analytical chemistry

Article

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Stabilization of Proteins and Noncovalent Protein Complexes during Electrospray Ionization by Amino Acid Additives

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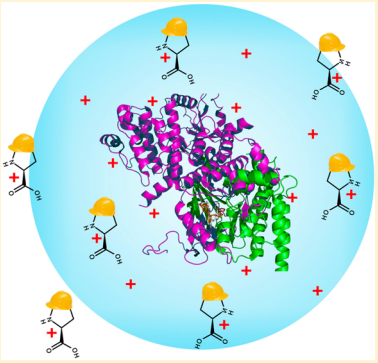
Supporting Information

ABSTRACT: Ionization of proteins and noncovalent protein complexes with minimal disturbance to their native structure presents a great challenge for biological mass spectrometry (MS). In living organisms, the native structure of intracellular proteins is commonly stabilized by solute amino acids (AAs) accumulated in cells at very high concentrations. Inspired by nature, we hypothesized that AAs could also pose a stabilizing effect on the native structure of proteins and noncovalent protein complexes during ionization. To test this hypothesis, here we explored MS response for various protein complexes upon the addition of free AAs at mM concentrations into the electrospray ionization (ESI) solution. Thermal activation of ESI droplets in the MS inlet capillary was employed as a model destabilizing factor during ionization. Our results indicate that certain AAs, in particular proline (Pro), pose considerable positive effect on the stability of noncovalent protein complexes in ESI-MS without affecting the signal intensity of protein ions and original protein−ligand equilibrium, even when added at the 20 mM concentration. The data suggest that the degree of protein stabilization is primarily determined by the osmolytic and ampholytic characteristics of AA solutes. The highest stability and visibility of noncovalent protein complexes in ESI-MS are achieved using AA additives with neutral isoelectric point, moderate proton affinity, and unfavorable interaction with the native protein state. Overall, our results indicate that the simple addition of free amino acids into the working solution can notably improve the stability and accuracy of protein analysis by native ESI-MS.



The preservation of a native structure for proteins and noncovalent protein complexes during ionization is a common problem in biological mass spectrometry (MS). Traditional biological buffers such as tris, phosphate, phosphate-buffered saline (PBS), etc. are nonvolatile and therefore very poorly compatible with electrospray ionization (ESI),[1] which is the most popular ionization routine in protein MS studies. The presence of nonvolatile salts in the protein solution even at micromolar (μM) concentrations can cause the formation of abundant salt clusters, nonspecific protein adducts as well as severe ion suppression.[2−5] To mitigate this problem volatile salts such as ammonium acetate or ammonium bicarbonate are most often employed to buffer protein solutions in ESI-MS.[6−8] Unfortunately, volatile salts have quite poor buffering strength compared to nonvolatile physiological buffers. As a result, the pH value of protein surrounding in ESI droplets can undergo significant changes during ionization process, which is one of the major factors affecting the stability of proteins and noncovalent protein complexes in ESI-MS.[9−12]

Solute amino acids (AAs) in the cells of living organisms can reach exceedingly high concentrations of up to several moles per liter without disturbing vital cellular functions.[13,14] Some intracellular AAs, in particular proline,[15] have been established to play an important role in the regulation of osmotic balance.[16,17] Furthermore, several AAs solutes have been found to pose a significant stabilizing effect on the native structure of proteins and cell components, both in vivo[18] and in vitro.[19,20] The stabilizing effect results from the substantial increase in the free energy of the unfolded protein state due to the presence of these AAs, while the native state remains relatively unaffected and functional.[21]

Given the stabilizing effect of AAs on protein structure in neutral solutions, here we hypothesized that AA solutes could also benefit the stability of proteins and protein complexes during the ionization process. Several recent studies by Campopiano, Clarke, and co-workers have demonstrated that the addition of free AAs into the protein solution at low millimolar (mM) concentrations does not cause any apparent dissociation of protein−protein and protein−ligand interactions but also efficiently reduces sodium adduction and improves the signal-to-noise ratio of protein ions in ESI-MS.[22−25] Furthermore, owing to their ampholytic properties AA solutes at low mM concentrations have been shown to stabilize the pH of a protein solution during ESI-MS.[26] In this study, we explored the effect of AA additives on the behavior of proteins and noncovalent protein−ligand complexes in ESI-MS upon thermal activation of ESI droplets inside the MS inlet capillary. Our data indicate that certain AA additives are not only

