Anal Bioanal Chem (2014) 406:577–586
DOI 10.1007/s00216-013-7491-z

RESEARCH PAPER

Using electrospray laser desorption ionization mass spectrometry to rapidly examine the integrity of proteins stored in various solutions

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Received: 14 September 2013 / Revised: 5 November 2013 / Accepted: 6 November 2013 / Published online: 18 December 2013

Abstract Electrospray laser desorption ionization mass spectrometry (ELDI/MS) allows the rapid desorption and ionization of proteins from solutions under ambient conditions. In this study, we have demonstrated the use of ELDI/MS to efficiently examine the integrity of the proteins stored in various solutions before they were further used for other biochemical tests. The protein standards were prepared in the solutions containing buffers, organic salts, inorganic salts, strong acid, strong base, and organic solvents, respectively, to simulate those collected from solvent extraction, filtration, dialysis, or chromatographic separation. Other than the deposit of a drop of the sample solution on the metallic sample plate in an ELDI source, no additional sample pretreatment is needed. The sample drop was then irradiated with a pulsed laser; this led to desorption of the analyte molecules, which subsequently entered the ESI plume to undergo post-ionization. Because adjustment of the composition of the sample solution is unnecessary, this technique appears to be useful for rapidly evaluating the integrity of proteins after storage or prior to further biochemical treatment. In addition, when using acid-free and low-organic-solvent ESI solutions for ELDI/MS analysis, the native conformations of the proteins in solution could be detected.

Keywords Electrospray laser desorption ionization mass spectrometry · ELDI · Protein integrity · Native conformation

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Introduction

In many circumstances, proteins are stored in different solutions prior to their further use. For example, (a) protein fractions from extraction, filtration, dialysis, or chromatographic separation are stored in various buffers, salts, or organic solvents; (b) integral membrane proteins are extracted and purified in detergent-containing solutions [1]; (c) water-soluble peripheral membrane proteins are stored in high-salt or high-pH solutions for better extraction from membrane pellets [2]; (d) the recoveries of certain types of proteins from inclusion bodies are improved by solubilizing the proteins in high-pH solutions or in detergents (e.g., urea, guanidine hydrochloride, Triton-X 100) [3]; (e) edible proteins from animal muscle are solubilized in alkaline aqueous solutions [4]; and (f) after being captured by immunomagnetic nanoparticles, proteins are sometimes processed and stored in alkaline solutions [5]. Evaluating the structures and integrity of proteins in the purification procedures or after storage is always important. Mass spectrometry is indeed a useful tool for detecting protein molecules; however, various compositions of solutions make the detection of proteins time consuming.

Since its development in 1984, electrospray ionization mass spectrometry (ESI/MS) has played an essential role in

characterizing biomolecules, including proteins, peptides, and metabolites [6–8]. For the practical analysis of protein or peptide mixtures, ESI/MS is usually coupled with liquid chromatography [9–11], but it can also be used in a standalone manner to characterize proteins or peptides in the solutions [12]. For this purpose, ESI/MS has been used to examine the integrity of proteins—that is, to assess their degrees of denaturation or decomposition, as caused by proteases, acids, bases, organic solvents, or chemical contaminants [13]. Typically, however, sample pretreatment must be performed prior to ESI/MS analysis to prevent ion suppression effects, to remove interference, or to increase the signal intensity of the protein ions. For example, alkaline protein solutions must be adjusted or diluted using electrospray solutions that typically contain organic solvents and organic acids. Although it is possible to generate protein ions having polarity opposite to that of the proteins present in solution, the number of protein ions tend to be lower than that obtained when the ESI polarity is the same as the net charge of the proteins in solution [12]. Thus, to detect proteins from alkaline solutions, either the ESI/MS system should be switched to the negative ion mode or pH adjustment and buffer dilution must be performed. In addition to the effects of alkaline solutions, interference from buffers and detergents are constant problems in ESI/MS analyses of protein samples; typically, these chemical components must be removed prior to analysis [14–18]. Moreover, the presence of moderate amounts of inorganic salts in a sample solution will decrease the stability of the electrospray and significantly affect the intensity of the analyte ion signals, due to ion suppression effects and adduct ion formation [19, 20]. Desalting processes are, therefore, required to obtain good-quality ESI mass spectra; such sample pretreatment procedures are, however, tedious and will make these analyses very time-consuming [14–18]. The development of analytical techniques for characterizing proteins directly from various sample solutions would make it possible to screen large numbers of sample solutions in an efficient manner.

Electrospray laser desorption ionization (ELDI), a two-step desorption ionization process, allows desorption and ionization of chemical and biological compounds directly from sample solutions under ambient conditions [21–30]. It has been applied to characterize non-volatile analyte molecules directly from the surfaces of samples. Irradiating the sample surface with a pulsed laser leads to desorption of the analyte molecules, which subsequently enter the ESI plume to undergo post-ionization [21–30]. In contrast to conventional ESI, the sample introduction and ionization processes in ELDI are two separate events; this feature allows independent control of each set of condition, thereby enhancing the potential use in various practical chemical or biochemical applications. For example, the status of ongoing chemical and biochemical reactions occurring in organic solutions can be monitored continuously using ELDI/MS [27]. In addition, reactive-ELDI provides additional analytical capabilities by incorporating gas phase reactions prior to ELDI/MS measurement [28].

