Amino Acid Clusters Formed by Sonic Spray Ionization

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A novel ion source based on the principle of sonic spray ionization has been built and used to optimize mass spectrometric conditions for generating amino acid clusters. The ion source employs a simple pneumatic spray operated at extremely high nebulizing gas flow rates. Several factors that affect the performance of the cluster source are identified, and information from these observations provides insights into the mechanisms of gas phase ion formation. Serine is used as a model system in optimizing instrumental and sample parameters to maximize cluster ion formation. The sonic spray results for this oligomer compare favorably with electrospray data, showing an order of magnitude better signal intensity and excellent signal-to-noise ratios. The performance of the system for the protonated serine octamer includes a limit of detection of 10 nM and a linear dynamic range of 4 orders of magnitude. Ion formation was observed to go into saturation above 1 mM. This result and data on pH, electrolyte concentration, and solvent composition are interpreted as supporting a charge residue model of sonic spray ionization. Other amino acids can be substituted for serine in the octamer, with a strong chiral preference in favor of homochiral cluster formation in the cases of threonine and cysteine. These amino acids show a preference for substitution of more than two serine molecules. Phenylalanine, asparagine, tryptophan, and tyrosine also substitute into the serine octamer; however, the process yields only two incorporations and only small chiral effects.

There is currently strong interest in the interactions of biological molecules to form more or less strongly bound and long-lived cluster species. Such entities play key roles in biological processes and are studied in solution using a wide variety of analytical and physical methods.[1] The development of electrospray ionization[2] has provided a tool that allows biomolecular clustering[3−7] to be studied under controlled conditions for model systems.

There is much debate over the degree to which such experiments sample the species present in solution and even more uncertainty about the biological significance of the results of such model studies. Nevertheless, a great deal of attention continues to be devoted to these experiments, as disclosed in recent reviews,[8−12] and it is clear that electrospray ionization (ESI) is the only widely used ionization technique that is able to give mass spectrometric information that may be relevant to solution-phase structures. Clustering studies in various systems have relied on electrospray data: the systems include solvated metal cations, enzyme−substrate complexes, and solvent clusters.[8,11,13−18] Among systems of current interest is the guanine quartet, in which strong correlations between ESI and solution-phase magic number clustering have been reported,[19−21]and the clusters of amino acids, among which serine, in particular, shows strong homochiral clustering,[22−27] which has been suggested to be implicated in the

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propagation of homochirality in the course of biomolecular evolution.[27] Serine displays abundant singly protonated octameric ions in its electrospray ionization mass spectrum. These octamers are homochiral, and when electrospraying binary mixtures of serine and another amino acid up to two of the serine molecules in this cluster can be substituted by the other amino acid, including cysteine, asparagine, or threonine.[26,27] These substitution reactions show strong chiral preferences.[27]

Despite the value and growing use of ESI for the study of intermolecular complexes in solution, it suffers from drawbacks with regard to the transmission of noncovalent, solution-phase ions into the gas phase. The ESI method is often assumed to be a particularly soft ionization method (i.e., a method that generates ions with low internal energy); yet, recent studies[28] reveal that small cations (transferred to the gas phase by ion evaporation) gain considerable amounts of internal energy in the ESI process. Such internal energies may be sufficient to alter or even destroy solution-phase molecular clusters. Atmospheric pressure chemical ionization (APCI), the other common spray technique, is even less likely to be successful at preserving cluster structures because of the high temperatures that are applied. Another disadvantage of ESI and other atmospheric pressure ionization methods is the possible formation of secondary clusters in the atmospheric interface. These clusters would be generated in the gas phase, and there would be no necessary correlation with solution-phase structures.

Sonic spray ionization (SSI) is a little-used spray ionization technique developed by Hirabayashi et al. in the mid 1990s.[29,30] In the original method a methanol/water solution was sprayed from a fused-silica capillary with a gas flow coaxial to the capillary. Ions, as well as charged droplets, are produced at atmospheric pressure, and ion intensities depend strongly on the flow rate of the nebulizing gas. The technique was developed originally for interfacing liquid chromatographs and capillary electrophoresis instruments to mass spectrometers. However, its most significant application has been to the analysis of extremely thermolabile molecules, such as catecholamines and other neurotransmitters.[31−33] The main advantage of the technique is its simplicity, which follows from the fact that neither heating nor high voltage is used in ion formation. As a consequence, ions of very low internal energy are generated. Unfortunately, as a result of the low ion internal energy, excessive clustering often occurs, and this is detrimental to the analytical performance of the method for molecular characterization. For example, the signal due to a particular compound generally occurs in the form of several different adducts, lowering the sensitivity of the method and making spectra harder to interpret. A further disadvantage of the technique is the favored formation of ions in low charge states as a consequence of a process that yields statistical charge distributions. Uncharged droplets represent the most probable situation and do not contribute to mass spectrometric analysis. Formation of multiply charged ions has been reported in cases in which a high voltage was applied to the source housing; however, this experimental arrangement is not substantially different from conventional ESI sources operated at high nebulizing gas flow rates and is not of interest in this study.

