resolution. Each spectrum was produced by accumulating data collected from 64 laser shots. Time-to-mass conversion was achieved by internal calibration using matrix peaks (m/z 379.34 for protonated α -cyano-4-hydroxycinnamic acid or m/z 225.12 for protonated sinapinic acid), and peaks from bradykinin, insulin B chain, or insulin as internal standards.

The nylon membranes investigated were nylon-66 filter membrane, with 0.45- μ m pore size and 50- μ m thickness (Sartorius, purchased from Anspec, Ann Arbor, MI), and Zetabind with 0.45- μ m pore size and 50- μ m thickness (Cuno Laboratory Products, purchased from Life Science Products Inc., Denver, CO). Standard protein samples were obtained from Sigma Chemical Co. (St. Louis, MO). Silver nitrate was obtained from EM Science (Gibbstown, NJ). SpoIID protein was supplied by Dr. L. R. Kroos, Department of Biochemistry, Michigan State University.

Membranes were fixed to probe tips as follows: one side of a double-sided adhesive strip was first applied to a piece of nylon membrane. The membrane was cut into 2 $\times$ 2 mm sections which could then be fixed to a stainless-steel probe tip. Protein samples (1–2 μ L volumes, see text for absolute protein amount) were applied to the membrane fixed to a probe tip, dried, and washed by immersing in either deionized water or aqueous 0.1% TFA for 15–20 seconds at room temperature. After drying, 2.0 μ L of a saturated matrix solution of either α -cyano-4-hydroxycinnamic acid (Aldrich Chemical Co., Milwaukee, WI) or sinapinic acid (Aldrich) in 1:1 acetonitrile/aqueous 0.1% TFA was applied to the membrane and air dried.

Mercurial labeling experiments were performed by first drying the protein solution on the membrane fixed to a probe tip, then adding 2.0 μ L of a solution that provides for a fivefold molar excess of p-hydroxymercuribenzoate (pHMB) (Sigma) over the free sulfhydryl groups. After 10–30 minutes of reaction time, excess reagents were removed by immersing the probe tip in a 0.027% ammonium hydroxide solution.

Enzymatic digests were performed by adding a 0.05 M ammonium bicarbonate buffered (pH = 8.0) enzyme solution [providing for a 2% (w/w) enzyme-substrate ratio] to a protein sample adsorbed on a membrane fixed to the MALDI probe tip. Probe tips were incubated for 6–20 hours in sealed vials above a

small volume of 0.05 M ammonium bicarbonate buffer solution to prevent drying of the reaction mixture droplet on the membrane. Endoproteinase Glu-C, chymotrypsin, and endoproteinase Asp-N were purchased from Sigma.

Results and Discussion

The types of membranes most commonly used for protein immobilization in automated protein sequencing or SDS-PAGE electroelution are either nitrocellulose (NC) [7], PVDF [8], positive charge-modified nylon [9, 10], or glass fiber [11]. These membranes can be classified as being uncharged (PVDF [8]), anionic (NC [12], glass fiber [13]), or cationic (charge-modified PVDF [14], charge-modified nylon [9, 10], or modified glass fiber [13]). The choice of a particular membrane depends upon the requirements that must be met in a specific application because these membranes differ in their binding capacities, sample retention capabilities, and resistance to chemicals and/or solvents. Table 1 compares the relative binding affinity of nylon, nitrocellulose, and PVDF for radiolabeled sheep antirabbit IgG by percent protein retention after washing the immobilized analyte first with phosphate buffer, then with 10% SDS or a 10% SDS/6 M urea solution. Even using extreme solvent conditions for protein solubilization (10% SDS/6 M urea), no elution is observed from PVDF, and only partial elution is found to occur from nitrocellulose, suggesting very strong membrane-protein interactions; however, elution is nearly complete from the nylon-based membrane. From our experiments involving proteins dot-blotted on either nylon-66 or positive charge-modified nylon membranes, we also have found adsorbed protein to be retained (as determined by visual staining with amido black) after water or aqueous 0.1% TFA washing, but completely eluted using 1:1 acetonitrile/aqueous 0.1% TFA as a solvent. The latter solvent is one typically used to prepare MALDI matrix solutions. Protein elution from nylon-based membranes closely parallels that observed in reverse-phase chromatography of proteins in that increasing proportions of acetonitrile in the mobile phase favors sample elution. More strongly membrane-adsorbed proteins can be resolubilized by the addition of organic modifiers (isopropanol) and with ion-pairing reagents (TFA, H_3PO_4) such as those used

