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Surfactant effects on protein structure examined by electrospray ionization mass spectrometry

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Abstract

Electrospray ionization mass spectrometry (ESI-MS) has proven to be a useful tool for examining noncovalent complexes between proteins and a variety of ligands. It has also been used to distinguish between denatured and refolded forms of proteins. Surfactants are frequently employed to enhance solubilization or to modify the tertiary or quaternary structure of proteins, but are usually considered incompatible with mass spectrometry. A broad range of ionic, nonionic, and zwitterionic surfactants was examined to characterize their effects on ESI-MS and on protein structure under ESI-MS conditions. Solution conditions studied include 4% acetic acid/50% acetonitrile/46% H_2O and 100% aqueous. Of the surfactants examined, the nonionic saccharides, such as *n*-dodecyl-β-D-glucopyranoside, at 0.1% to 0.01% (w/v) concentrations, performed best, with limited interference from chemical background and adduct formation. Under the experimental conditions used, ESI-MS performance in the presence of surfactants was found to be unrelated to critical micelle concentration. It is demonstrated that surfactants can affect both the tertiary and quaternary structures of proteins under conditions used for ESI-MS. However, several of the surfactants caused significant shifts in the charge-state distributions, which appeared to be independent of conformational effects. These observations suggest that surfactants, used in conjunction with ESI-MS, can be useful for protein structure studies, if care is used in the interpretation of the results.

Keywords: conformation; denaturation; detergent; electrospray; mass spectrometry; surfactant

Surfactants play a significant role in protein chemistry with primary applications ranging from solubilization and stabilization of proteins to disaggregation of protein complexes and denaturation (Neugebauer, 1990, 1992; Bollag & Edelstein, 1991). Some surfactants can disrupt protein higher order structure, whereas others are used as aids in protein refolding. They are employed in both gel and capillary electrophoresis and enhance protein and peptide recoveries from synthetic membranes employed in electroblotting and in electroelution of proteins from gels (Fernandez et al., 1994). Membrane-bound proteins, in particular, require surfactant treatment for solubilization.

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Abbreviations: CMC, critical micelle concentration; ESI, electrospray ionization; ESI-MS, electrospray ionization mass spectrometry; MALDI, matrix-assisted laser desorption/ionization; NP40, Nonidet P40; CHAPS, 3-(3-cholamidopropyl)dimethylammonio-1-propane sulfonate; CTAB, cetyl trimethylammonium bromide; LDAO, laurel dimethylamine oxide; *n*-dodecyl glucoside, *n*-dodecyl-β-D-glucopyranoside; *n*-hexyl glucoside, *n*-hexyl-β-D-glucopyranoside; octyl glucoside, octyl-β-D-glucopyranoside; octyl thioglucoside, 1-*S*-octyl-β-D-thioglucopyranoside.

Matrix-assisted laser desorption/ionization or electrospray ionization have demonstrated molecular weight determinations for proteins greater than 100 kDa (Fenn et al., 1989; Smith et al., 1991; Chait & Kent, 1992; Feng & Konishi, 1993). ESI-MS, in particular, is increasingly being used to examine noncovalent complexes between protein subunits or proteins and their ligands. ESI-MS conditions can be mild enough that proteins retain their native conformations and enzyme-substrate complexes or subunit interactions can be observed (Ganem et al., 1991a, 1991b; Katta & Chait, 1991b; Baca & Kent, 1992; Ganguly et al., 1992; Drummond et al., 1993; Goodlett et al., 1993; Light-Wahl et al., 1993a, 1993b; Loo et al., 1993a, 1993b; Ogorzalek Loo et al., 1993). A number of investigators have taken advantage of the dramatic increase in charge state observed when some proteins are denatured to probe protein structure (Katta & Chait, 1991a, 1991b; LeBlanc et al., 1991; Loo et al., 1991, 1993b; Mirza et al., 1993). The applicability of mass spectrometry to biological problems would be greatly enhanced and expanded if methodologies were developed to analyze surfactant-containing protein solutions. Recent studies have explored surfactant methods in MALDI (Schey & Finch, 1993; Vorm et al., 1993). Surfactants may also be useful in mass spectrometry to probe fundamental processes in ESI due to their abilities to alter the surface tension of ESI-created microdroplets (Buchanan et al., 1993).

Unfortunately, without extensive removal of the surfactants, protein solutions containing some frequently used surfactants are less amenable to mass spectrometry, and the presence of certain surfactants has been reported to be incompatible with mass spectrometry. In many cases, ion signals from surfactant clusters dominate the mass spectra. In order to examine surfactant effects on proteins by ESI-MS, we needed first to evaluate a broad range of commonly used ionic and nonionic surfactants with the goal of finding suitable (i.e., non- or less-interfering) surfactants and experimental conditions for protein analysis by ESI-MS. The effects of surfactants on the degree of observed protein charging in ESI were also examined. Most of the investigations were performed with surfactant concentrations of 0.01%, 0.1%, and 1.0% weight:volume (w/v), typical concentrations used in biochemical applications.

Results and discussion

Behavior of surfactants with myoglobin in 4% acetic acid/50% CH_3CN/46% H_2O

Because most ESI-MS work is done in an acid/organic/water solvent mix, we examined ESI-MS performance in the presence of surfactants and 4% acetic acid/50% CH_3CN/46% H_2O. In order to evaluate signal suppression at various surfactant concentrations, 6 pmol/μL of horse heart myoglobin (M_r 16,951) in the above solvent with and without surfactant was continuously infused at 5 μL/min on the Vestec ESI mass spectrometer. Three effects must be considered in studying surfactant suitability: (1) surfactant background ions, which can obscure the protein signal; (2) suppression of the protein signal; and (3) adduct formation. A fourth effect, a shift in the charge envelope, is described below. Table 1 summarizes performance relating to effects 2 and 3 only. It was anticipated that protein signal suppression would be a problem for ESI-MS of ionic surfactant solutions based on the work of Kebarle and colleagues (Ikonomou et al., 1990), which pointed out that ESI sources yield ion currents proportional to electrolyte concentration for concentrations below 10^{-5} mol/L, but above this concentration, analyte sensitivities decrease with analyte ion and foreign electrolyte concentrations. If the ionic strength of a solution exceeds the concentration limit, all charge-carrying species must compete for the finite electrospray current.