Received: April 29, 2015
Accepted: June 25, 2015
Published: June 26, 2015

DOI: 10.1021/acs.analchem.5b01643
Anal. Chem. 2015, 87, 7433−7438

"compatible" with ESI-MS of proteins but also pose a significant stabilizing effect on the native structure of proteins and noncovalent protein complexes during ionization.

METHODS

ESI-MS Analysis. MS experiments were conducted on an LTQ linear ion trap mass spectrometer (Thermo Scientific, San Jose, CA, USA). Mass spectra were collected in m/z range 100−4000. The working solutions were injected at a flow rate of 3−30 μL/min. The ESI voltage was fixed to 3.0 kV. Nitrogen sheath gas pressure was 1.4 MPa. LTQ capillary temperature was varied from 150 to 450 °C. Other parameters were set to default LTQ instrument values.

Materials and Reagents. Lysozyme (from chicken egg white) and myoglobin (from equine heart) were purchased from Sigma-Aldrich (St. Louis, MO, USA) at 98% and 90% purity. *N,N′,N″*-Triacetylchitotriose (NAG_3) and *N,N′,N″,N‴*-tetraacetylchitotraose (NAG_4) at 98% purity were obtained from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). NH_4Ac and all the amino acids were purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). The deionized water used for the experiments was provided by ECIT chemistry facility. Working and stock solutions for all the studied proteins and protein complexes were prepared in 20 mM aqueous NH_4Ac solution (pH 6.9). The working concentration of 5 μM was used for the both studied proteins (lysozyme or myoglobin). AAs were added from stock solutions in 20 mM NH_4Ac directly prior to MS analysis.

RESULTS

ESI-MS of the Lysozyme-NAG_3 Complex. Figure 1a shows broadband ESI mass spectrum of a 20 mM NH_4Ac aqueous solution containing equal amounts (5 μM) of lysozyme protein (from chicken egg white; MW 14310 kDa) and tri-*N*-acetylchitotriose ligand (NAG_3; MW 627 Da) recorded at the LTQ capillary temperature of 250 °C. The charge state distribution (CSD) for both free lysozyme ions and lysozyme-NAG_3 complex ions spanned the range from 6+ (six protons attached) to 9+ (nine protons attached), 8+ being the dominant charge state in the both CSDs, which is consistent with earlier reports.[27] The signals corresponding to free NAG_3, including $(NAG_3)H^+$, $(NAG_3)Na^+$, and $(2NAG_3+H_2O)H^+$, could also be seen in the spectrum, albeit at a weaker intensity.

Figure 1b shows broadband ESI mass spectrum of the same protein−ligand solution recorded at the LTQ capillary temperature of 375 °C, other experimental conditions being identical. In contrast to 250 °C, the signal intensity for the lysozyme-NAG_3 complex relative to free lysozyme was greatly reduced at 375 °C. Also, the charge state at 10+ became evident in the CSD of free lysozyme ions at 375 °C.

ESI-MS of the Lysozyme-NAG_3 Complex with AA Additives. To explore the effect of AA additives on protein complex stability in ESI-MS, a set of identical lysozyme-NAG_3 solutions (5 μM−5 μM in 20 mM NH_4Ac) was spiked with different AAs at the concentration of 2 mM, and the broadband ESI mass spectra were recorded at 375 °C under the same instrument settings. Figure 1(c-g) shows the most representative spectra obtained using glycine (Gly), serine (Ser), proline (Pro), lysine (Lys), and histidine (His) additives. Glutamic acid and aspartic acid were not used because of their insufficient solubility in water. The addition of Gly to the protein−ligand solution (Figure 1c) did not induce any evident effect to the original spectrum (Figure 1b), showing very similar CSD and signal intensities. The addition of Ser to the lysozyme-NAG_3 solution (Figure 1d) resulted in partial protein unfolding during ESI, as manifested by the binary shape of protein CSD due to