Table 1. Characteristics of protein adsorption to immobilization membrane supports

Membrane type	Protein binding capacity ^a (μ g/cm ²)	Membrane retention ^b (percent) of sheep anti-rabbit IgG after	
		22-hour wash in 10% SDS	22-hour wash in 10% SDS + 6 M urea
Nylon	80	5.0	3.8
Nitrocellulose	80–100	40.2	7.8
PVDF	170–200	102.0	103.4

^a From Protein Blotting Handbook, BIO-RAD Bulletin 1721, catalog number 170 3980.

^b From 1991–1992 Millipore Product Catalogue.

in the reverse-phase liquid chromatographic analysis of peptides and proteins [15].

Effects on MALDI-MS Analyses Performed from Membranes

Initial MALDI-MS experiments were performed using picomole levels of protein adsorbed onto pieces of PVDF or nitrocellulose transfer membranes fixed to stainless-steel probe tips. A saturated solution of either α -cyano-4-hydroxycinnamic acid or sinapinic acid prepared in a 1:1 mixture of acetonitrile/aqueous 0.1% TFA was added as a droplet upon the surface of the membrane containing the adsorbed protein before and after an aqueous 0.1% TFA washing. Spectra recorded from samples applied to PVDF following aqueous 0.1% TFA washing showed that matrix ions were generated but no analyte signal was detected. However, samples that were applied to the PVDF and not washed showed some weakly detectable analyte signals. Our interpretation of these results is that the samples applied to PVDF and washed retain only a strongly adsorbed monolayer of protein on the membrane surface which did not resolubilize and cocrystallize with the matrix. However, samples that were applied to PVDF and not washed were likely to contain both protein adsorbed to the membrane surface as a monolayer, and more weakly associated secondary (or higher) levels of protein-protein adsorbed layers which could be resolubilized by the appropriate solvent. Protein adsorption to surfaces has been shown to involve both primary protein-surface, and secondary protein-protein interactions [16]. Samples applied to nitrocellulose membranes fixed to MALDI probe tips showed a similar behavior, although resolubilization of proteins from this surface occurred more readily than from PVDF. MALDI-MS data collected from experiments using samples initially adsorbed onto nylon-66 or positive charge-modified nylon membranes proved to be the most promising. Figure 2 compares MALDI mass spectra obtained after two identical samples were analyzed in either a conventional procedure or after initial adsorption onto a piece of nylon membrane. In the first case (Figure 2a), a mixture of ribonuclease A and trypsinogen (5 pmol each) in a 1:1 mixture of acetonitrile and aqueous 0.1% TFA saturated with α -cyano-4-hydroxycinnamic acid was applied to a stainless-steel probe tip and dried. A second sample was prepared by applying an equal amount of this protein mixture first to a membrane fixed to the stainless-steel probe tip, air drying, washing by immersing in distilled water for 20 seconds, then adding a 1:1 mixture of acetonitrile/aqueous 0.1% TFA saturated with α -cyano-4-hydroxycinnamic acid. Detection limits and resolution are not apparently affected by the membrane immobilization/washing procedure, and no change in mass accuracy is observed when analysis is performed directly from the nylon surface. However, there is a time shift (increase) in ion flight time when analysis by MALDI-TOF-MS is performed directly from the mem-

Figure 2. MALDI mass spectra of a ribonuclease A/trypsinogen mixture (5 pmol each) averaged from 64 laser shots. (a) Sample/matrix solution loaded on a stainless-steel probe tip; (b) sample first loaded onto nylon-66 membrane fixed to a stainless-steel probe tip, dried, washed with distilled water, dried, then matrix applied. Matrix was applied as a saturated solution of α -cyano-4-hydroxycinnamic acid in 1:1 acetonitrile/aqueous 0.1% TFA.