Table 1 summarizes the observed myoglobin ion signal for continuous infusion at surfactant concentrations of 1.0% in the acid/organic/water mix. A wide range of anionic, cationic, zwitterionic, and nonionic surfactants have been explored. The nonionics include polyoxyethylenes such as Tween 20, Thesit, Triton X, and NP40, and saccharides such as *n*-hexyl and *n*-dodecyl glucoside. Overall performance was quite poor at the 1.0% surfactant concentration for most surfactants studied. No protein signal was observed for the anionic, cationic, and zwitterionic surfactants, nor for the nonionic polyoxyethylenes. Only a few percent of the protein signal observable without surfactant was recovered when the nonionic saccharides were added. Under these conditions, *n*-hexyl glucoside and *n*-dodecyl glucoside were the best performers, yielding approximately 10% of the protein signal strength relative to no surfactant. It is important to point out that the signal-to-noise ratio for the glucosides was quite adequate for many protein studies.

Table 1. *Effect of surfactant on myoglobin ESI-MS sensitivity in 4% acetic acid/50% acetonitrile/46% H_2O*

	Detergent concentration[a]		
Detergent	1.0%	0.1%	0.01%
Anionic			
SDS	–	+	+
Taurocholate, sodium	–	+	+
Cholate, sodium	–	+++	+++++
Cationic			
CTAB	–	+	+++++
LDAO[b]	–	+	+++++
Zwitterionic			
CHAPS	–	++++	+++++
Nonionic			
Tween 20	–	++	+++++
Thesit	–	+	+++++
Triton X-100	–	+	++++
NP40	–	+	++++
n-Octyl sucrose	+/5	++	+++++
n-Dodecyl sucrose	+/5	++	++++
n-Dodecyl maltoside	+/5	+++	+++++
Octyl glucoside	+/5	+++	+++++
Octyl thioglucoside	+/5	+++	+++++
n-Hexyl glucoside	+/1	++++	+++++
n-Dodecyl glucoside	+/1	+++++	+++++

[a] Concentration in w/v. ESI-MS performance indicated by the symbols: – = 0% of the original intensity; +/5 = 2–5%; +/1 = 8–10%; + = <10%; ++ = 10–20%; +++ = 21–30%; ++++ = 30–60%; +++++ = >60%.

[b] LDAO is zwitterionic above pH 7.

The results are much better at lower surfactant concentrations. Table 1 shows data for continuous infusion with a 0.1% surfactant concentration, one of the most commonly used concentrations in protein chemistry. The best performer was *n*-dodecyl glucoside, yielding 70% of the signal obtained without surfactant; CHAPS and *n*-hexyl glucoside also provided good quality spectra, yielding intensities in the 30–60% range. No background interference from the surfactant was observed with 0.1% *n*-dodecyl glucoside. Figure 1 illustrates data obtained with and without 0.1% CHAPS, where about 35% of the original myoglobin signal is retrieved. Interfering CHAPS oligomers also appear. This spectral interference may present a problem for some samples. In the figures throughout this paper, 100% relative abundance is taken as the intensity of the protein charge state of maximum intensity in the absence of detergent. Abundances for spectra obtained from detergent-containing solutions reflect ion intensities relative to detergent-free solution.

At a 0.01% surfactant concentration, we observe that most surfactants are suitable for ESI-MS (Table 1). SDS and sodium taurocholate, both strong anionic surfactants with sulfonic acid groups, are notable exceptions. They performed poorly under all continuous infusion conditions examined, displaying extensive surfactant multimers and strongly suppressing the protein signal. They should be avoided for mass spectrometry. Excellent surfactants at this concentration included CHAPS, *n*-hexyl glucoside, *n*-octyl sucrose, and *n*-dodecyl maltoside, all of which

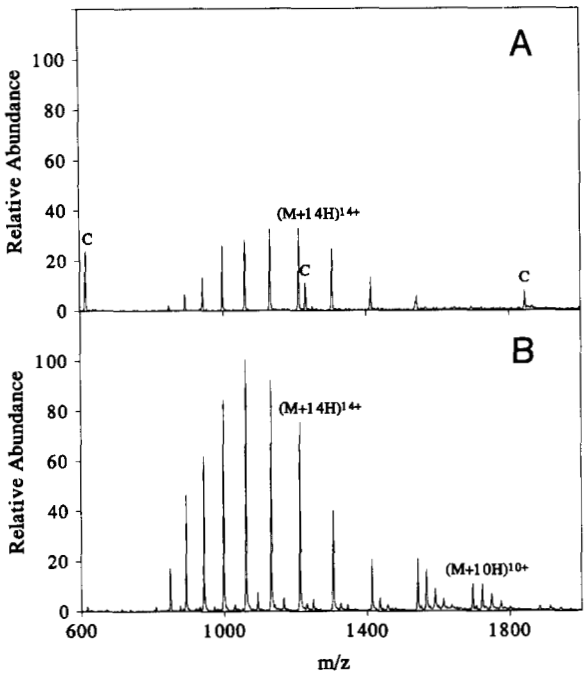

Fig. 1. ESI-MS of horse heart myoglobin (M_r 16,951) in 4% acetic acid/50% acetonitrile/46% H_2O with (**A**) and without (**B**) 0.1% CHAPS. C, CHAPS multimer. Spectrum B shows unidentified counterion adducts of approximately 265 Da.

yielded 100% of the signal obtained without surfactant. Clearly, there is quite a choice of possible surfactants at concentrations in the 0.01% range, although the nonionic saccharides are the best choice due to their lack of interfering ions and absence of adduct formation. The superior performance of the nonionic saccharides is consistent with the prediction that nonionic surfactants will not compete aggressively with the analyte for limited ion current. However, nonionic polyoxyethylene surfactants appear to contain enough sodium ions to limit analyte sensitivity. Chemical background is low with the nonionic saccharides because the surfactants are not particularly efficient at ionizing and may contain less Na^+ than the polyoxyethylene mixtures.