Herein, we report the use of ELDI/MS under ambient conditions to rapidly produce high-quality mass spectra of proteins stored in the solutions at different pH, organic solvent contents, buffers, salts, or detergents. Comparatively, positively charged proteins ions can be directly detected through ELDI/MS without the need for any sample pretreatment. In addition, by adjusting the composition of the ESI solution in an ELDI/MS analysis, the conformations of proteins stored in solutions with different pH was rapidly determined.

Material and methods

The chemicals and protein standards including cytochrome c, myoglobin, urea, guanidine·HCl, tetrabutylammonium bromide (TBAB), ammonium sulfate (AMS), glycerol, sodium dodecyl sulfate (SDS), 3-[(3-cholamidopropyl)dimethylammonio]-1-propane sulfonate (CHAPS), octyl phenol ethoxylate (Triton X-100), and various buffers and salts were purchased from Sigma and Aldrich (Milwaukee, WI) and used without further purification. The organic solvents (HPLC grade) were purchased from Merck (Darmstadt, Germany); acetic acid (reagent grade) was purchased from J. T. Baker (Phillipsburg, NJ). Distilled deionized water, obtained using a Milli-Q plus system (Millipore; Molsheim, France), was used to prepare the sample solutions. The stock solutions each contained sodium dihydrogen phosphate, sodium acetate, sodium carbonate, and tris(hydroxymethyl)methylamine (25 mM). The pH of the buffered solutions was adjusted by adding HCl (1 M) or NaOH (1 M). The stock protein solutions were diluted in different buffer solutions to obtain protein concentrations of 10^{-4} M.

A drop of the protein sample solution (ca. 5 μL) was deposited through a micropipette onto a stainless-steel sample plate [5 (L)×2 (W)cm^2], which was then positioned on an acrylic plate placed on a XYZ stage and set in front of the sampling capillary of an ion trap mass spectrometer (Esquire 3000 plus, Bruker Daltons). The surface of the sample droplet was irradiated with a pulsed Q-switched Nd:YAG laser (MINILITE I, Continuum, CA, USA) under ambient conditions. The laser was operated at a wavelength of 266 nm, a frequency of 10 Hz, a pulsed energy of approximately 250 μJ, and a pulse duration of 5 ns; the laser beam was focused through an objective lens to create a spot size of approximately 5×10^{-3} cm^2. The strongest ion signal was obtained at an incident laser angle of approximately 45°.

The laser-ablated molecules were post-ionized in an electrospray plume. The capillary electrosprayer was aligned parallel to and approximately 3.5 mm above the acrylic sample plate. The ESI plume was directed toward the ion sampling orifice (i.e., parallel to the sample plate). The electrospray needle was held at +4 kV, with the sampling cone voltage in

the ion trap mass spectrometer maintained at −0.5 kV. The ESI solution (containing 50 % water/MeOH and 0.1 % acetic acid) was delivered through a capillary by a syringe pump with flow rate of 150 μL/h. The ions generated in this manner were detected using the ion trap mass analyzer. The mass spectra were recorded at a scan rate of approximately 2 s/scan. Figure 1 displays the ELDI/MS setup for the direct analysis of proteins in solution.

Results and discussion

In previous studies, we found that fine droplets could be generated through laser desorption of sample solutions containing fine carbon powders [26, 27, 29]. The laser energy absorbed by the carbon powders was subsequently transferred to the solvent medium and analytes for further desorption, and the liquid used to dissolve the analyte appeared to play no role during such an ELDI/MS analysis, except as an energy-transfer medium [26, 27, 29]. Small chemical compounds in various solutions, including those for which the liquid medium was viscous, hydrophilic, hydrophobic, volatile, or contained nonvolatile organic solvents, were successfully detected by this ELDI/MS. There are, however, several shortcomings when mixing carbon powders with sample solutions: the carbon powder might end up stuck to the MS inlet or inside the mass analyzer, potentially damaging the instrument; the analyte might adsorb permanently onto the surface of the carbon powder, thereby decreasing the detection sensitivity; and an appropriate amount of carbon powder must be measured and added to the sample solution, increasing the analysis time. Therefore, we wished to develop a technique for detecting proteins in various solutions without the need for added carbon powder. Unfortunately, in the absence of carbon powder, the sample solutions were transparent to the UV-laser, resulting in no desorption during laser irradiation [26, 27].

In this study, we examined the capability of ELDI/MS to directly characterize proteins stored in various solutions. However, when we deposited a small sample droplet onto a stainless-steel plate, spread it to form a thin film, and then irradiated it with a UV-laser, we found that the UV-laser energy was absorbed by the stainless-steel plate to produce numerous fine droplets of the sample solution (Fig. 1b). If the sample solution was not spread into a thin film on the plate, large droplets were produced during laser irradiation, and their ionization efficiency during ELDI process was extremely low.