brane fixed to the probe tip (about 200 ns for an ion of mass 379.3). A similar increase in ion flight times has been observed by others during analyses of samples immobilized on PVDF membranes fixed to the probe tip [6]. The increase in ion flight times is likely the result of the membrane acting as an insulating layer, causing the desorbed ions to experience a decreased accelerating potential difference in the ion source. Alterations of the effective electric fields would therefore require the use of either internal standards, or external standards analyzed from a membrane, for accurate time-to-mass conversion. These results show that protein samples adsorbed onto nylon membranes are effectively retained during water washings and can be solubilized later from the membrane and incorporated into growing matrix crystals by using an appropriate organic solvent.

Removing Interfering Contaminants from Membrane-Adsorbed Proteins for Subsequent MALDI-MS Analysis

Removal of water-soluble contaminants from low-level protein samples can be performed after the analyte is immobilized on nylon-66 or Zetabind membranes. This

can be useful for samples containing, for example, a high concentration of sodium or potassium salts which often result in peaks with multiple shoulders toward higher mass, or a varying degree of suppression of the MALDI-MS signal. To illustrate the effect of removing an interfering cation from a protein sample, we intentionally spiked samples of insulin with a silver salt. Silver nitrate was chosen because each protein-silver ion adduct results in a mass shift of 107.9 u. Figure 3 compares the MALDI mass spectra obtained from (1) a 10-pmol sample of insulin containing a tenfold molar excess of AgNO_3 after application to a Zetabind membrane fixed to the probe tip, and (2) a similarly prepared sample that was dried and then immersed in aqueous 0.1% TFA for 20 seconds before adding matrix. The sample that was not washed produced signals corresponding to the protonated molecule plus species containing up to four silver atoms. The most abundant ion in these experiments was the protonated molecule $(M + H)^+$. The ions containing silver have several possible interpretations. For example, the ion containing one silver could be a simple silver ion adduct of the analyte, $(M + \text{Ag})^+$, or it could represent the silver salt of the analyte in the protonated form (which would have the same mass-to-charge ratio value), $((M - H +$

$\text{Ag}) + H)^+$. Another option would involve a protein molecule in which a silver atom has been incorporated (inserted into a disulfide bond) in a protonated form $((M + \text{Ag}) + H)^+$. We assume that, because multiple additions of silver atoms result in ions that retain a net charge of +1, these "silver ion adducts" are formed by H^+/Ag^+ exchange, followed by protonation, leading to the ion descriptions listed in Figure 3. Clearly, they are not $(M + n(\text{Ag}^+))$ species. In contrast, the spectrum recorded from the sample washed in TFA showed only one minor silver adduct. Higher molar ratios of silver ion to insulin caused disulfide bond reduction with the formation of similar multiple silver adducts for both the α - and β -chains of insulin. Multiple adduct formation also can be observed in MALDI-TOF mass spectra from proteins contaminated by alkali halide salts with the effect of producing spectra containing multiple high mass shoulders, or spectra skewed at the trailing edge depending on the mass of the analyte. This can lead to decreased mass accuracy from poorly assigned peak centroids. These results show that acid washing of picomole levels of protein adsorbed on membranes, to facilitate salt removal, can be performed with minimal losses of the analyte.

Analyte Concentration in the Presence of MALDI-MS Interfering Contaminants

Figure 4a shows a MALDI mass spectrum of a DNA-binding regulatory protein, SpoIIID (MW = 10,802; $(M + H)^+_{\text{obs}} = 10,808$), that activates or represses the transcription of several genes involved in the sporulation of *Bacillus subtilis* [17]. In this instance, elution of SpoIIID from a polyacrylamide gel required solubilization with 6 M guanidine $\cdot$ HCl, a medium that is not compatible with successful MALDI-MS sample preparation. Analysis of the diluted sample using conventional MALDI-MS sample preparation techniques provided no useful data. This failure was likely due either to interference by the guanidine, insufficient sample concentration, or both. However, after the sample was applied to a nylon-66 membrane fixed to a probe tip, and washed (repeated four times), useful mass spectra were recorded, showing the use of an immobilization membrane for the concentration of analyte in the presence of a contaminant that interferes with MALDI-MS analysis.