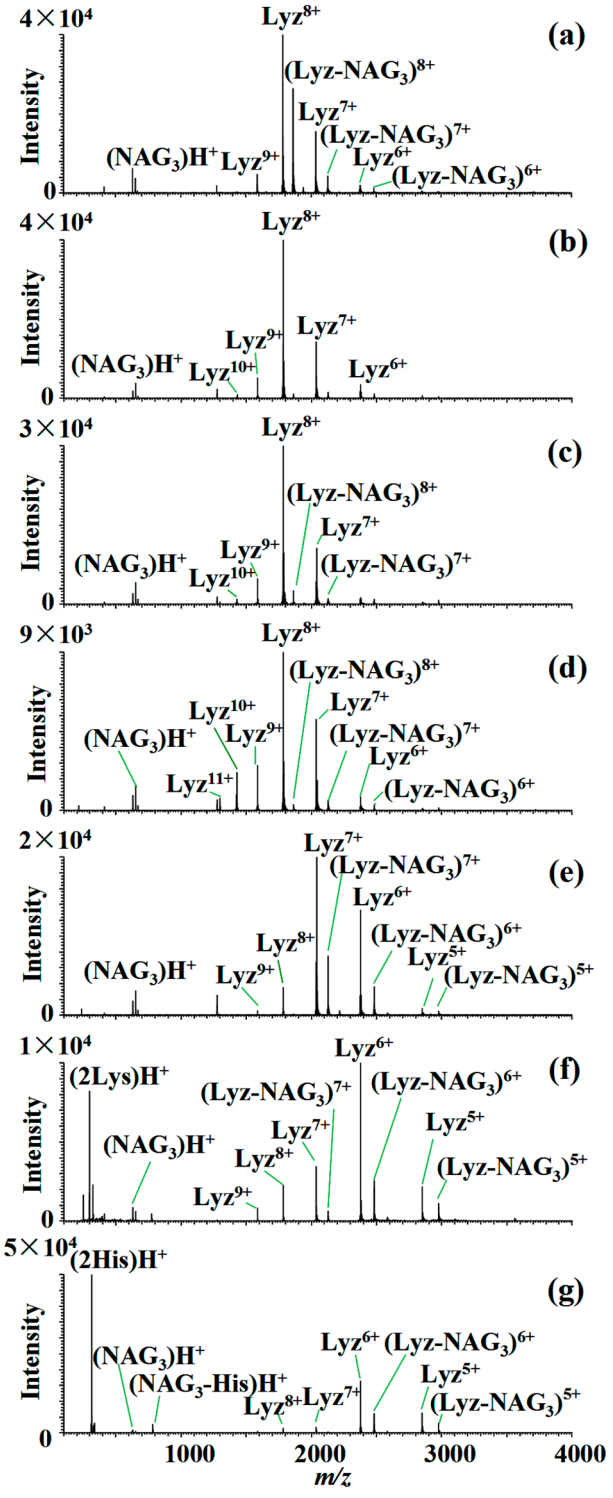


Figure 1. ESI mass spectra of a 20 mM NH_4Ac aqueous solution containing equal amounts (5 μM) of lysozyme protein (Lyz) and the NAG_3 ligand recorded under different conditions: (a) at the LTQ capillary temperature of 250 °C, (b) at the LTQ capillary temperature of 375 °C, (c-g) at the LTQ capillary temperature of 375 °C using (c) 2 mM glycine (Gly), (d) 2 mM serine (Ser), (e) 2 mM proline (Pro), (f) 2 mM lysine (Lys), and (g) 2 mM histidine (His) additives.