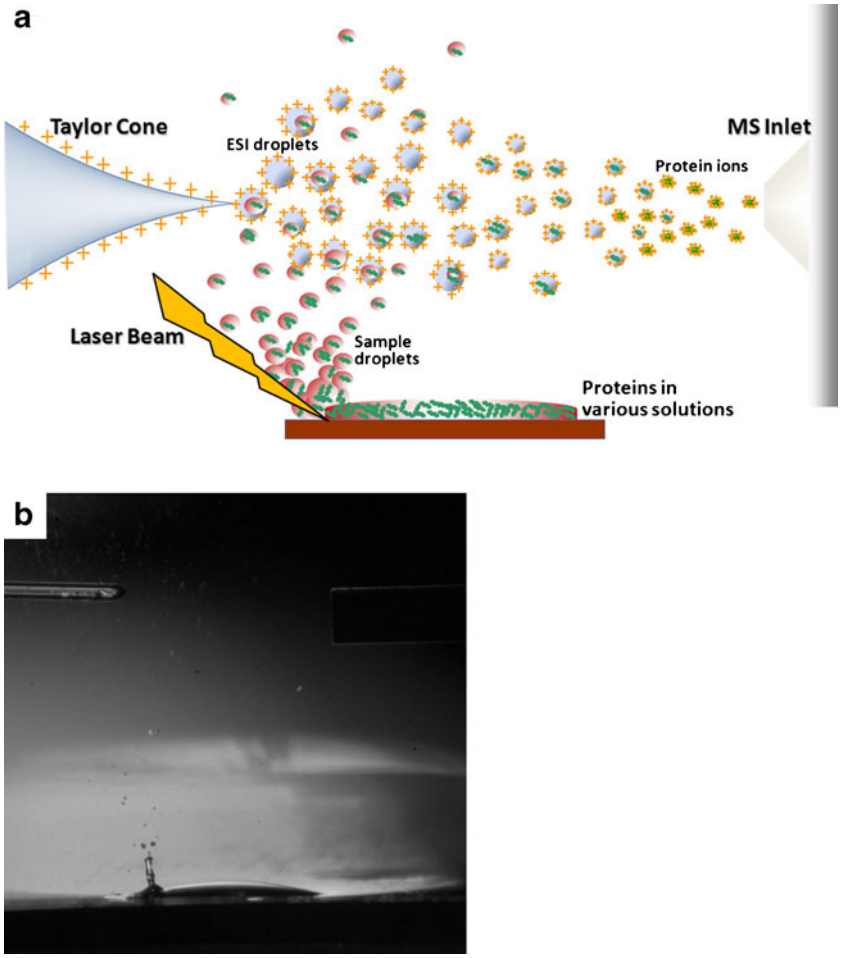

Fig. 1 (**a**) Schematic representation and (**b**) photograph of the desorption and ionization processes during ELDI

It seems the formation of fine sample droplets is a prerequisite for subsequent post-ionization of the analytes in the ESI plume. In this study, the energy required to form analyte-containing droplets arose from the absorption of laser energy by the stainless-steel sample plate. Similar phenomena have been observed in fused-droplet ESI (FD-ESI), another two-step ESI method [31–34]. In FD-ESI, an ultrasonic nebulizer is used to disperse an aqueous sample solution into a fine mist of droplets; the resulting neutral aerosol is then fused with the charged droplets generated by electrospraying an acidic organic solvent.

Figure 2 presents positive ion mode ELDI mass spectra and respective deconvoluted mass spectra of cytochrome c dissolved in aqueous solutions with pH ranging from 0 to 14. To post-ionize the proteins in the droplets generated through laser desorption, we used an ESI solution containing MeOH and water (50 %, *v*/*v*) and acetic acid (0.1 %). We detected signals for cytochrome c ions in all of the solutions having pH values of less than 13. Typically, proteins are multiamphiphilic species that can form net-positively or -negatively charged gaseous ions under a variety of ESI conditions; in this study, we manipulated the composition of the ESI solution for protein post-ionization in the acidic range to enable the formation of positively charged protein ions for analysis in the positive ion mode. Thus, the cytochrome c (p*I* = 10.0) molecules desorbed from the buffer solutions (pH from 0 to 13) were fused with the acidic ESI plume (pH 2.7) to form positively charged ESI droplets; the proteins in the droplets were subsequently ionized through the ESI process to generate positively charged protein ions.

In Fig. 2c–g, the cytochrome c ion is the predominant ion present in the deconvoluted mass spectra. In addition, we detected a protein/phosphate adduct ion from the phosphate buffer solution at pH 2.2 (Fig. 2b) and a protein/chloride adduct ion from the 1 M HCl solution at pH 0 (Fig. 2a). The presence of phosphate in the protein solution fortunately did not significantly suppress the signal of the cytochrome c ion; this situation differs from that of conventional ESI/MS, where the protein ion is heavily suppressed by phosphate in the sample solution. A possible explanation for this phenomenon is that phosphate and HCl were diluted during the fusion process (the desorbed sample droplets fused with charged ESI droplets) [22, 29]. This "dilution" of matrices in the sample solution during fusion is an important feature of two-step ionization. Many factors (e.g., salts, organic solvent) that have the potential to interfere with ion signals will be diluted when the fine sample droplets are fused with the electrospray droplets. The protein ions were readily detected in the solutions containing sodium acetate and citrate (Fig. 2c, d, respectively). Tris, another organic compound that interferes with the detection of proteins in conventional ESI/MS, also did not affect the detection of the protein when using ELDI/MS (Fig. 2e, f). The results obtained here will agree with those from our previous studies using FD-ESI/MS [31–34].