In order to evaluate performance with a different ESI interface, 23 pmol/μL bovine ubiquitin (M_r 8,565) in 4% acetic acid/50% acetonitrile with various surfactants was examined on the Fisons/VG mass spectrometer. This instrument relies on pneumatically assisted electrospray. Studies employing 1.0% concentrations of *n*-dodecyl glucoside and *n*-hexyl glucoside yielded 13% and <2%, respectively, of the protein signals without surfactant. At the 0.1% level, *n*-dodecyl glucoside, octyl glucoside, *n*-hexyl glucoside, *n*-dodecyl sucrose, CHAPS, CTAB, LDAO, and sodium cholate were evaluated, yielding 40, 20, 20, 20, 10, 2, 0, and 0%, respectively, of the signal obtained without surfactant. With 0.01% surfactant concentrations of *n*-dodecyl glucoside, *n*-hexyl glucoside, and CTAB, signals equal to 50%, 100%, and 40%, of those without surfactant were obtained, respectively. Although there were some differences in response between surfactants on the Vestec and VG mass spectrometers, the same general trends were observed in both. The pneumatically assisted electrospray instrument tended to yield more detergent clusters than the electrospray instrument.

The possibility that ESI charge-state distributions could be affected by surfactant addition was considered. It was predicted that surfactants would shift charge-state distributions to lower charge as the ESI droplets required less surface charge density to overcome the lowered surface tension. Instead, 1% solutions of *n*-dodecyl sucrose, octyl glucoside, octyl thioglucoside, and *n*-hexyl glucoside in 4% acetic acid/50% acetonitrile increased the protein charge state of maximum intensity and shifted the charge envelope 2–4 charges higher. Protein charge distributions were shifted to lower charge only for the 0.1% LDAO solutions and the LDAO behavior may reflect proton transfer to unoxidized amine impurities. Currently, we have no definitive explanation for this charge-shifting behavior. Denaturation has been shown to lead to higher charging (Katta & Chait, 1991a, 1991b; LeBlanc et al., 1991; Loo et al., 1991, 1993a; Mirza et al., 1993). However, we consider the possibility of further denaturation to be unlikely under these conditions because the myoglobin should already be completely denatured in a 4% acetic acid/50% acetonitrile solution and this same effect is also observed with peptides as small as 6 residues, which are unlikely to have higher-order structure (data not shown). An alternate hypothesis is that the smaller droplets evaporate faster, yielding higher charging (Fenn, 1993). Charge distributions will be discussed further in the following sections.

Behavior of surfactants with ubiquitin in 100% H_2O

Electrospray in 100% aqueous solutions is of interest for the study of noncovalent interactions and protein conformation. Moreover, because critical micelle concentrations have been measured in aqueous solutions, we can determine if there is a relationship between surfactant performance in ESI and CMC. The 100% aqueous studies were carried out by continuous infusion of a 23-pmol/μL solution of bovine ubiquitin (M_r 8,565) in water. Ubiquitin was selected for these studies because of the ease with which it could be sprayed from aqueous solutions.

At 1% concentrations, only 1 surfactant of those studied, *n*-dodecyl glucoside, consistently yielded significant ubiquitin ion signals on both mass spectrometers, providing 20% of the signal obtained without surfactant. Performance is better at a 0.1% surfactant concentration, primarily with the saccharide-based nonionics (see Table 2 for results on the Vestec mass spectrometer). Again, *n*-hexyl glucoside and *n*-dodecyl glucoside were the best performers, yielding 30–60% of the signal obtained without surfactant. In a subsequent study performed under conditions that were believed to be the same, octyl glucoside performed almost as well as *n*-hexyl glucoside, demonstrating that there appear to be significant run-to-run variations with some of the surfactants. Based on the CMCs for *n*-hexyl and *n*-dodecyl glucoside (Table 2), it appears that CMC is not a good predictor of how well a surfactant will perform in ESI-MS experiments. The surfactant concentration of 0.1% exceeded *n*-dodecyl glucoside's CMC, but was below that of *n*-hexyl glucoside, yet both surfactants performed similarly. On the Fisons/VG mass spectrometer, extensive detergent clusters were observed for *n*-hexyl glucoside at concentrations of 0.01% and above, but not for *n*-dodecyl glucoside, also in contrast to predictions based on their CMCs.