DOI: 10.1021/acs.analchem.5b01643
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Table 1. Average Charge State (ACS) and Relative Signal Intensity Ratios for the Lysozyme-NAG_3 (Lyz-NAG_3) Complex and Free Lysozyme (Lyz) Ions with Different AA Additives Recorded at the LTQ Capillary Temperature of 375 °C[a]

AA	PA	HI	pI	I(Lyz-NAG_3)/I(Lyz)	ACS (Lyz)	ACS (Lyz-NAG_3)
	N/A	N/A	N/A	$(7.4 \pm 0.5) \times 10^{-2}$	7.73 ± 0.02	6.85 ± 0.04
Gly	211.6	−0.4	5.97	$(8.4 \pm 1.8) \times 10^{-2}$	7.79 ± 0.01	6.89 ± 0.08
Ala	214.S	1.8	6.01	$(12.3 \pm 0.5) \times 10^{-2}$	7.46 ± 0.04	6.84 ± 0.02
Ser	216.5	−0.8	5.68	$(8.9 \pm 0.9) \times 10^{-2}$	7.90 ± 0.02	7.16 ± 0.43
Val	218.3	4.2	5.97	$(14.5 \pm 1.7) \times 10^{-2}$	7.24 ± 0.01	6.77 ± 0.03
Pro	222.4	−1.6	6.48	$(27.4 \pm 0.3) \times 10^{-2}$	6.76 ± 0.02	6.67 ± 0.03
Lys	228.7	−3.9	9.74	$(24.5 \pm 3.0) \times 10^{-2}$	6.45 ± 0.02	5.93 ± 0.01
His	230.5	−3.2	7.59	$(41.0 \pm 1.7) \times 10^{-2}$	6.06 ± 0.01	5.78 ± 0.02

[a]Literature values are provided for the proton affinities (PA, kcal/mol), hydropathy indexes (HI), and isoelectric points (pI) of corresponding AAs.[28]

the onset of 10+ and 11+ charge states. Almost no signal enhancement for the lysozyme-NAG_3 complex due to the addition of Ser was observed indeed. Unlike Gly and Ser, the addition of Pro to the protein−ligand solution (Figure 1e) resulted in the significant increase of the signal intensity ratio between the lysozyme-NAG_3 complex and free lysozyme ions (Table 1). Besides that, the addition of Pro also induced a shift of CSD for the both free lysozyme and lysozyme-NAG_3 complex ions toward lower charge states (from 6+ to 8+), 7+ being the most abundant charge state. Similar to Pro, the addition of Lys to the protein−ligand solution (Figure 1f) also induced the increase of the signal intensity ratio between the lysozyme-NAG_3 complex and free lysozyme ions as well as the shift of protein CSD toward lower charge states. Quantitatively, Lys induced a stronger CSD shift but weaker signal enhancement for the protein−ligand complex than Pro (Table 1). Apart from the signals corresponding to lysozyme-NAG_3, free lysozyme, and free NAG_3, the Lys dimer signal was observed at a high intensity in the ESI-MS of the Lys-spiked solution (Figure 1f). The AA signal was even stronger upon the addition of His, resulting in partial suppression of the protein signal (Figure 1g). The addition of arginine (Arg), which is more basic than His and Lys, resulted in the dominant observation of Arg and total suppression of protein signals.

The relative abundance of the lysozyme-NAG_3 signal and the average charge states of lysozyme and lysozyme-NAG_3 ions using different AA additives are summarized in Table 1. Overall, the average protein charge state was found to be in good agreement with the AA proton affinity. The higher the proton affinity of AA the lower is the average charge state for both lysozyme-NAG_3 and lysozyme. The observation of the lysozyme-NAG_3 signal was favored by more hydrophilic AAs: Pro, Lys, and His.

The enhancement of the protein complex signal in ESI-MS became evident starting from ca. 500 μM concentration of AA additives and reached saturation at ca. 2−3 mM (Figure S-1). The stabilizing effect of AA additives was also clear when lysozyme-NAG_3 was electrosprayed from pure water instead of 20 mM NH_4Ac, albeit protein signals produced from the pure water solution were ca. 2 orders of magnitude weaker compared to the protein signals produced from 20 mM NH_4Ac (Figure S-2).