Figure 2a reveals that cytochrome c dissolved in strong HCl solution (pH 0) could be detected using this ELDI approach. Surprisingly, we even detected signals for cytochrome c ions from concentrated (6 and 12 M) HCl solutions (Fig. 3a, b, respectively). In addition to the predominant signals of the molecular ion, however, we also detected a signal for the chloride adduct of the protein ion in the deconvoluted mass spectrum as a result of the extremely high concentration of HCl in the system. Signals for some other protein-like ion

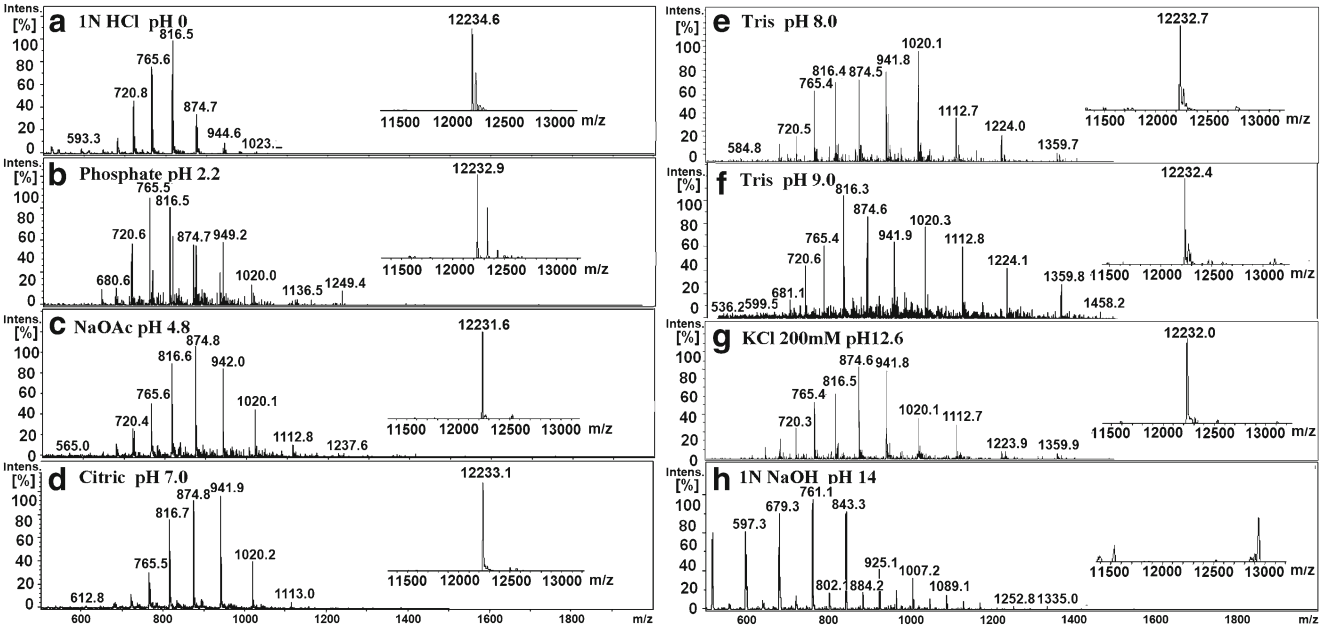

Fig. 2 Positive-ion ELDI mass spectra of cytochrome c dissolved in buffer solutions at various values of pH. *Insets*: Deconvoluted mass spectra of the cytochrome c ions. ESI solution: 50 % MeOH containing 0.1 % acetic acid

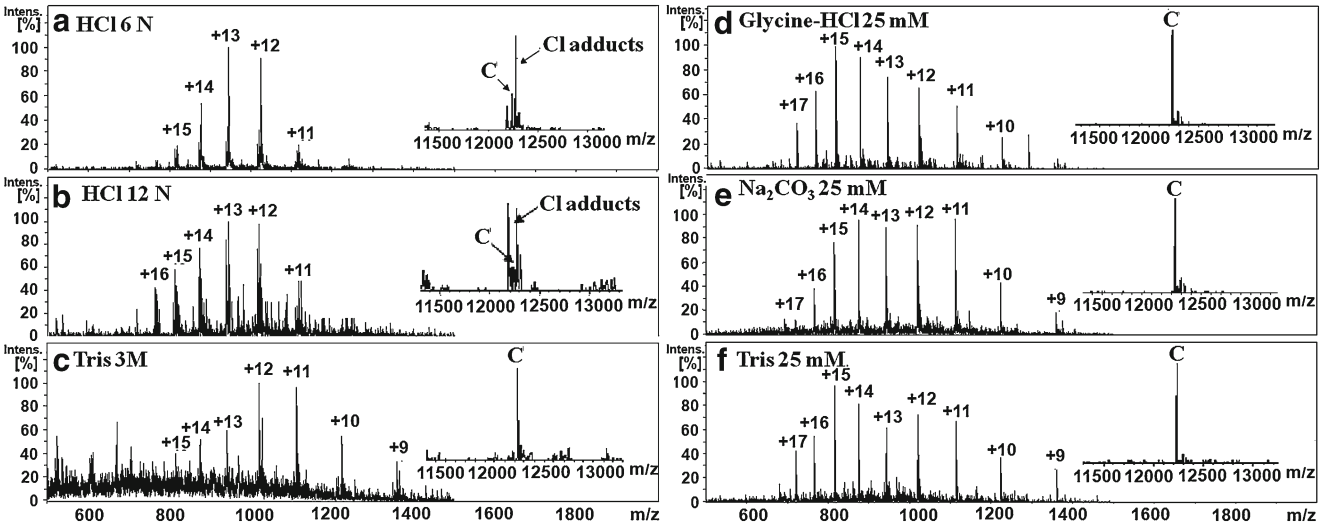

Fig. 3 Positive-ion ELDI mass spectra of cytochrome c dissolved in (**a**), (**b**) extremely high concentration of HCl, (**c**) high concentration of Tris buffer, (**d**)–(**f**) different buffer solutions at the same pH (pH 9.0). *Insets*: Deconvoluted mass spectra of the cytochrome c ions. ESI solution: 50 % MeOH containing 0.1 % acetic acid