As was also observed for the acid/organic/water solvent system, at the 0.01% concentration, many surfactants appear to be suitable for ESI-MS analyses (see Table 2 for results on the

Table 2. *Effect of surfactant on ubiquitin ESI-MS sensitivity in H_2O*

Detergent	Detergent concentration[a]		
	CMC (%)[b]	0.1%	0.01%
Anionic			
SDS	0.2	+	+
Taurocholate, sodium	0.2	+	+
Cholate, sodium	0.4	+	++
Cationic			
CTAB	0.04	+	+++
Zwitterionic			
LDAO[c]	0.03	+	+++
CHAPS	0.3	+	++
Nonionic			
Tween 20	0.007	+	++++
Thesit	0.005	+	++++
Triton X-100	0.01	+	++++
NP40	0.02	++	++++
n-Octyl sucrose	1.0	+++	++++
n-Dodecyl sucrose	0.02	+++	++++
n-Dodecyl maltoside	0.01	++	++++
Octyl glucoside	0.4	+++	+++++
Octyl thioglucoside	0.3	++	+++++
n-Hexyl glucoside	7.0	++++	+++++
n-Dodecyl glucoside	0.005	++++	+++++

[a] Concentration in w/v. ESI-MS performance indicated by the symbols: + = <10% of the original intensity; ++ = 10–20%; +++ = 21–30%; ++++ = 30–60%; +++++ = >60%.
[b] Sources: Neugebauer (1990, 1992).
[c] LDAO is cationic below pH 3.

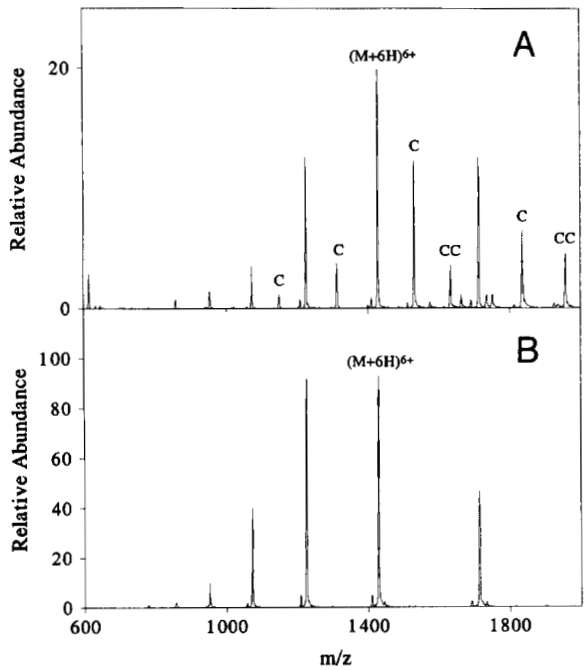

Fig. 2. ESI-MS of bovine ubiquitin (M_r 8,565) in H_2O with (**A**) and without (**B**) 0.01% CHAPS. C, Ubiquitin + CHAPS complex; CC, ubiquitin + 2 CHAPS complex.

Vestec mass spectrometer). The saccharide-based nonionics appear best, but some performance can be achieved with the polyoxyethylene nonionics. However, the polyoxyethylenes yield significant background ions and form adducts with the ubiquitin molecule, complicating the spectra acquired with these surfactants. As with the acid/organic/water mix, the SDS and sodium taurocholate perform poorly under all conditions. The spectrum for 0.01% *n*-dodecyl glucoside in 100% aqueous solution showed little loss in sensitivity and showed little interference from background ions. Figure 2 illustrates CHAPS at the 0.01% concentration. CHAPS was a reasonable performer in the acid/organic/water mix, but only 20% of the signal obtained without surfactant was retrieved here. Surfactant adducts with CHAPS are pronounced in water. Thesit forms adducts with the protein ions as do the other polyoxyethylene surfactants. In Figure 3, the singly charged polymer background and the adducts are observed. Because Thesit is polydisperse, the adducts give rise to many peaks in the spectrum. Clearly this background will be a problem for low-level protein components.

Analyte signal suppression was reduced when the surfactant–protein solutions were delivered by injection loop or autoinjector, versus continuous infusion. The difference was attributed to dilution of the surfactant with the running solvent and possible interaction of the surfactant with the PEEK (polyetherether ketone) tubing. Evidence of detergent interaction with the tubing was provided by the time dependence of mass spectra obtained during elution of the sample plug. Generally, the mass spectra obtained during the first 0.5–1.0 min of elution showed much less protein signal suppression, adduct formation, and detergent background than spectra obtained later in the elution

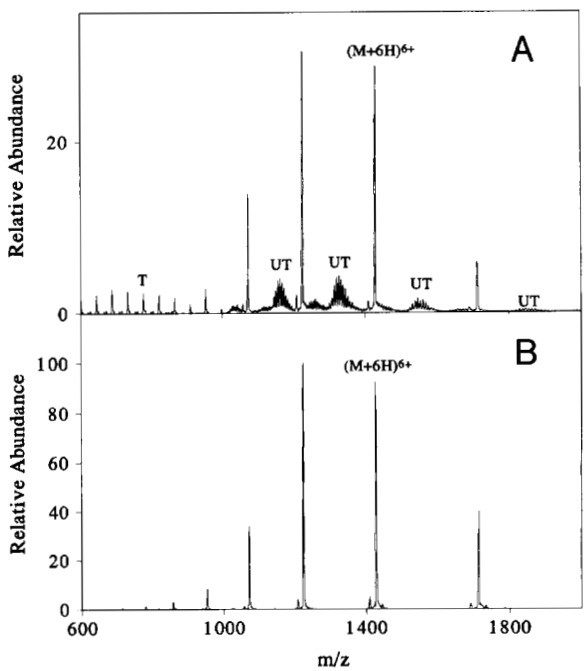

Fig. 3. ESI-MS of bovine ubiquitin in H_2O with (**A**) and without (**B**) 0.01% thesit. T, Thesit multimer ions; UT, ubiquitin + thesit complexes.

profile. Signal suppression increased with multiple injections of the surfactant/protein mix; e.g., a subsequent injection might not show the high protein signals at the early portion of the elution profile. This behavior suggests that higher surfactant concentrations can be tolerated in flow injection for a single injection when using PEEK tubing. It may also explain the discrepancy between our studies, which found severe protein signal suppression with 0.01% SDS delivered by continuous infusion, and those of Kay and Mallet (1993), which reported 54% of cytochrome *c* signal to be recovered with 0.01% SDS in an injection of 20 pmol of protein. Because of our observations, measurements for the figures and tables in this manuscript were performed by continuous infusion, under conditions where we believe steady-state signal levels were measured. The tubing was well-rinsed between measurements to elute any adsorbed detergent.