The fact that sonic spray ionization favors cluster ion formation suggests that it might be appropriate as a method to generate amino acid cluster ions. This assumption was explored in detail in this study using a home-built sonic spray source. In interpreting the results, attempts were made to distinguish the possibility of clustering during the spray process from the possibility of observing weakly bound clusters present in the nascent solution phase. Important instrumental parameters having effects on cluster formation were identified and optimized using L-serine as a model compound. Herocluster ions, including mixtures of other amino acids with serine, were also examined, and the results are compared to ESI data, especially with regard to chiral effects. Insights into the sonic spray ionization process are reported.

EXPERIMENTAL SECTION

A sonic spray source was built following the Hirabayashi design but instead of an aluminum orifice of 0.4-mm diameter, a coaxial fused-silica capillary with internal diameter of 0.25 mm was used. A detailed cross-sectional view of the source is shown in Figure 1. The smaller difference between the o.d. (0.2 mm) of the sample capillary and the i.d. of gas capillary (0.25 mm) was expected to provide higher linear gas velocities at similar mass flow rates. The source was operated at a nitrogen nebulizing gas pressure of 1.2×10^6 Pa. Liquid sample was introduced at a flow rate in the range of $1-50$ μL/min. Electrospray spectra were recorded using the same ion source operated in a pneumatically assisted electrospray mode. The nebulizing gas pressure was 1.0×10^5 Pa, and the sample flow rate was $1-3$ μL/min. A high voltage of $2.3-2.7$ kV was applied on the infusion syringe tip using a copper alligator clip. Experiments for the comparison of two techniques (ESI and SSI) were carried out without changing source geometry and instrumental settings, except for the spray high voltage and nebulizing gas pressure, both of which were optimized.

All spectra were recorded using a Thermo Finnigan LCQ quadrupole ion trap mass spectrometer. The standard ESI source was replaced by the sonic spray source, and this was carefully aligned to the atmospheric interface to achieve the highest sensitivity. Alignment was carried out by maximizing the signal intensity due to the singly charged protonated serine octamer at 841 Th (Th = 1 Dalton/unit charge[34]), using 20 μL/min 0.01 M L-serine in MeOH/water 1:1 solution. The optimal spray position was found to be approximately the same for both ionization methods, so a single position was used in subsequent experiments. Optimal instrumental parameters were found to be dependent on the solvent composition and the sample flow rate. Standard instrumental settings used for pure methanol, methanol−water, and aqueous solutions of L-serine are summarized in Table 1.

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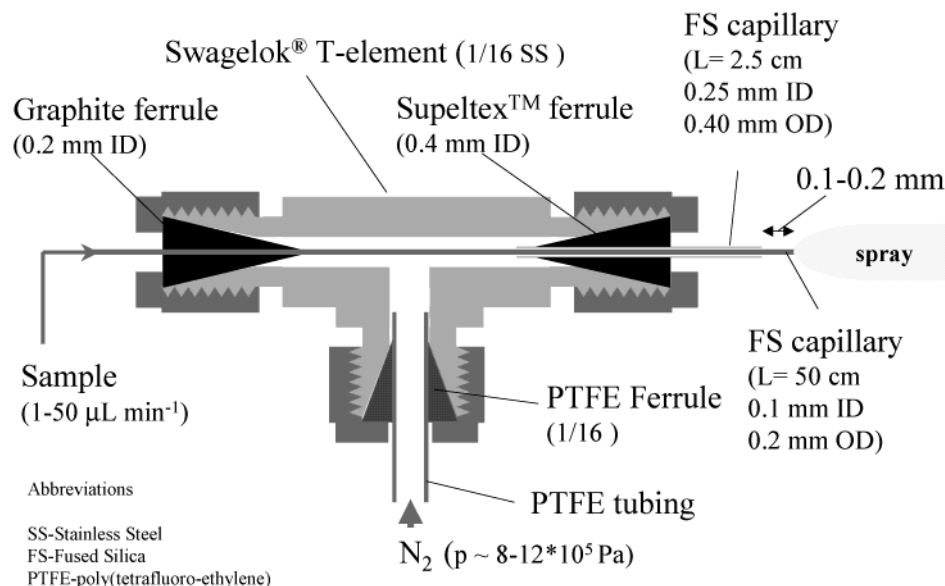(34) Cooks R, G.; Rockwood A. L. *Rapid Commun. Mass Spectrom.* **1991**, *5*, 93.