signals were also evident in the spectra of the 6 and 12 M HCl samples, presumably because some of the amide linkages of the proteins were cleaved in the strongly acidic solutions. In contrast, we detected no protein ions from the solution in 1 M NaOH (pH 14.0); instead, the mass spectrum featured predominant sodium cluster ions, namely $Na^+(NaOH)_m(H_2O)_n$ and $Na^+(NaOAc)_m(H_2O)_n$ species (Fig. 2h) [35, 36]. The failure to detect protein ions stored in the strongly basic solution suggested that the structure of the protein was destroyed or that the signal of the protein ion was heavily suppressed by basic species (in this case, NaOH).

To further examine the effect of pH on the protein ion signal, we compared the ELDI mass spectra of cytochrome c solutions prepared in different buffer solutions at the same pH (pH 9.0; Fig. 3d–f) with those obtained at different values of pH in the same buffer. Again we detected the signal for the cytochrome c ion in all of the ELDI mass spectra, regardless of the nature of the buffer (Fig. 3c–f). Since we observed the signal for the protein ion in all of the solutions at different values of pH (see Fig. 2), it seems that the pH has no obvious effect on the detection of cytochrome c ions when using this ELDI approach.

Although it appears that the pH and buffer have little effect on the detection of the protein ions in solutions, the ELDI mass spectra in Fig. 2 present cytochrome c ions in their denatured form. This phenomenon arose because the cytochrome c ions were formed through post-ionization in an ESI plume generated from an acidic MeOH/water solution. Our results suggest that regardless of the type of solution in which the protein was stored, contact with acetic acid and MeOH in the ESI plume during post-ionization changed the protein's conformation. It has been reported that the use of 20 % MeOH/water for ESI results in ELDI/MS signals of the native conformation of cytochrome c ion [28]. Figure 4 displays ELDI mass spectra and respective deconvoluted mass spectra of myoglobin in buffer solutions at various values of pH when using an acid-free MeOH solution (20 % by volume) as the ESI solution. Native and denatured states, corresponding to the holo- and apo-forms, of Mb cannot only be discerned by their charge state distribution, but also by mass due to the loss of the heme group. Based on the calculated molecular weight and charge distribution, we could deduce the conformation of the myoglobin ion. For the sample solutions having values of pH between 5 and 7, the mass spectra featured predominant signals for native myoglobin ions (Fig. 4d, f, j, l), while denatured myoglobin ions were detected when the pH of the sample solution was less than 4.4. These conformations information are delightfully in accordance with those of previous studies [37]. This strongly suggests that ELDI/MS is a direct, rapid, useful, and efficient tool to study the conformations of proteins stored in solutions at various values of pH, which is not achieved by other ionization methods.

The ability to use ELDI/MS to assess the structures of proteins stored in solutions at various pH arises from several unique features of the techniques: (a) droplets containing protein molecules are generated from aqueous sample solutions under laser irradiation (Fig. 1b); (b) mixing the myoglobin-containing droplets with the ESI solvent droplets containing 20 % MeOH does not induce denaturation of myoglobin much; and (c) ESI proceeds from the droplets formed after fusing the myoglobin droplets with the ESI solvent droplets to generate myoglobin ions. These features make ELDI/MS a useful technique to study the integrity and conformations of proteins stored in various solutions.

Organic solvents are commonly used to extract and precipitate proteins from solutions during sample preparation. Organic solutions are also used as mobile phases for chromatographic separation, often in eluting gradients. To study the effects of