There are several considerations involved in surfactant selection for mass spectrometry. Obviously the protein must be soluble in the surfactant and the surfactant must not seriously suppress the protein ion signal. Beyond these primary concerns is the need to minimize background ions arising from the surfactant. All of the surfactants produce some background ions, but in many cases, the background can be tolerated, particularly at 0.1% or lower surfactant concentrations. Background ions are relatively limited for the nonpolymeric surfactants. Dimers were observed with most of the surfactants listed in Table 3 at 1% concentration, but spectral congestion was generally not as much of a problem as signal suppression. Singly and doubly charged CHAPS oligomers extending out to high *m/z*, however, added significantly to spectral congestion. Thesit, a polymeric surfactant, contributes ions primarily in the range from 400 to 900 *m/z*. Oligomer background ions were more apparent on the Fisons/VG mass spectrometer (pneumatically assisted ESI) than on the Vestec (no nebulizing gas), an observation that we attribute to differences in the instruments' ESI interfaces. Broad, unresolved background signals below 500 *m/z* were usually observed for 1% concentrations of the nonionic glucoside, maltoside, and sucrose detergents on the Vestec. They may reflect solvent clusters indicating an unstable electrospray under these conditions (see Table 3 for a summary of background ions and other observations on surfactant use).

The charge shifts noted for proteins with some of the surfactants in acid/organic solutions were also apparent in water solutions. They were observed with *n*-hexyl glucoside, *n*-octyl sucrose, octyl glucoside, *n*-dodecyl sucrose, and *n*-dodecyl glucoside with the extent of the charge shift increasing as surfactant concentration is raised. The relationship, if any, of surface tension to charging is currently being explored. It is clear that not all of the charge shift correlates to surface tension alone, because charge shifts for *n*-dodecyl glucoside increase with increasing surfactant concentrations even above the CMC, where surface tension is independent of surfactant concentration.

Surfactants as denaturants

Some surfactants are quite effective in denaturing proteins. Figure 4 illustrates mass spectra of myoglobin under slightly acidic conditions (in 0.01% acetic acid/H_2O) with (upper) and without (lower) 0.01% CTAB, a strongly denaturing surfactant. The dominant ion in the spectrum obtained without surfactant corresponds to the myoglobin-heme complex (M_r 17,568) (Katta & Chait, 1991b; Loo et al., 1993a), whereas the surfactant spectrum shows primarily apo-myoglobin (M_r 16,951) in higher charge states; only a small amount of the binary myoglobin-

Table 3. *Background ions and other observations from ESI-MS of detergent solutions*

Detergent	M_r	Background ions and observations
SDS	288	1+, 2+, 3+-charged Na^+-attached SDS multimers extending to high *m/z*. Protein signal severely suppressed; SDS-protein adducts observed at low SDS concentrations
Taurocholate (sodium salt)	515 (free acid)	1+, 2+-charged Na^+-attached multimers; $[M + Na]^+$, $[M - H + 2Na]^+$ at *m/z* 538, 560, where M = free acid. Forms adducts with proteins
Cholate (sodium salt)	409 (free acid)	Singly sodiated and protonated multimers of cholic acid extend to high *m/z*
CTAB	284 (amine)	$[M + H]^+$, $[2M + Br + 2H]^+$ at *m/z* 285, 649 where M = cetyl trimethylamine. Denaturing detergent
LDAO	229	$[M + H]^+$, $[2M + H]^+$ and ions at *m/z* 263, 487. Zwitterionic at pH >7, cationic at pH <3
CHAPS	615	Singly charged protonated multimers extend to high *m/z*. Forms adducts with proteins, particularly in 100% aqueous solutions or at low temperatures; e.g., unheated spray chamber. Nondenaturing detergent effective at breaking protein-protein interactions
Tween-20	1,228 (ave.)	Polymer background *m/z* 500–1,200. Severe signal suppression. Forms adducts with proteins
Thesit	583 (ave.)	Polymer background *m/z* 400–900
Triton X-100 (reduced)	631 (ave.)	Polymer background *m/z* 500–1,000. Severe signal suppression. Forms adducts with proteins
Nonidet P40	603 (ave.)	Polymer background *m/z* 400–1,000. Severe signal suppression. Forms adducts with proteins
n-Octyl sucrose	468	$[M + Na]^+$, $[M + K]^+$ at *m/z* 491, 507
n-Dodecyl sucrose	524	$[M + Na]^+$, $[M + K]^+$ at *m/z* 547, 563
n-Dodecyl maltoside	511	$[M + Na]^+$, $[2M + Na]^+$ at *m/z* 534, 1,045
Octyl glucoside	292	$[M + Na]^+$, $[2M + Na]^+$ at *m/z* 315, 608. 1% solution gives intense background for *m/z* < 500
Octyl thioglucoside	308	$[M + Na]^+$, $[2M + H]^+$, $[2M + Na]^+$ at *m/z* 331, 618, 640. 1% solution gives intense background for *m/z* < 500
n-Hexyl glucoside	264	$[M + Na]^+$, $[2M + Na]^+$ at *m/z* 287, 551
n-Dodecyl glucoside	348	$[M + Na]^+$