Temperature Profile of the Lysozyme-NAG_3 Complex Signal with and without the Pro Additive. The signal intensity ratio between the lysozyme-NAG_3 complex and free lysozyme ions in ESI-MS of the lysozyme-NAG_3 solution (5 μM−5 μM in 20 mM NH_4Ac) was profiled within a broad range of the LTQ capillary temperatures (150 °C−450 °C), with and without the 2 mM Pro additive (Figure 2). Without

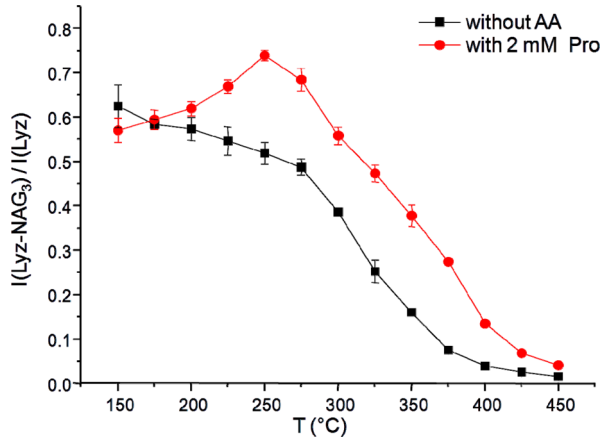

Figure 2. Signal intensity ratio between lysozyme-NAG_3 complex ions and free lysozyme ions in ESI-MS at different LTQ inlet temperatures with and without the Pro additive. Three independent measurements were performed at each temperature.

Pro, the lysozyme-NAG_3 complex signal was fairly stable below 200 °C but rapidly decreased starting from 200 to 450 °C. No lysozyme-NAG_3 signal was observed at 450 °C. With Pro, the relative intensity of the lysozyme-NAG_3 complex signal below 200 °C was very similar to the relative intensity of the lysozyme-NAG_3 complex without Pro. However, the subsequent (>200 °C) intensity decrease of the lysozyme-NAG_3 complex with Pro occurred at a much slower rate than without Pro. Partial preservation of the complex could be observed even at the highest allowed LTQ inlet temperature of 450 °C.

ESI-MS of the Lysozyme-NAG_4 Complex with and without AA Additives. Another lysozyme complex, with the NAG_4 ligand (MW 830 Da), revealed a similar characteristic ESI-MS response to the addition of AAs as that described for the lysozyme-NAG_3 system. The behavior of the lysozyme-NAG_4 system is summarized in Table S-1.

ESI-MS of Myoglobin with and without AA Additives. To extend the investigation, we also explored the effect of AA additives on the thermal stability of myoglobin during ESI-MS. Myoglobin contains a noncovalently bound heme group in its hydrophobic pocket. Upon vibrational activation, the heme moiety becomes a much weaker bound and can be readily lost by the protein. Myoglobin with the preserved heme moiety is referred to as holomyoglobin. Myoglobin without heme is commonly referred to as apomyoglobin. Figure 3a shows the broadband ESI mass spectrum of a 20 mM NH_4Ac aqueous solution containing 5 μM myoglobin recorded at the LTQ capillary temperature of 150 °C. The spectrum is dominated by the signals of holomyoglobin at 8+ and 9+ charge states