Figure 1. Schematic cross section of sonic spray ion source. Liquid sample is pneumatically sprayed by the coaxial gas flow. The cross section of the gas flow in this source is restricted to 0.017 mm^2

Table 1. Standard Conditions Used for Recording Sonic Spray Mass Spectra of Amino Acids

	solvent		
	methanol	MeOH/water 1:1	water
concn (M)	0.001	0.01	0.01
flow rate (μL/min)	3	30	20
gas flow rate (L/min)	6	9	10
capillary temperature(°C)	25	80	100
tube lens offset (V)	80	80	80
capillary voltage (V)	−40	−40	−40
injection time (ms)	100	100	100
pH	4.5	6.2	6.5

Automatic gain control was turned off to ensure identical conditions in the acquisition of both types of spectra, and generally, a 100-ms injection time was used for both types of ionization experiments. Sample concentrations were 10 mM for serine and other amino acids, unless otherwise specified. The pH of the solutions was adjusted by adding appropriate volumes of 0.1 M HCl or 0.1 M NH$_3$. Product ion tandem mass spectrometry (MS/MS) was performed using procedures and operating conditions described elsewhere.[22]

RESULTS AND DISCUSSION

Cluster Ion Formation in SSI and ESI. Figure 2 compares mass spectra of 10 mM L-serine in methanol/water 1:1 recorded using electrospray ionization and sonic spray ionization, respectively. The concentration of 10 mM is high for electrospray ionization; however, this concentration is in the saturation range for both techniques (see below). Thus, at this concentration level, the maximum achievable signal intensities can be compared. Relatively high analyte concentrations are also needed to study clustering by ESI. There are two main differences between the two spectra shown in Figure 2. One is the absolute abundance of the protonated octamer at 841 Th. Sonic spray gives 10−15 times

higher signal intensities at similar baseline noise levels when compared to ESI for this cluster ion. Note again that the same instrumental settings were used in both cases, except for spray voltage and nebulizing gas flow rate, which are the two parameters that establish the difference between sonic spray and electrospray. A second significant difference lies in the relative abundances of other serine oligomeric ions relative to the protonated octamer. In the case of electrospray, the monomer and octamer have very similar abundances to the octamer, and additional clusters are also observed. By contrast, ions other than the octamer are virtually absent from the sonic spray spectrum. This remarkable result indicates either strong discrimination in the sonic spray experiment against these species or the virtually exclusive formation of the octameric ion or precursors that lead to it. The former assumption is not likely, since other species with similar molecular mass and chemical characteristics do not show any suppression in sonic spray spectra.[28] Furthermore, by elevating the heated capillary temperature in the atmospheric interface and increasing the tube lens offset, the peak ratios in sonic spray spectra become closer to ESI values with regard to the higher relative abundances of the monomer and dimer ions. Although a typical ratio of monomer/dimer/octamer in ESI is 90:20:100, in sonic spray, it is 0:1:100. Using a heated capillary temperature of 130 °C and tube lens offset of 200 V, the latter ratio is shifted to 70:90:100. These two parameters are well-known to influence the internal energy of the ion population. It is also noteworthy that the published ESI spectra[22] of L-serine in the same concentration range always contain high-intensity monomer and lower intensity dimer peaks, whereas in sonic spray, the monomer/dimer ratio is the opposite.