Enzymatic Digestions Performed on Membrane Immobilized Proteins

To use MALDI-MS to obtain structural information from biological samples at low levels, we have performed microscale chemical modifications of proteins immobilized on nylon-based membranes. Both chemical and enzymatic digestions have been reported for proteins on PVDF, NC, and glass fiber [14, 18, 19]. Figure 4b shows the MALDI mass spectrum of an

Figure 3. MALDI mass spectra of bovine insulin (10 pmol) in the presence of a tenfold molar excess of silver nitrate. (a) Sample applied to Zetabind membrane fixed to the probe tip, dried, then matrix solution applied; (b) sample applied identically as in a, but the probe tip was immersed in a aqueous 0.1% TFA solution for 20 seconds and dried before adding matrix solution (a saturated solution of α -cyano-4-hydroxycinnamic acid in 1:1 acetonitrile/aqueous 0.1% TFA).

Figure 4. MALDI mass spectra of a DNA-binding regulatory protein, SpoIIID, recorded from sample applied to Zetabind membrane fixed to a probe tip (a) after four sequential application/washing/drying steps to facilitate guanidine removal and concentration before adding matrix solution; (b) after four sequential application/washing/drying steps followed by a 6-hour Asp-N endoproteinase digestion on the membrane before adding matrix solution (see Table 2 for peptide mass assignments). Matrix was a saturated solution of α -cyano-4-hydroxycinnamic acid in 1:1 acetonitrile/aqueous 0.1% TFA.

endoproteinase Asp-N digestion of SpoIIID (approximately 1 μ g based on staining after separation by SDS-PAGE) performed on a Zetabind membrane fixed to a probe tip. In this instance, peaks for which assignments have been made (Table 2) represent peptides generated from cleavage at aspartic acid residues. Chymotrypsin digestions were performed on β -lactoglobulin at the milligram level in vials (in the presence and absence of denaturant), or at the microgram level on protein adsorbed to Zetabind membrane (in the

absence of denaturant). Poor chymotryptic yields were obtained on the membrane in the absence of denaturant. Because the major cleavage sites for chymotrypsin are at peptide bonds C-terminal to aromatic or large hydrophobic residues which tend to be buried in the protein interior, poor digestion yields in the absence of denaturants are likely to be the result of limited exposure of the cleavage sites. Because chaotropic reagents such as guanidine $\cdot$ HCl (2 M), typically used with chymotrypsin, fail to readily dry and cause sample elution from the membrane, digestions employing enzymes requiring denaturants will be limited. Alternatively, enzymes cleaving at residues found at the surface of macromolecules such as Asp-N endoproteinase, Lys-C endoproteinase, clostripain, *Staphylococcus aureus* protease V8, or trypsin are expected to provide more complete coverage in the absence of denaturants and are better suited for these analyses.

Chemical Derivatization of Membrane-Adsorbed Proteins

Site-directed derivatization of the free sulfhydryl group in β -lactoglobulin (MW = 18,366) with pHMB was performed at the picomole level on sample first immobilized on Zetabind membrane fixed to a probe. MALDI mass spectra (Figure 5) recorded from unmodified protein produced a signal at m/z 18,375 corresponding to the protonated molecule $(M + H)^+$ and one partially resolved "lagging" shoulder at m/z 18,683 ($M' + H)^+$. Spectra recorded from β -lactoglobulin modified with pHMB showed a minor leading shoulder with a mass corresponding to the underivatized protein $(M + H)^+$ and two signals that parallel results obtained from the native protein, but with mass shifts (321 u) indicative of p-mercuribenzoate labeling of the single free sulfhydryl group $((M - H^+ + [pHMB - OH^-]) + H)^+$. Because the two resolved signals obtained from analysis of native β -lactoglobulin by MALDI-MS were both shifted in the presence of the organomercurial, the "lagging" shoulder likely represents an homologous contaminant. Bovine β -lactoglobulins are known to exhibit genetic polymorphism with as many as four variants, one having bound

Table 2. Assignment of aspartyl peptides produced from the endoproteinase Asp-N digestion of SpoIIID on Zetabind membrane fixed to a MALDI-MS probe tip