DOI: 10.1021/acs.analchem.5b01643
Anal. Chem. 2015, 87, 7433−7438

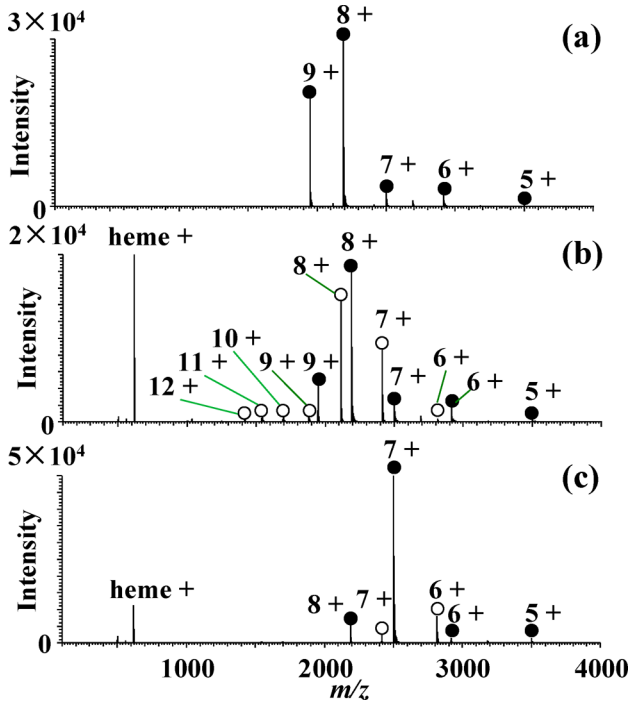

Figure 3. Effect of the Pro additive on the thermal stability of myoglobin during ESI-MS: (a) ESI-MS of myoglobin without the Pro additive at the LTQ capillary temperature of 150 °C, (b) ESI-MS of myoglobin without the Pro additive at the LTQ capillary temperature of 450 °C, (c) ESI-MS of myoglobin with the Pro additive at the LTQ capillary temperature of 450 °C. Holomyoglobin signals are labeled with close circles, and apomyoglobin signals are labeled with open circles.

without notable signals of apomyoglobin. The ESI mass spectrum of the same protein solution recorded at the LTQ capillary temperature of 450 °C showed a notable presence for apomyoglobin and free heme (Figure 3b), revealing complex dissociation. Also, the occurrence of a new band in CSD at higher charge states indicates partial protein unfolding. The ESI mass spectrum of the same protein solution spiked with 2 mM Pro recorded at the LTQ capillary temperature of 450 °C showed the dominant signal for holomyoglobin at the 7+ charge state with only a minor signal for the free heme (Figure 3c). With 2 mM His, the spectrum showed the dominant signal for the 7+ holomyoglobin charge state, albeit the signals for apomyoglobin and the free heme were more intense than with Pro, indicating higher degree of protein unfolding (Figure S-3a). With 2 mM Ser, the spectrum was dominated by apomyoglobin and the free heme signals with only minor contribution from holomyoglobin (Figure S-3b), indicating nearly complete complex dissociation.

DISCUSSION

The obtained results indicate either partial or full dissociation occurring for all the studied noncovalent protein complexes upon thermal activation (>300 °C) inside the LTQ capillary. Furthermore, partial protein unfolding in heated ESI droplets is also evident from the onset of higher protein charge states at high temperatures of the LTQ capillary (Figure 1b, Figure 3). Interestingly, in our previous study we observed a notably higher degree of thermally induced protein unfolding in ESI droplets,[29] which could be due to the use of spraying capillary with a larger inner diameter (200 μm). The increase of a spraying capillary diameter results in a larger initial size and, therefore, longer lifetime of ESI droplets.[10] As a consequence, proteins residing inside ESI droplets are exposed to thermal activation for a longer period of time, which causes a higher degree of unfolding.

The addition of AAs to the spraying solution influences both the CSD and thermal stability of noncovalent protein complexes in ESI-MS. The gradual shift of protein CSD toward lower charge states with the increase in proton affinity of the AA additive (Table 1) is consistent with the mechanism recently proposed by Ogorzalek Loo et al.[30] According to this mechanism, solutes with high proton affinity added at a high concentration to the protein solution can carry away a significant share of protons from the ESI droplet via the ion ejection process, thus reducing the charge available to the protein and driving the shift in protein CSD. Consistent with the mechanism proposed by Ogorzalek Loo et al. and our observations, Clarke and Campopiano have recently reported a noticeable shift to lower charge states in the ESI-MS of bovine serum albumin (BSA) promoted by the addition of 5 mM Lys or His into the working protein solution (200 mM aqueous NH_4Ac).[25]