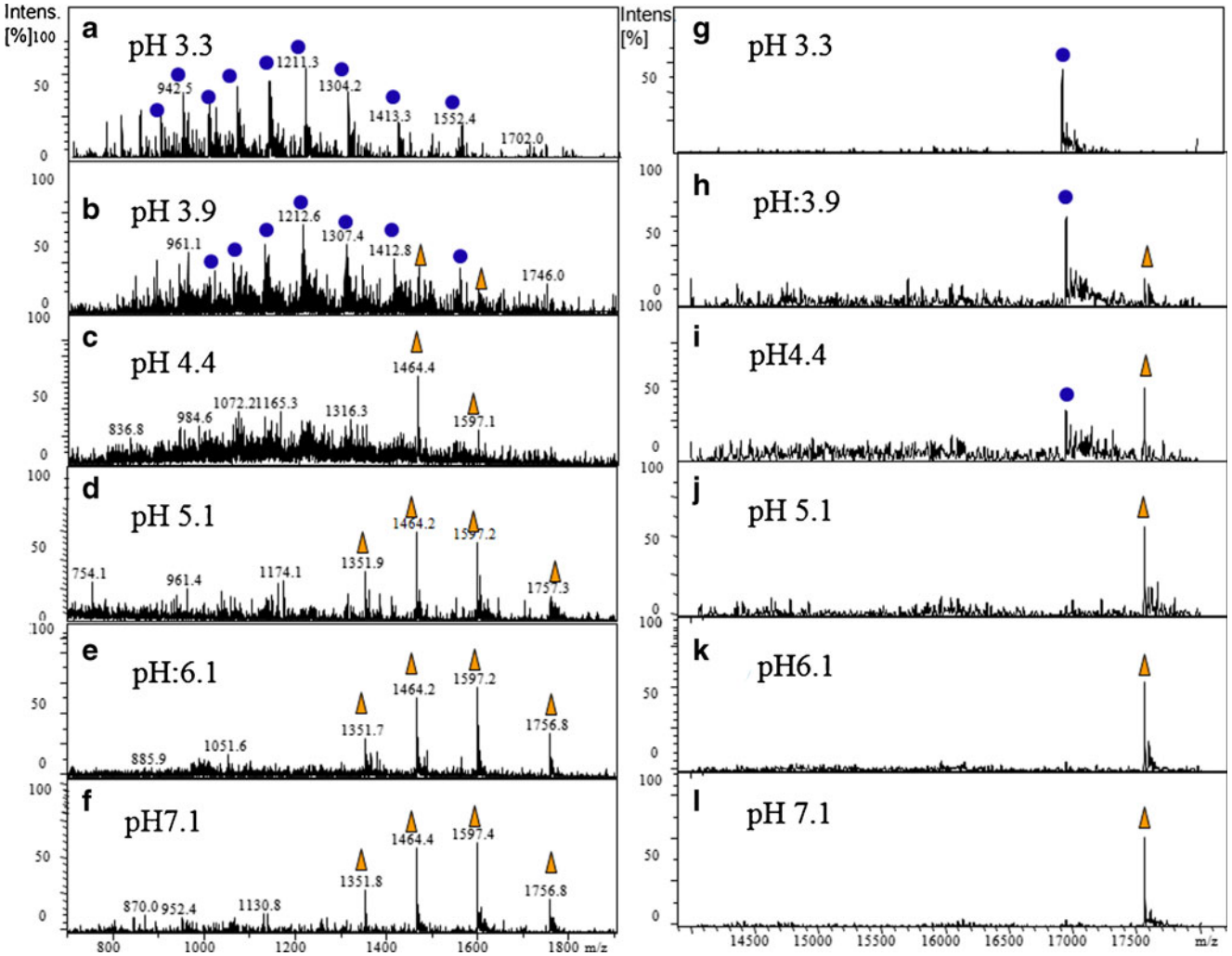

Fig. 4 (**a**)–(**f**) Positive-ion ELDI mass spectra of myoglobin in buffer solutions at various values of pH. (**g**)–(**i**) Deconvoluted mass spectra of the myoglobin ions. ESI solution: 20 % MeOH/water (*v*/*v*). *Blue circles* denatured protein; *yellow triangles* native protein

organic solvents on the signals of protein ions during ELDI/MS analyses, we prepared protein solutions by dissolving cytochrome c (10^{-4} M) in IPA, EtOH, MeOH, ACN, DMF, and DMSO, respectively. In these cases, the ESI solution was 50 % MeOH with 0.1 % acetic acid. After applying each sample solution to the stainless-steel plate, we subjected it to a pulsed UV laser and analyzed it using ELDI/MS equipped. Figure 5 displays the ELDI mass spectra of the samples; surprisingly, we detected signals for the protein ions from all of the solvents.

IPA is a water-miscible solvent that is commonly used in lipoprotein precipitation, peptide and protein extraction, and as a crucial reagent in Eastern blot analysis [38, 39]. Figure 5a presents the positive-ion ELDI mass spectrum of cytochrome c dissolved in IPA. Although the polarity index of IPA is only 3.9, signals for the cytochrome c ion were still obtained through ELDI/MS.

In addition to their use as reagents for protein precipitation, EtOH, MeOH, and ACN are commonly employed in mobile phases for solvent-gradient HPLC/MS. Baseline drift of the total ion chromatogram upon increasing the ratio of organic solvent is, however, a common problem for HPLC/MS. Since we detected signals for the protein ions from all of these solvents (Fig. 5b–d), it appears that ELDI/MS might be an alternative useful technique when combined on-line with HPLC for protein separation and characterization. Comparing to conventional HPLC/ESI source, the ions in ELDI source were produced through post-ionized by consistent electrospray solution; it has potential to dissolve the baseline drift problem.

DMSO and DMF are common solvents for preparing stock solutions because of their high polarity (allowing the dissolution of a wide range of chemical compounds), low chemical reactivity, and low vapor pressure. They are also useful solvents for the ESI analysis of hydrophobic compounds, as well as being LC-compatible and capable of improving chromatographic resolution during the analysis of intact proteins [40–43]. Furthermore, the charge enhancement of protein ions by DMSO and DMF has been demonstrated [40–43]. Figure 5e, f displays ELDI mass spectra of cytochrome c dissolved in 100 % DMF and