Peptide	Span	$(M + H)_{calc}^+$	$(M + H)_{obs}^+$	Sequence
1	40-60	2422.7	2421.6	DLT---L(D)
2	61-81	2529.9	2528.9	DYH---K(D)
3	51-81	3655.2	3654.5	DLA---K(D)
4	61-93	3855.3	3853.8	DYH---S
5	3-39	4295.1	4294.6	DYI---K(D)
6	1-39	4563.5	4565.9	MHD---K(D)
7	40-81	4933.7	4932.9	DLT---K(D)

Figure 5. High-mass region of MALDI mass spectrum of β -lactoglobulin (200 pmol) immobilized onto Zetabind membrane fixed to a probe tip (a) showing the unmodified singly protonated molecule; (b) the pHMB derivative prepared by adding a fivefold molar excess of reagent (over free sulfhydryl) to the immobilized protein and reacting for 10 minutes and drying before adding matrix solution. Mass shifts of 321 u for both the major unmodified protonated molecule and the partially resolved lagging shoulder suggest a heterogeneous sample lot. Matrix was a saturated solution of sinapinic acid in 1:1 acetonitrile/aqueous 0.1% TFA.

carbohydrate [20]. Despite the heterogeneous sample, the mass shift of 321 u observed in Figure 5 confirms that β -lactoglobulin contains one accessible free sulfhydryl group, and site-specific chemical derivatization of membrane-immobilized proteins can be used to elucidate structural information.

Conclusions

The advent of MALDI-TOF-MS allows analysis of biological samples at the picomole level. Part of the challenge in successfully applying the MALDI-MS technique lies in the development of methodologies for low-level sample handling. One approach involves protein immobilization by adsorption onto synthetic supports at some stage during sample preparation as is routinely performed in automated Edman sequence analysis.

Because sample preparation in MALDI-MS requires conditions such that both sample and matrix be initially soluble in a particular solvent or combination of solvents, we sought a membrane support on which

protein can be adsorbed and efficiently eluted under mild conditions (e.g., acetonitrile/water mixtures) typical in MALDI-MS sample preparation. By protein dot-blotting/staining experiments, we have found protein adsorption to PVDF, nitrocellulose, and glass fibers to be quite strong and apparently irreversible under mild conditions (and only partially reversible under harsh conditions). Poor release of protein precludes efficient use of these popular transfer membranes as a support for sample immobilization in MALDI-TOF-MS. We have demonstrated, however, the feasibility of direct MALDI-MS analysis of biological samples first immobilized on nylon-based membranes. The success of this class of membranes has largely been due to the reversibility of protein adsorption to the nylon-based surface. Use of Zetabind has allowed us to develop methods to facilitate the removal of water-soluble contaminants and to perform microscale chemical and enzymatic modifications of the immobilized protein to obtain structural information.

Although SDS-PAGE is one of the most powerful protein separation techniques, molecular weight accuracy is currently limited to 5–10%. Furthermore, many proteins that are not fully denatured (and do not bind a constant weight ratio of SDS), are glycosylated, or are very basic (e.g., histones) show anomalous migration patterns and do not exhibit a linear relationship of molecular weight to protein standards [21]. Combining SDS-PAGE and MALDI-TOF-MS would then be an obvious extension to MALDI-MS analysis from membrane supports because proteins separated in a polyacrylamide gel can be blotted onto a membrane and analyzed directly after elution or chemical modification. Positive charged nylon has been used in the electrotransfer of proteins from SDS-PAGE gels [9, 10, 12, 22]. These types of membranes have the advantage of a high binding capacity (480 $\mu\text{g}/\text{cm}^2$ as compared to 80 $\mu\text{g}/\text{cm}^2$ for nitrocellulose) and give consistently better results than nitrocellulose in the electroelution of proteins from SDS gels [9]. However, positively charged membranes display a tenacious binding of common anionic dyes such as Coomassie brilliant blue or amido black 10B which will require development of alternative means of sample visualization. Our laboratory is investigating methods of visualizing proteins on positive charged nylon supports with negative staining procedures using anionic dyes and reversible positive-type stains that do not interfere with subsequent analysis by MALDI-MS.

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