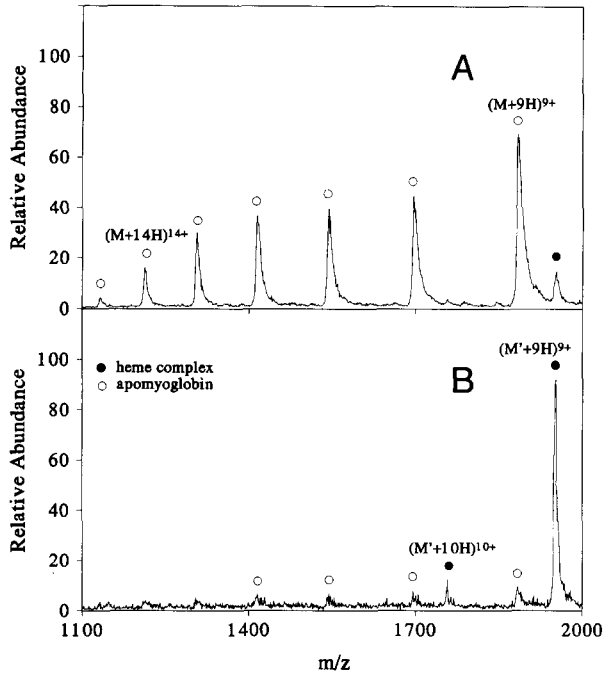

Fig. 4. Horse-heart myoglobin in 0.01% acetic acid/H_2O with (**A**) and without (**B**) 0.01% CTAB.

heme complex is apparent. This result is consistent with denaturation of myoglobin by the CTAB. We did not observe CTAB adducts on the myoglobin ions, but the interaction may have been too weak to yield a complex in the mass spectrum. Even though noncovalent interactions are often observed in ESI-MS, experimental conditions need to be very gentle in order to observe some complexes. Even under the most gentle conditions, many complexes are not observed or only a small percentage of the complexes known to exist in solution are observed. Thus, not observing detergent-myoglobin complexes does not prove that an interaction does not exist in solution.

A similar experiment compared spectra of bovine ubiquitin in H_2O with and without 0.01% CTAB; addition of CTAB eliminated the ubiquitin dimer ion contribution, albeit at a serious loss in signal intensity overall. Ubiquitin dimer was stable to less denaturing detergents such as *n*-dodecyl glucoside.

We have also used the less denaturing surfactant CHAPS to break up protein-protein interactions in noncovalent complexes of concanavalin A dimer and tetramer (102 kDa) (Light-Wahl et al., 1993b; Loo et al., 1993b). In that study, concanavalin A was sprayed from 5 mM ammonium acetate at pH 6. Con A has been reported to be a dimer at pH 5.5 and a tetramer at pH >7. Figure 13 of that paper (Loo et al., 1993b) shows that, under gentle interface conditions, the noncovalent dimer is the primary species observed in the mass spectrum; monomer and tetramer are also observed. In the presence of 0.01% CHAPS, however, the dimer and tetramer are greatly reduced (see Fig. 14 of Loo et al., 1993b). This behavior is consistent with the detergent properties of CHAPS; it is considered to be an effective, but mild, detergent and is often used to disaggregate proteins to their monomeric forms without denaturation.

Significant differences were observed in the ESI-MS charge distributions of cytochrome *c* in water heated off-line with and without 0.01% CHAPS (Fig. 5). Similar observations were noted for lysozyme in 4% acetic acid/50% CH_3CN/46% H_2O boiled off-line with and without 0.01% CHAPS (Fig. 6). These differences are attributed to a reduction in the folding rate (LeBlanc et al., 1991; Mirza et al., 1993) in the presence of this surfactant. Because CHAPS is a mild, nondenaturing detergent, differences in protein charge-state distributions could only be observed by first denaturing the protein and then monitoring its folding.

Electrospray spectra of bovine ubiquitin in H_2O with and without 0.01% SDS are illustrated in Figure 7. The ubiquitin spectra acquired without detergent differ slightly from those acquired on another day under the same conditions (Figs. 2, 3)—the charge state of maximum intensity has shifted by about 1–2 charge states. This small variation in charge-state distributions is not unusual for ESI-MS and illustrates the small day-to-day shifts in ESI-MS charge distributions. Conformational studies that are based on ESI-MS spectra must be measured at the same time in order to be compared. We also regard a shift of about 2 charge states in a protein spectrum to be the minimum shift that we can consider significant for charge-state arguments without careful controls. In the ubiquitin spectra of Figure 7, the charge state of maximum intensity shifts to higher charge (lower *m/z*) by 2 charge states for the SDS-containing solution, consistent with denaturation (Katta & Chait, 1991a; Loo et al., 1991). Multiple SDS adducts were observed for the lower charge states of ubiquitin in the surfactant solution, consistent with an interaction between surfactant and protein. This charge shift could also be explained by the shift to higher charge state observed with increasing surfactant concentration, discussed earlier, and so the observation raises some important points that

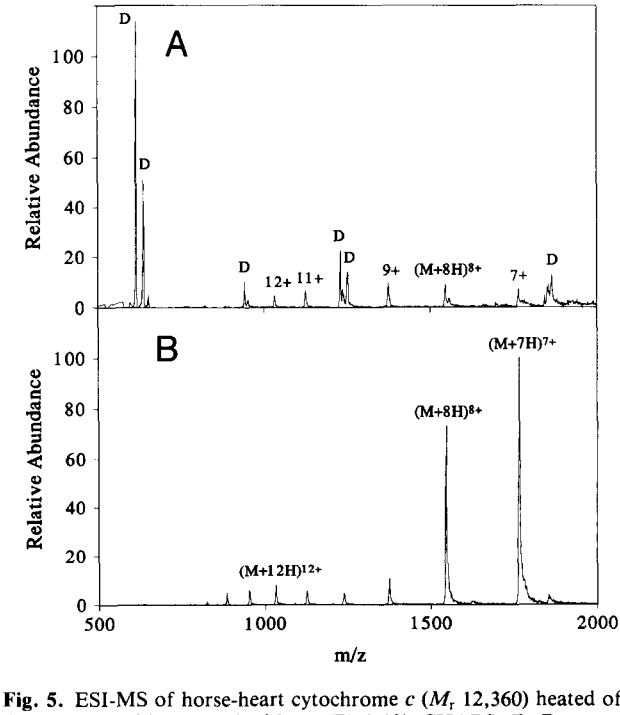

Fig. 5. ESI-MS of horse-heart cytochrome *c* (M_r 12,360) heated off-line in H_2O with (**A**) and without (**B**) 0.1% CHAPS. D, Detergent-related ions.