There are two important conclusions that arise from a consideration of the above data. The simpler is that sonic spray generates ions with considerably lower average internal energies than does ESI. The second is that serine molecules could be present in the solution phase as the octamer (or higher oligomer). This point is taken up in a later section.
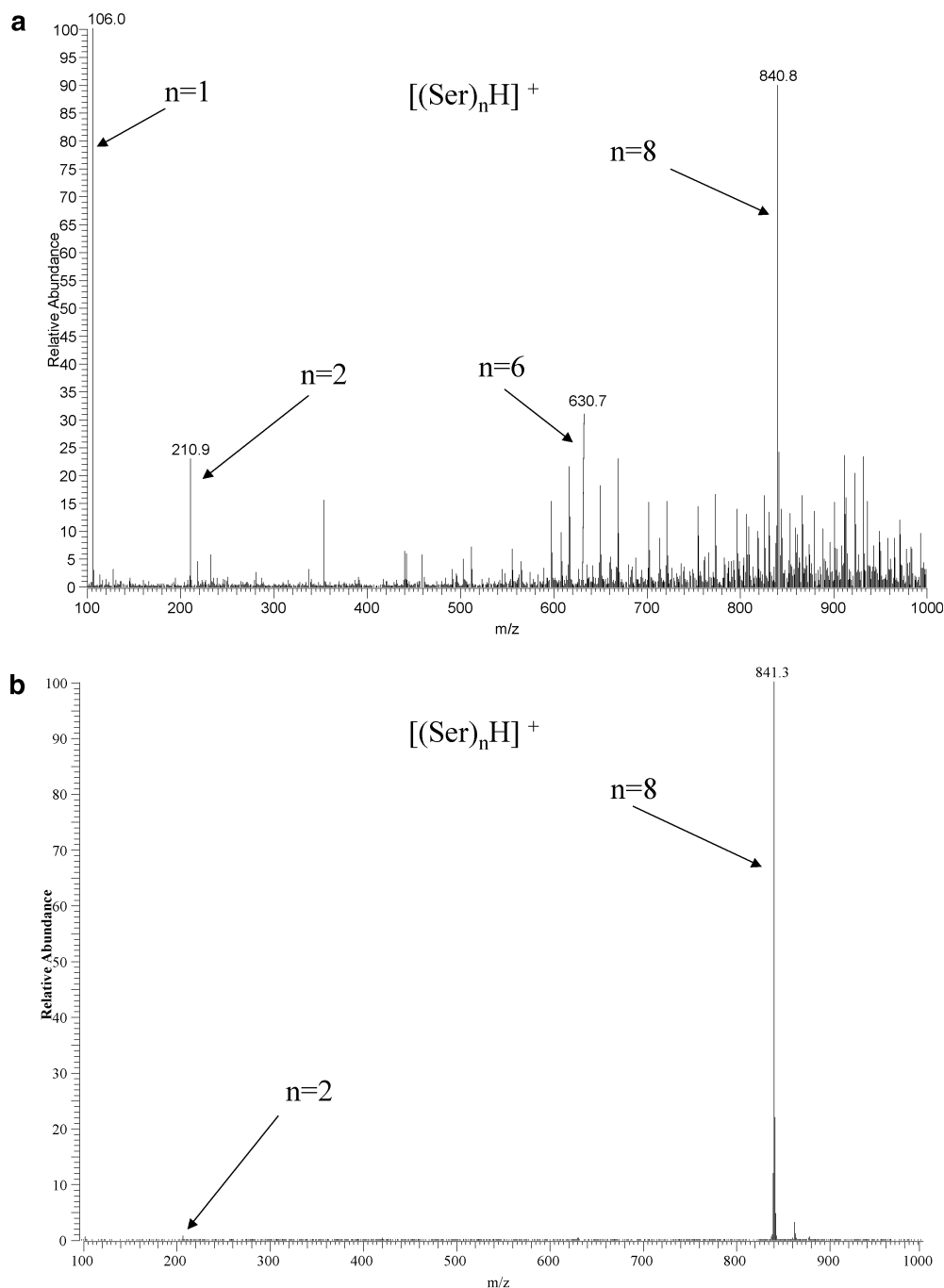
Figure 2. (a) Electrospray and (b) sonic spray spectra of a 0.01 M L-serine solution in methanol/water 1:1. Spectra were acquired using the source shown in Figure 1 at a sample flow rate of 5 μL/min. Nebulizing gas flow of 9 L/min and 0.4 L/min rate was applied for sonic spray and electrospray, respectively. In the electrospray mode, 2.3 kV was applied, but the system was grounded in sonic spray mode. The heated capillary temperature was 80 °C. Instrumental settings were the same in sonic and electrospray mode. The signal intensity of the protonated octamer ion was 2.8×10^5 in the electrospray mode and 1.6×10^7 in sonic spray. The baseline noise was on the order of 10^2 in both cases.

Sonic Spray Ionization Mechanism. The formation of charged droplets in sonic spray is tentatively attributed to statistical charge distribution during droplet formation in the pneumatic spray.[35] Charging can be ascribed to microscopic fluctuations in the ion concentrations in the liquid phase. According to this model, the average charge of the droplet $<|q|>$ is proportional to the square root of ion concentration in solution. To test this relationship, NaCl, a strong electrolyte, was added to

the solution at different concentrations. As shown in Figure 3, the total ion signal (more than 90% of which consists of the sodiated serine octamer ion at 863 Th) increases linearly with electrolyte concentration in the range from 0 to 1 mM. This result is in agreement with the prediction of the statistical charging model.

Variations in pH also show strong effects, the lowest protonated octamer abundance being observed at pH 5.8, which corresponds to the isoelectric point of serine (pH 5.68) (Figure 4). It is

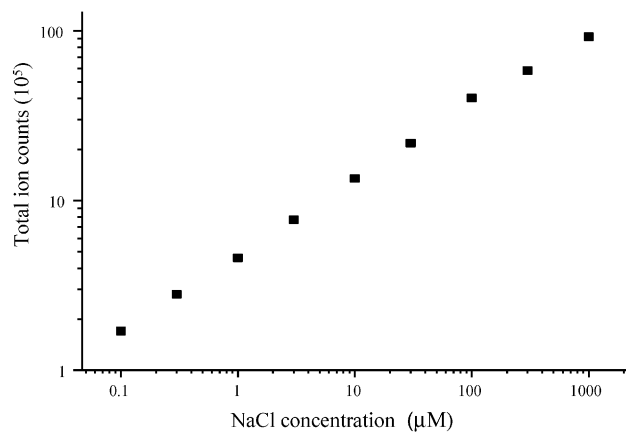(35) Dodd, E. E. *J. Appl. Phys.* **1953**, *24*, 73−80.