The proposed mechanism for the reduction of protein charging via the ejection of protonated AAs from mature ESI droplets implies that the abundant gas-phase ions of AAs should be produced during ionization. Indeed, the signals of free AAs and AA adducts were readily revealed in the low mass range of ion detection (m/z 50−500). Basic AAs, in particular Arg and His, were also highly visible in the high mass range of ion detection, severely suppressing the protein signal (Figure 1g). The signal intensity and concomitant suppression effect of basic AAs increased in the order Lys < His < Arg, in accordance with the increasing proton affinity (Table 1). Apparently, basic AAs are poorly compatible with the sensitive protein analysis by ESI-MS and mostly represent interest as model systems to address the mechanistic aspects of protein ionization in the presence of AAs. In contrast, AAs with lower proton affinities were usually not observed in the high mass range of ion detection (m/z 200−2000) and did not pose any measurable suppression effect on the intensity of the protein signal recorded in the absence of AAs even at the 20 mM concentration (Figure S-4), exhibiting very good compatibility with the sensitive ESI-MS detection of proteins and protein complexes.

While the magnitude of the CSD shift induced in protein ions by the addition of AA correlates with proton affinity of the corresponding AA, the stability of noncovalent protein complexes appears to be more sensitive to the isoelectric point (pI) of the AA additive (Table 1). AAs are well-known for their ampholytic properties: when added at a mM concentration into a μM protein solution, AAs adjust solution pH to their pI value. For example, the estimated pI for His is very close to the physiological pH of ca. 7.3 (Table 1). Accordingly, His revealed a strong stabilizing effect on the structure of a native protein complex during ESI-MS despite its suppressing influence on the net protein signal intensity (Figure 1f). The stabilizing effect was also revealed for other AAs with neutral pI (Table 1), even when the original 20 mM NH_4Ac buffer was replaced with pure water. Our data indicate that neutral AAs exhibit a strong buffering capacity in ESI-MS when added at the mM level (Figure S-1). The stability of the lysozyme-NAG_3 complex due to AA additives was even more notable when electrosprayed from pure water instead of 20 mM

DOI: 10.1021/acs.analchem.5b01643
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NH_4Ac (Figure S-2). Therefore, low-volatility AA additives may conceivably provide supplemental buffering of native proteins analyzed from the aqueous solutions of volatile salts such as NH_4Ac. Contrasting behavior was observed for Ser, which has the lowest pI value out of the studied AAs (Table 1). The addition of Ser forced the acidic shift for the original pH of the lysozyme-NAG_3 solution, thus causing partial unfolding of lysozyme (Figure 1d). It is important to note, however, that the pK_a and pI values of AAs are sensitive to temperature. Therefore, it is possible that the relative pI order is not preserved throughout the entire range between 25 and 450 °C.[31] Other factors might be also important. For example, the apparent destabilization of the lysozyme-NAG_3 complex upon Ser addition could possibly be related to the destabilizing hydrogen-bonding interaction imposed by the OH group of Ser.

Among the AA additives studied here, Pro showed the optimal behavior for the analysis of proteins and protein complexes by ESI-MS. First, solute Pro did not suppress the intensity of protein ions even when added at 20 mM concentration, which greatly favors Pro over AAs with higher proton affinity, such as Lys, His, and Arg (Figure 1). Second, Pro revealed a strong stabilizing effect on the integrity of noncovalent protein complexes during ESI-MS, which is likely related to its neutrality and high buffering strength. The effect of the Pro additive is particularly vivid from the comparative analysis done for the myoglobin-heme complex (Figure 3, Figure S-3). Third, the addition of Pro did not notably affect the protein−ligand binding equilibrium at low temperatures. Thus, the original signal intensity ratio for the lysozyme-NAG_3 complex ions and free lysozyme ions in the ESI-MS of the lysozyme-NAG_3 mixture at low LTQ capillary temperatures (weak thermal activation) was not notably altered with the addition of Pro (Figure 2). The stability of the protein−ligand complex as well as the lack of protein-Pro adducts in ESI-MS indicate that Pro does not directly interact with protein complexes during ionization. Overall, these behaviors underscore the beneficial role of the Pro additive in the native ESI-MS analysis of proteins and noncovalent protein complexes.