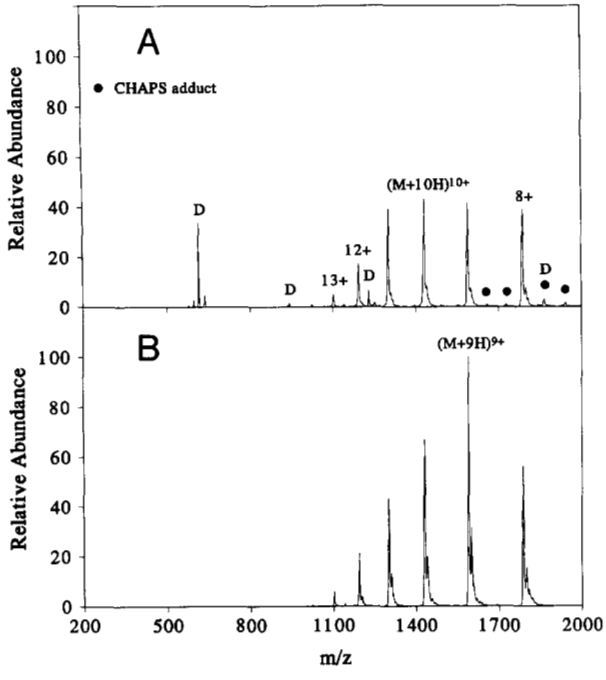

Fig. 6. ESI-MS of hen lysozyme (M_r 14,306) boiled off-line in 4% acetic acid/50% acetonitrile/40% H_2O with (**A**) and without (**B**) 0.1% CHAPS. D, Detergent-related ions.

should always be considered when framing denaturation arguments around ESI-MS data.

Charge-state distributions can be affected by many things, including interface voltages and the presence of counterions (Mirza & Chait, 1993). Thus, one tries to hold most parameters constant during an experiment, varying only the parameter being explored. Even so, there can be some question as to whether an ESI-MS charge shift observed is properly interpreted as a denaturation or as something else, perhaps an additional site charging without a higher-order structural change. When the denaturation is accompanied by a change in mass, as in the myoglobin/apomyoglobin data of Figure 4, or the concanavalin data (Loo et al., 1993b), the interpretation is clear. When no mass change occurs, further care must be exercised. One guide in interpretation is to look for the appearance of a bimodal charge distribution, or a change in the relative contribution of charge states in a bimodal distribution.

Under this guide, the SDS data are still inconclusive. The cytochrome *c* data in Figure 5, however, illustrate clear changes in a bimodal charge distribution. After heating in the absence of detergent, a bimodal charge distribution is observed, showing low (7+–8+) and high (10+–14+) charge states. Most of the intensity is in the low charge states, indicating that only some of the cytochrome *c* unfolded fully upon heating or that the cytochrome *c* unfolded fully upon heating, but is quickly folding. In the presence of detergent, the bimodal distribution is mostly gone and the 7+–8+ charge states are not significantly more intense than 9+–12+, indicating that the protein is either refolding more slowly in the detergent solution or denatures more readily in the presence of detergent.

Figure 6 illustrates similar, although less compelling data. Charge distributions for disulfide-intact denatured versus native lysozyme differ by only a few charge states (Loo et al., 1991) and so the charging effects for which we look are more subtle than those observed in the cytochrome *c* and myoglobin studies. Here it is argued that the 10+ and 11+ charge states are enhanced in the presence of detergent because the protein refolds more slowly. The data look like 2 overlapping charge distributions of similar intensities (maxima at perhaps 11+ and 9+) in the detergent-containing solution going to primarily a single distribution peaking at 9+ in the detergent-free solution.

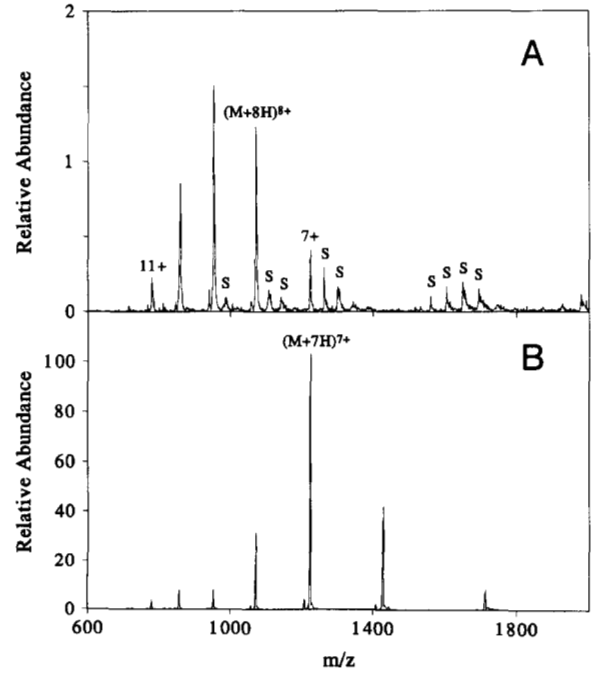

Fig. 7. ESI-MS of bovine ubiquitin in H_2O with (**A**) and without (**B**) 0.01% SDS. SDS-protein adducts are labeled S.