Figure 3. Dependence of total ion current on strong electrolyte concentration. L-Serine (0.01 M) was sprayed at different concentrations of NaCl. Spectra contain sodium adducts of serine clusters with the general composition $(Ser_8Na)_n^{n+}$.
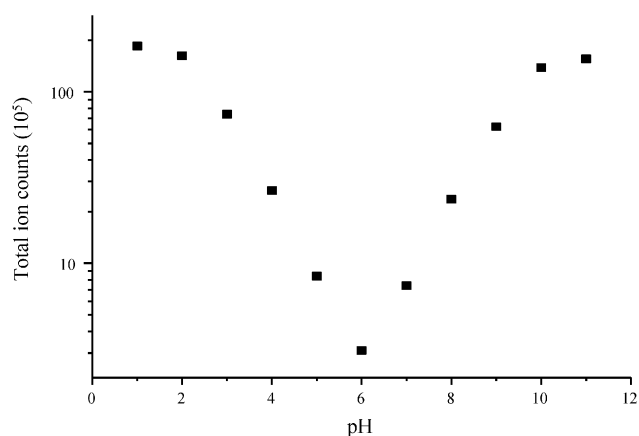

Figure 5. Dependence of total ion current on solvent composition for 0.01 M L-serine showing an increase in signal with increasing methanol concentration.

Figure 4. pH dependence of total ion current in sonic spray for 0.01 M L-serine. The minimum is close to the isoelectric point of serine (pH 5.89). The signal intensity corresponds to positive ions over the entire pH range.

sions are much less likely as a result of the low degree of charging of the droplets, so shrinking of the droplets by solvent evaporation is the only reasonable route to nanodroplet formation. It is only because of the extremely high gas flow that this process is efficient enough to yield nanodroplets in sufficient quantity (and with small enough size) to give good ion currents.

Formation of gaseous ions from nanodroplets in ESI is generally assumed to follow either the charge residue model[37−39] or the ion evaporation model,[40] depending mostly on the mass of the ion. Since the average charge on droplets of similar size can be assumed to be considerably lower in SSI than in ESI, ion evaporation in the traditional sense is much less likely. Hence, the main route to gaseous ion formation in SSI is assumed to be the charge residue model, which should make the ionization efficiency highly dependent on the volatility of the solvent. Using different methanol/water ratios, the total ion abundance behaves in a fashion consistent with these considerations (Figure 5). A contributing factor, which also explains the data of Figure 5, might be the lower surface tension of methanol; i.e., surface tension determines the number of droplets formed in the spray and ion abundance is proportional to this value.

Another parameter that has a large influence on evaporation efficiency is the flow rate of the nebulizing gas. By increasing the gas flow rate, ion abundance increases and then goes into saturation, indicating complete evaporation above a certain flow rate (Figure 6). This saturation effect occurs at gas flow rates that are higher in the case of water than in methanol, which is in good agreement with its higher surface tension and rate of evaporation. Together, these results support—although they do not prove— the charge residue model for SSI.

The temperature of the heated capillary at the atmospheric interface also has an effect on the evaporation efficiency of droplets produced by sonic or by electrospray. The capillary temperature dependence of total ion abundance at various flow rates is shown in Figure 7. At high sample flow rates, when the evaporation

noteworthy, that above the isoelectric point, the protonated octamer was still observed. Since ammonia was used for pH adjustment, this phenomenon can be explained tentatively by the formation of serine octamer ammonium adduct, which dissociates in the gas phase to ammonia and the protonated serine octamer as a result of the relative gas phase basicities of the two systems. At the isoelectric point, there is the highest concentration of zwitterionic serine species in the solution; however, the lowest signal intensity was observed. This phenomenon could be interpreted as evidence for the octamer being nonzwitterionic (consisting of uncharged serine molecules bound by H-bonds). A more plausible explanation is that the availability of positive charges in solution increases both as the pH is increased and as it is decreased, and since only cationized clusters can be detected by the mass spectrometer in positive ion mode, increasing the concentration of cations increases the ionization efficiency of serine octamers.

Nanodroplet formation in ESI occurs via sequential coulomb explosions of highly charged droplets accompanied by continuous solvent evaporation.[36] In the case of sonic spray, coulomb explo-

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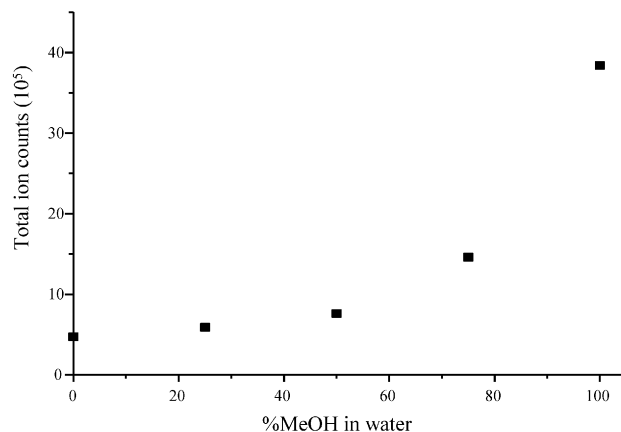
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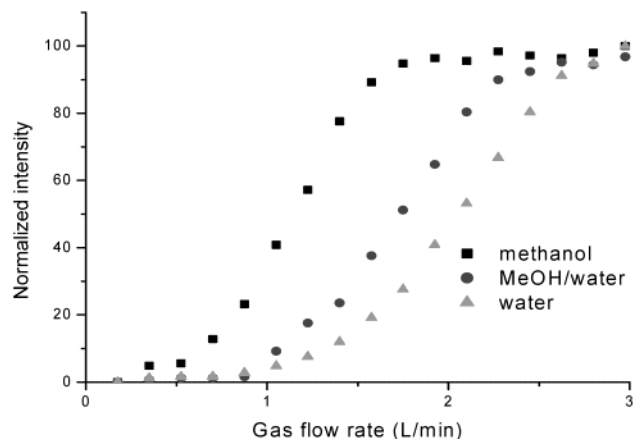(40) Iribarne, J. V.; Thomson, B. A. *J. Chem. Phys.* **1976**, *64*, 2287−2294.