The beneficial effect of Pro on protein stability in ESI-MS well agrees with the fact that Pro is commonly accumulated by living cells in molar concentrations as one of the major naturally occurring protecting osmolytes.[14,21] Apart from the control of cellular osmosis, it is well established that protecting osmolytes play an important role in stabilizing the structure of proteins and cell subunits both *in vivo*[18] and *in vitro*.[19,20] The stabilizing effect results from the substantial increase in the free energy of the unfolded protein state due to the presence of protecting osmolytes, while the native state remains relatively unaffected and functional.[21] The importance of this mechanism is that osmolytes act primarily on the denatured state in bringing about stabilization. The interaction of protecting osmolytes with the native state is greatly unfavorable, causing preferential exclusion of osmolytes from the hydration shells of the protein.[20] This mechanism provides the means for stabilizing cellular proteins against the denaturing stress without interfering with their functional activity. We therefore suggest that it is the interplay of ampholytic and osmolytic properties of solute Pro that yields the enhancement of protein stability in ESI-MS.

We speculate that another factor contributing to the enhancement of protein stability in ESI-MS by AA additives might be related to the gradual charge depletion of ESI droplets due to the ejection of free AA cations occurring inside the MS inlet interface. The charge depletion results in the reduced extent of protein charging during ionization (Figure 1). In the case of charged ligands (e.g., NAG), the reduction in protein charging minimizes Coulombic repulsion between the protein and the ligand, thus stabilizing the integrity of the complex. We found that gas-phase lysozyme-NAG_3 ions preferentially dissociated via the loss of protonated NAG_3 moiety and the stability of complex ions was decreased with the charge state.

In addition to protecting the stability of protein complexes during ionization, certain AA additives have been also found to have a notable desalting effect on protein ions in ESI-MS. In a recent report, Clarke and Campopiano demonstrated that several AAs, such as Ser and Ala, added to the ESI spray solution at mM concentration greatly reduced the adverse effects of sodium adduction to large proteins, e.g., BSA (66 kDa) and protein complexes, including serine palmitoyl transferase (SPT, a 92 kDa homodimer), enolase (a 93 kDa homodimer), and alcohol dehydrogenase (ADH, a 148 kDa tetramer).[25] The reduction in ion sodium ion adduction occurred with no loss of the noncovalent protein−protein interactions and allowed significant peak narrowing (~10-fold) as well as an increased signal-to-noise ratio (~4-fold) of protein ions. Interestingly, the effectiveness of AA additives at reducing sodium adduction was found to roughly trend with their gas phase sodium affinities.[25] This correlation suggests a mechanism whereby the free AAs directly sequester sodium cations from the ESI solution, similar to the mechanism of proton ejection by basic AA additives.

CONCLUSION

Overall, our study demonstrates the use of free amino acid additives as a simple yet efficient approach to increase the stability of proteins and noncovalent protein complexes in native ESI-MS. Importantly, the stabilizing effect is achieved without deteriorating the ESI-MS visibility of protein ions and the original protein−ligand equilibrium in solution. Amino acid additives can be applied as protein protectants in the studies using direct infusion ESI-MS as well as in combination with chromatographic separation, e.g., capillary electrophoresis or isoelectric focusing. The stabilization effect of AA additives on protein structure in ESI-MS discovered in this study together with the earlier discovered desalting effect of AAs on protein ions in ESI-MS strongly indicate the power of this approach to improve the quality of biological MS analysis. Of specific interest for future studies are the unusually low charge states of protein complex ions resulting from the interaction with certain AA additives, as the low charge states commonly reveal higher "nativity" in ESI-MS.

ASSOCIATED CONTENT

Supporting Information

Figures and table as noted in text. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.analchem.5b01643.

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The authors declare no competing financial interest.

DOI: 10.1021/acs.analchem.5b01643
Anal. Chem. 2015, 87, 7433−7438

■ ACKNOWLEDGMENTS

The work was supported by the National Natural Science Foundation of China (NNSFC) (No. 21225522), National key scientific instrument development projects (Nos. 2011YQ14015008, 2011YQ170067), Program for Changjiang Scholars and Innovative Research Team in University (PCSIRT) (No. IRT13054), and Science and Technology Planning Project from the Department of Science and Technology of Jiangxi Province (No. 20124ACB00700).

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DOI: 10.1021/acs.analchem.5b01643
Anal. Chem. 2015, 87, 7433−7438