Conclusions

Under the range of conditions explored, nonionic saccharide surfactants, especially *n*-dodecyl glucoside, yielded the strongest ESI signals without serious chemical background, whereas SDS and sodium taurocholate performed poorly. The analyte signal suppression observed for ionic surfactants as compared to nonionic is consistent with a competition between surfactant and analyte ions for ESI current. Protein signal levels with various surfactants were somewhat ESI interface-dependent, although general trends were similar between instruments. Limited data suggest that detergents may also be used with capillary style interfaces (Loo et al., 1993b). The amount of surfactant oligomer background observed was interface-dependent. These studies also indicated that critical micelle concentration does not appear to be a good predictor of how well a surfactant will perform in aqueous ESI-MS experiments.

Octyl glucoside, octyl thioglucoside, *n*-hexyl glucoside, and *n*-dodecyl sucrose at 1.0% concentrations in 4% acetic acid/50% acetonitrile shifted myoglobin ions to higher charge states, in contrast to predictions based on droplet surface tension. LDAO in acid/organic solution shifted myoglobin ions to lower charge states. Charge shifts with surfactant addition were also observed

in H_2O. This effect on charge state was also observed for very short peptides and is consistent with an effect on droplet evaporation rate (Fenn, 1993).

Several of the surfactants examined in this study should be useful for mass spectrometric analysis of peptides and proteins that are difficult to solubilize, including membrane proteins (Barnidge et al., 1994), in coupling separations such as micellar electrophoresis to ESI-MS (Matsubara & Terabe, 1992; Varghese & Cole, 1993), and in improving protein recoveries from analytical or purification steps preceding mass spectrometry. This study, in applying surfactants to protein denaturation and protein–protein interactions, demonstrates that surfactants can be used in conjunction with ESI-MS to study higher-order protein structure. Strong examples of detergent-induced conformational effects uncovered in these studies include the effect of CTAB on myoglobin and CHAPS on concanavalin A dimers and tetramers. The study identifies surfactants and conditions suitable for ESI-MS of proteins, describes the unusual effect of detergents on charge populations, and provides a hypothesis to explain the effect. In light of this effect on charge distributions, care must be exercised in drawing conclusions regarding protein structure and denaturation from ESI-MS studies in the presence of detergent. Identification of conformation changes requires the presence of a bimodal charge-state distribution, a change in quaternary structure, or confirmation by other analytical methods, including CD, analytical ultracentrifugation, light scattering, or NMR studies.

Materials and methods

Positive ion ESI-mass spectra were acquired on a Vestec 201 single quadrupole mass spectrometer (Houston, Texas) employing the Vestec atmospheric pressure/vacuum interface (Allen & Vestal, 1992; Andrews et al., 1992). This interface employs nozzle-skimmer lens elements (in contrast to interfaces that use glass capillary [Whitehouse et al., 1985] or metal capillary [Chowdhury et al., 1990] ion-transport devices) and utilizes a heated chamber to assist in droplet desolvation. For the studies reported here, the spray chamber was maintained at 56 °C and the electrospray needle was bent to enable off-axis sampling of the spray. Countercurrent and coaxial gas flows typically used for droplet desolvation with other ESI interfaces are not applied in this interface. We have observed that this mass spectrometer generally displays lower charged (higher m/z) ions compared to protein mass spectra acquired with other instruments and that it discriminates more against low molecular weight components relative to higher molecular weight components.

Additional studies were performed on a Fisons/VG Platform single quadrupole mass spectrometer (Danvers, Massachusetts). The Platform pneumatically assisted electrospray interface (Bruins et al., 1987; Covey et al., 1988) also employs a nozzle-style inlet. Nitrogen nebulizing gas flows coaxial to the electrospray needle, which sprays into nitrogen bath gas in a heated (60 °C) source chamber.

Two solvent compositions selected to reflect typical conditions for ESI-MS of proteins were used: (1) 4% acetic acid/50% CH_3CN/46% H_2O and (2) 100% H_2O. Samples were introduced to the ESI source by continuous infusion (5–8 μL/min) or by flow injection, either manually (10- or 20-μL injection loop volume) or via autosampler (5-μL injection loop volume) at 5–20 μL/min.

No obvious difficulties were encountered in forming a stable spray at these detergent concentrations in either the acid/organic or 100% aqueous solutions, although the unresolved background observed below m/z 500 for 1.0% solutions of some of the nonionic detergents on the Vestec may reflect an unstable spray, as discussed earlier.

Triton-X 100 (reduced) was obtained from Aldrich, (Milwaukee, Wisconsin). NP40, sodium taurocholate, and CHAPS were supplied by Sigma Chemical Co. (St. Louis, Missouri). CTAB, sodium cholate, LDAO, *n*-octyl sucrose, *n*-dodecyl sucrose, *n*-dodecyl-β-D-maltoside, *n*-dodecyl glucoside, *n*-hexyl glucoside, and Thesit were purchased from Calbiochem (La Jolla, California). Octyl glucoside was purchased from Millipore Corporation (Bedford, Massachusetts) and octyl thioglucoside was obtained from Pierce (Rockford, Illinois). Tween-20 was obtained from Mallinckrodt (Paris, Kentucky) and SDS was obtained from Gibco BRL Life Technologies (Gaithersburg, Maryland). Surfactant samples were used without further purification. Stock surfactant solutions were prepared as 10% w:v and diluted further prior to analysis, except for *n*-dodecyl glucoside and CTAB, which were prepared as 2% stock solutions. The *n*-dodecyl glucoside stock solution was warmed slightly prior to removal of aliquots to completely dissolve the surfactant.

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