Figure 6. Relative total ion abudance at different solvent compositions as a function of nebulizing gas flow rate. Each curve is normalized. The increase in ionization efficiency can be attributed either to the increasing number of droplets formed or the increased solvent evaporation efficiency. Absolute maximum abundances correspond to values shown in Figure 5.
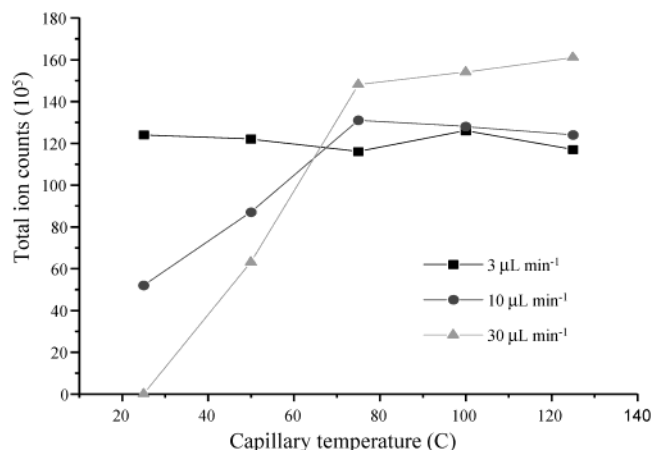

Figure 7. Capillary temperature dependence of total ion abundance at different sample flow rates. At low sample flow rate, ion formation appears to occur in the atmospheric region by gas-phase processes, and consequently, the heated capillary temperature has no role in the evaporation process. Note that the opposite occurs at higher sample flow rates.

process is not completed in the atmospheric pressure region of the source, the total ion current is highly dependent on capillary temperature, indicating that evaporation continues in the capillary. However, at low sample flow rates, the ionization process is independent of the capillary temperature, indicating that under these conditions, it is not droplets, but already formed gas-phase ions, that enter the interface.

Analytical Performance in Cluster Formation. Analytical performance of the SSI method was evaluated, but only in the restricted sense that amino acid clustering was studied using data for serine and the closely related amino acid, threonine. Sonic spray spectra of threonine at two concentrations are shown in Figure 8. The structural similarity of threonine to serine is reflected in similar clustering behavior in both the positive and the negative ion modes (Figure 9). The protonated octamer of threonine is the most abundant peak in the positive ion sonic spray spectrum of L-threonine recorded at a concentration of 0.2 mM; however, at higher concentrations, other clusters, even the less

stable odd-numbered clusters, occur in relatively high abundances (Figure 8 a,b). Throughout the concentration range from 0.1 μM to 0.01 M, L-threonine spectra contain a greater variety of types of clusters with higher abundances than do the L-serine spectra. Abundances of the odd-numbered clusters show a higher than linear dependence on concentration, and this underscores the difference in their origin from the even-number clusters that increase linearly with concentration and appear to be fragments of the octamer. The negative ion spectra of both amino acids show less pronounced clustering than do the positive ion data. This is seen in the lower abundances observed for the most abundant clusters and in the occurrence of a larger variety of cluster ions of significant abundance. The most prominent cluster in the threonine spectra recorded using both ionization methods corresponds to the singly deprotonated hexamer. Remarkably, this ion, like most of the other negatively charged clusters, contains significant contributions from doubly charged species. Evidence for doubly charged species is found in the presence of odd-mass ions (half-integral isotopic m/z values), and in some cases, in fragmentation behavior recorded in MS/MS spectra that includes tell-tale fragment ions of greater mass/charge ratio than the selected cluster ions themselves. The signal intensity in the negative ion mode is 2−3 orders of magnitude lower than in the positive mode, even though the spray is expected to generate equal quantities of positive and negative ions. Although it is by no means unusual for negative ion mass spectra to contain much lower signals and to be less informative than positive ion spectra in other forms of mass spectrometry, such a situation makes the negative ion data a much less reliable source of information on the species present in the original solution than are the positive ion data.

The linear dynamic range and limit of detection for serine determination by sonic spray ionization using the protonated serine octamer are summarized in Table 2. As is shown in Figure 10, signal intensity goes into saturation with increasing analyte concentration. Saturation is ascribed to the ionization process itself (vide supra), since it is dependent only on the concentration, not on the nature of the amino acid. The saturation phenomenon is not caused by space-charging in the ion trap; neither is it due to detector saturation: this is shown by the fact that when the signal intensity was lowered to 10% of its saturation value by changing the capillary position, the saturation phenomenon was observed to occur at exactly the same concentration values.