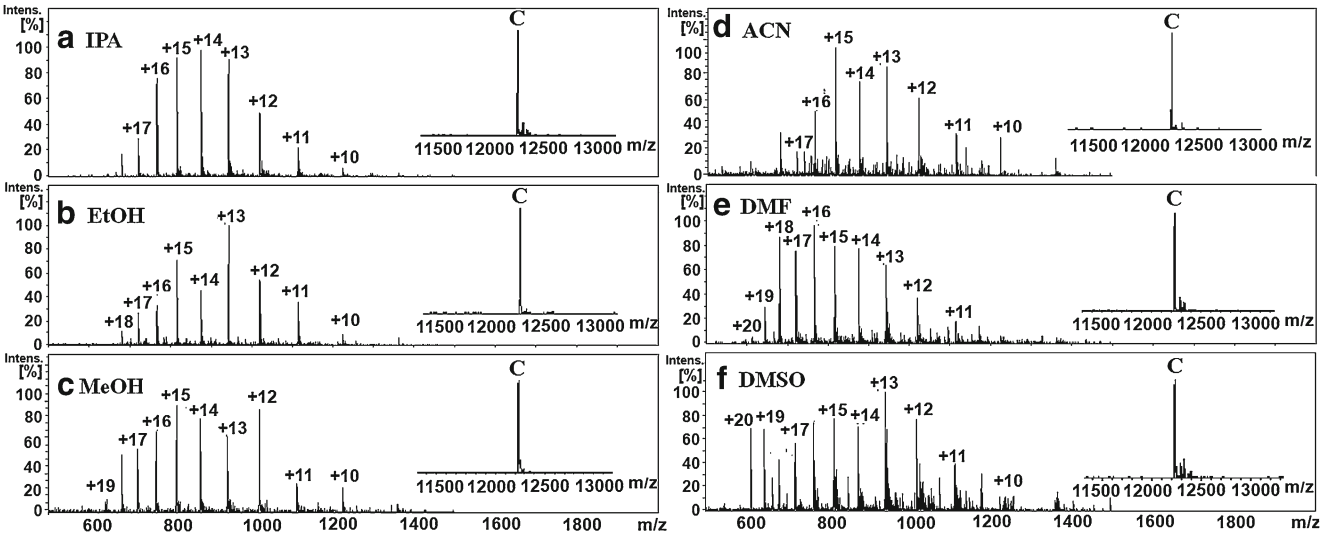

Fig. 5 Positive-ion ELDI mass spectra of cytochrome c dissolved in various organic solvents. *Insets*: Deconvoluted mass spectra of the cytochrome c ions

DMSO, respectively. The highest charge state of cytochrome c shifted from 18+ or 19+ (in other organic solvents, Fig. 5a–c) to 20+ in DMSO and DMF (Fig. 5e, f).

Another attractive feature of ELDI for protein analysis is its high tolerance toward salts and detergents. In previous studies, we demonstrated the very high salt tolerance [especially for inorganic salts (e.g., NaCl, NaH_2PO_4)] of ELDI/MS and FD-ESI/MS when using acidic MeOH as the ESI solution for protein analysis [21–34]. Because the solubility of inorganic salts in acidic MeOH (ESI solution) is at least ten times lower than that in water, somehow the salt molecules or ions would be excluded from the charged MeOH droplets during ionization. This is one possible reason why the presence of inorganic salts in sample solutions did not affect the detection of proteins through ELDI/MS and FD-ESI/MS analysis. It is also possible that less-polar analyte molecules were positioned on the surfaces of the neutral sample droplets, whereas the salts were stabilized through increased solvation within the droplets. The analyte molecules presented on the droplets' surfaces would then be easier to extract into the charged MeOH droplets during ionization. Therefore, in this study, we also examined the effects of the salts and detergents that are commonly used for protein storage on the ELDI/MS detection of proteins.

Here, we prepared solutions containing cytochrome c and various types of salts and detergents (urea, guanidine · HCl, glycerol, SDS, CHAPS, Triton X-100). Urea and guanidine · HCl are used widely as chaotropic agents for protein denaturing, solubilizing inclusion bodies, and eluting highly hydrophobic proteins [44, 45]. Protein solutions containing these detergents often require additional cleanup steps prior to analysis through conventional ESI/MS [14–18]. ELDI/MS analysis, however, exhibited extremely high tolerance. Figure 6