Detailed examination of the ionization mechanism gives a plausible explanation for the saturation phenomenon. In a pneumatic spray method such as sonic spray, droplets are generated at the spray point, and after their formation, no further fission occurs. The droplets shrink only by solvent evaporation, and finally, a charged or uncharged molecule or nanocrystal of nonvolatile analyte remains as an end product. Thermodynamically unstable charged nanocrystals then dissociate into stable forms, many of which will occur as magic number clusters. According to this scenario, the signal intensity should increase with concentration until all the droplets formed contain enough molecules to form magic number clusters. Further increases in concentration may yield heavier clusters, which either show less fragmentation or fragment to give ions other than the stable magic number cluster. At lower concentrations, the spectrum is expected to show
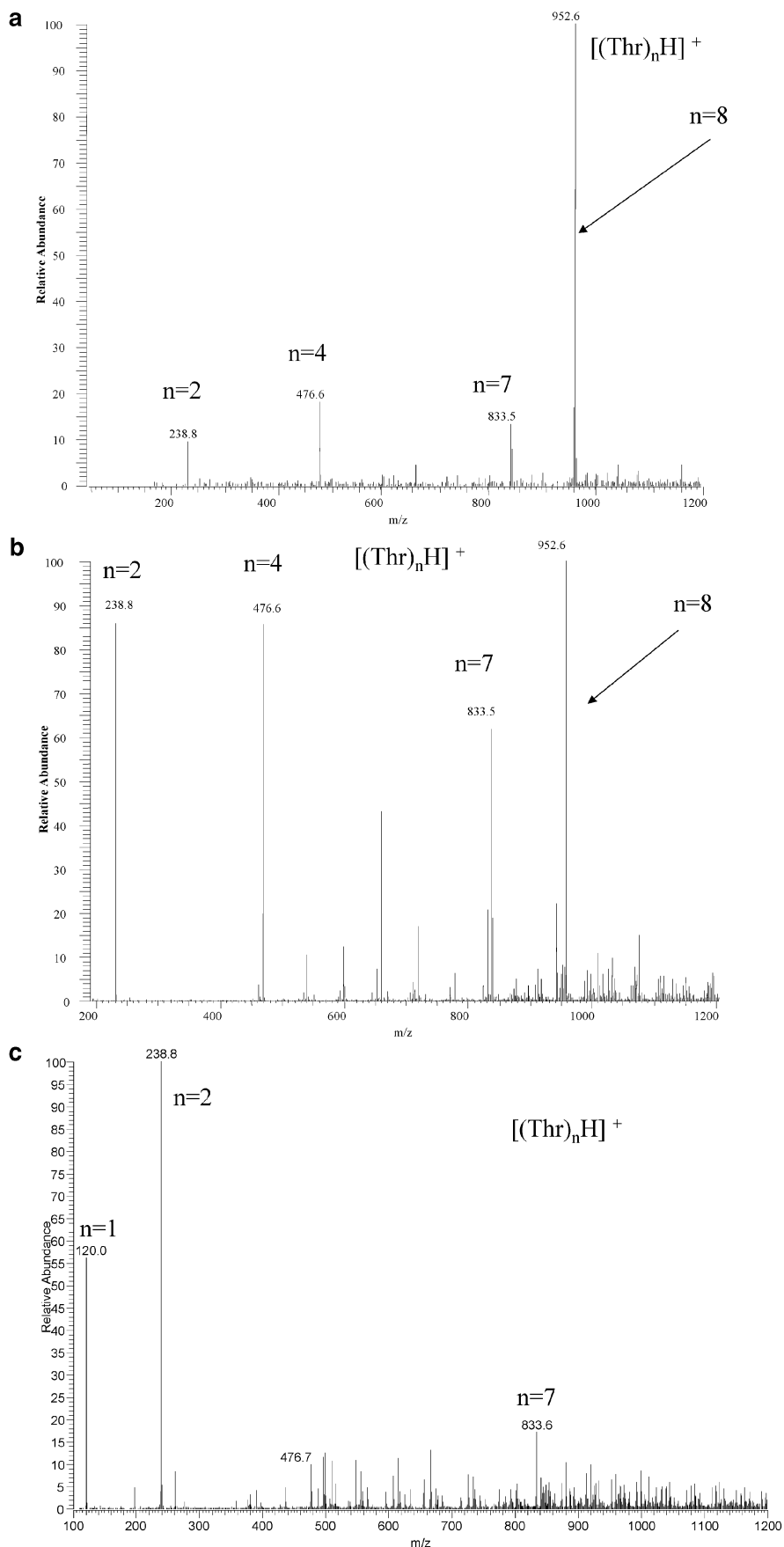