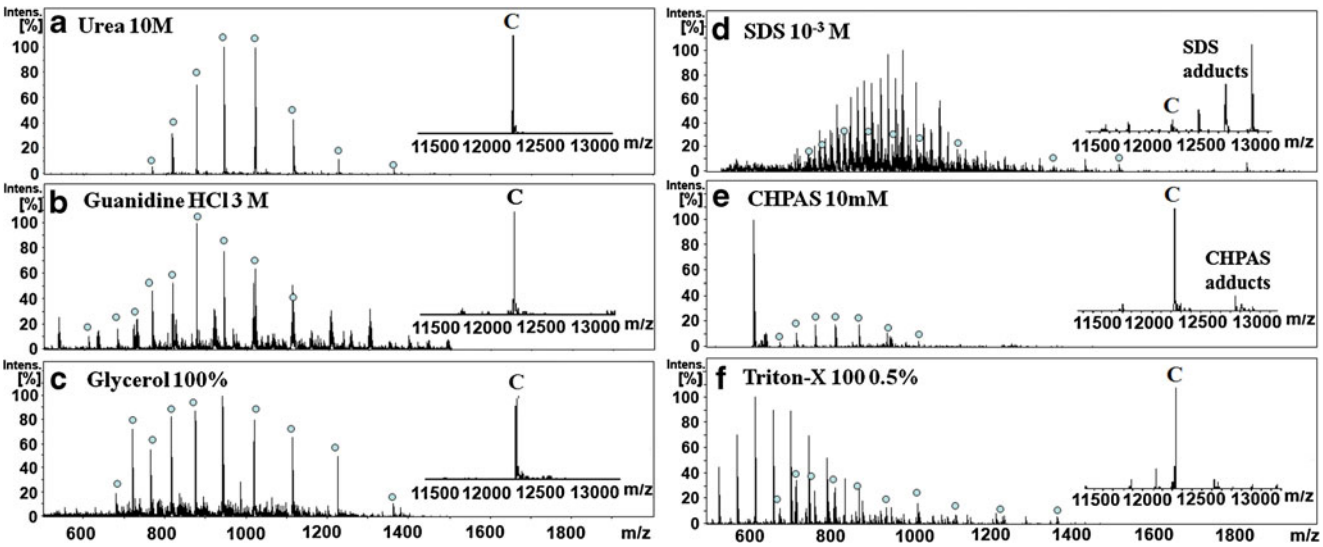

Fig. 6 Positive-ion ELDI mass spectra of cytochrome c in solutions spiked with various detergents and salts. *Insets*: Deconvoluted mass spectra of the cytochrome c ions. *Blue circles* signals for cytochrome c ions

display positive-ion ELDI mass spectra of cytochrome c in solutions spiked with various detergents and salts. Surprisingly, the protein signals remain unchanged even in the solution with 10 M urea (Fig. 6a), higher than other ionization methods, presumably because of the lack of solubility of urea in MeOH. In contrast, the removal of guanidine·HCl, which is soluble in both water and MeOH, through the ELDI process was not as favorable as that of urea. Nevertheless, we could still obtain reasonably good signal intensity for the cytochrome c ions from the solution containing 3 M guanidine·HCl when using ELDI/MS (Fig. 6b).

Figure 6c shows the ELDI mass spectrum of cytochrome c stored in glycerol. As generally known, glycerol is a widely used reagent to stabilize proteins in aqueous solution [46]. Nevertheless, the presence of high concentrations of glycerol in sample solutions can strongly affect the ESI/MS performance and decrease the intensities of the signals of analyte ions [47]. In this study, we obtained strong ELDI/MS signals for cytochrome c ions even when the samples were stored in 100 % glycerol. Again, we suspect that this phenomenon arose because the glycerol molecules were diluted during the fusion process.

The presence of detergent in protein solutions is another troublesome factor for conventional ESI/MS analysis. Integral membrane proteins are typically extracted from their native environments using detergent-containing solutions. In addition, the recoveries of certain types of proteins in aqueous solutions can be improved by solubilizing the protein extracts in detergent solutions. Three types of detergents (ionic, zwitterionic, nonionic) are commonly used for this purpose. SDS, an anionic detergent, is one of the most common additives used in bioanalytical assays. Figure 6d reveals that cytochrome c-SDS adducts were evident in the ELDI mass spectrum when the SDS concentration reached 10^{-3} M. Because SDS has high solubility in both water (sample solution) and MeOH (ESI solution), ELDI/MS is not particularly successful at removing its interference. Indeed, the deconvoluted mass spectrum barely revealed the presence of cytochrome c ions and protein-SDS adducts ions.

CHAPS is a zwitterionic detergent commonly used as a non-denaturing solvent during protein purification; it is particularly useful when purifying membrane proteins. CHAPS is also used in conjunction with nonionic detergents (e.g., Triton X-100) to solubilize membrane proteins in their native state. The mass spectrum in Fig. 6e reveals that the signal for cytochrome c ions interfered in the presence of 10 mM CHAPS, but cytochrome c signal is predominated in the deconvoluted mass spectrum, with the presence of minor protein-CHAPS adducts signal. Triton X-100 is a nonionic detergent that features a hydrophilic polyethylene oxide group and a lipophilic or hydrophobic hydrocarbon unit. Although the signals of the protein ions were disrupted by the presence of Triton X-100 at 0.5 % (Fig. 6f), unambiguous characterization of the cytochrome c ion was possible after deconvolution. Because most detergents are also soluble in MeOH, the detergent tolerance of ELDI/MS was greater than that of ESI/MS presumably because of the diluting effect during the infusion of the fine sample droplets into charged-MeOH ESI droplets. Further studies will be necessary, however, if we are to better understand the mechanisms behind the removal of salt during ELDI/MS.

Conclusion

Positive ion mode ELDI/MS under ambient conditions can be used to detect proteins dissolved in solutions of various compositions. The three-dimensional conformations of the structures of the proteins in solution can be preserved during analysis if the composition of the electrospraying solution is chosen properly. In contrast to the often time-consuming approaches used for analyses in the field of protein biochemistry, ELDI/MS can be a rapid and practical tool for assessing the structures and stabilities of protein molecules in solution. Positively charged proteins ions can be detected directly through ELDI/MS without the need for any sample pretreatment, even when the proteins are dissolved in alkaline solutions, concentrated HCl solutions, or less-polar, moderately polar, or highly polar organic solvents. Furthermore, the salt and detergent tolerance of ELDI/MS is higher than that of conventional ESI/MS, thereby making direct analysis possible. In conclusion, ELDI/MS has great potential for use in the rapid screening of proteins—for their existence and integrity—in various environments. This feature can be used to follow the progress of biochemical procedures and protein purification steps, and to assess their effectiveness. Semi-quantitative information and detection limits of the ELDI/MS for detecting protein in various solutions would be studied discussed in future studies.

Acknowledgments This study was supported financially through a grant (NSC 99-2923-M-110-007-MY3) from the National Science Council, Taiwan.

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