Figure 8. Positive ion sonic spray spectrum of (a) 0.2 mM (b) 10 mM L-threonine in methanol/water 1:1 and (c) positive ion electrospray spectrum of 0.2 mM L-threonine in methanol/water 1:1. As in the case of serine, the most intense peak is due to the octamer (and its metaclusters) at m/z 953; however, the simple protonated dimer (m/z 239), tetramer (m/z 477), heptamer (m/z 834), and doubly charged nonamer (m/z 655.5) also occur as abundant ions. Electrospray spectra show only intense monomer and dimer ions.
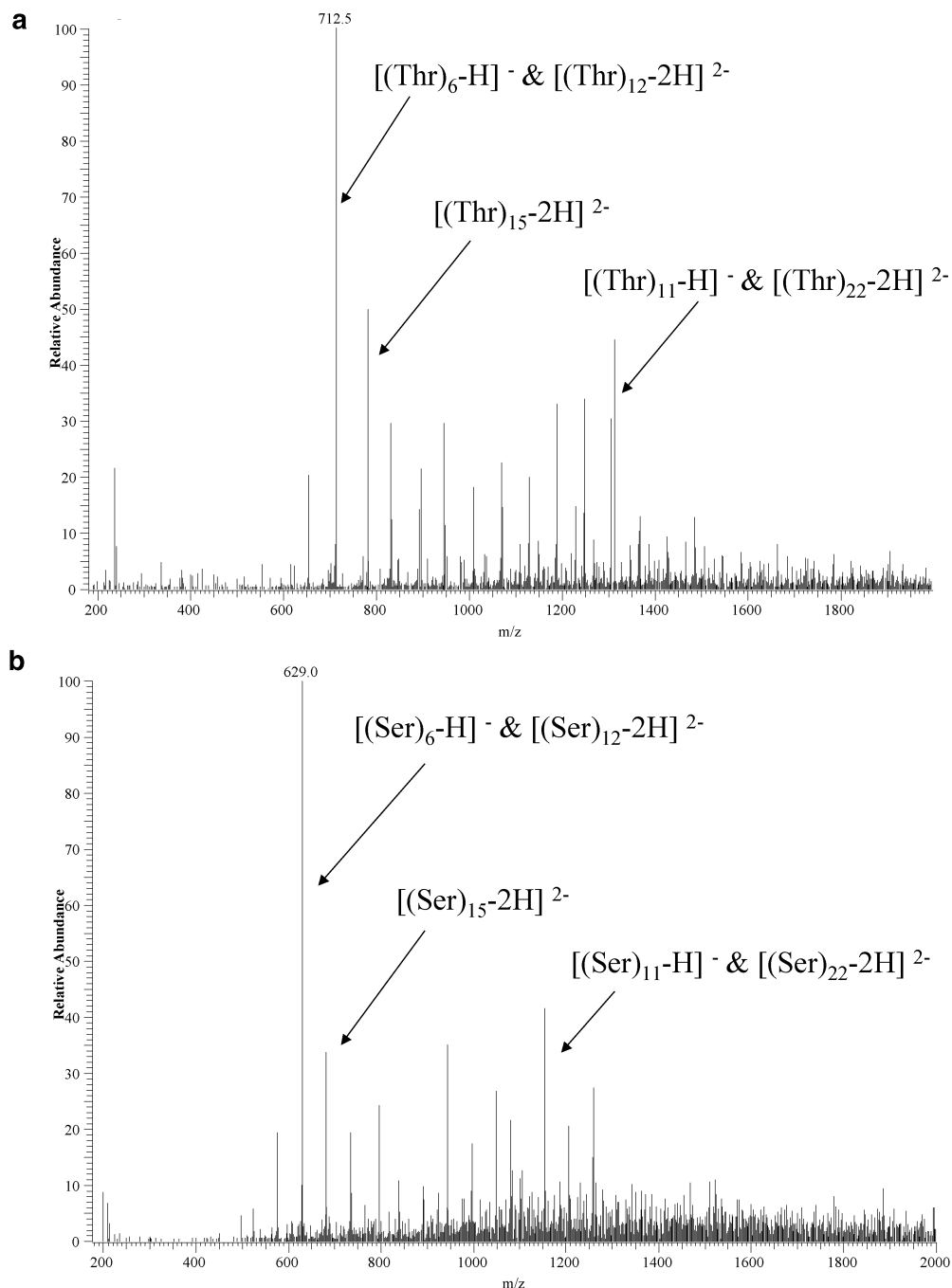
Figure 9. Negative ion sonic spray spectra of (a) 0.01 M L-threonine (b) 0.01 M L-serine in methanol/water 1:1. Sample flow rate of 5 μL/min and nebulizing gas flow of 9 L/min was applied. Heated capillary temperature was set to 80 °C.

Table 2. Analytical Performance in Sonic Spray Ionization

cluster ion	charge number[a]	linear response (M)	limit of detection (3:1 S/N ratio), M	cluster/monomer ratio
serine octamer	1	$10^{-7}-10^{-3}$	3×10^{-8}	$>4000-50$
threonine octamer	1	$10^{-6}-10^{-3}$	4×10^{-7}	$200-80$
cysteine dodecamer	2	$10^{-5}-10^{-2}$	5×10^{-6}	$100-20$
phenylalanine undecamer	2	$10^{-5}-10^{-2}$	4×10^{-6}	$150-5$

[a] Major component, since higher order metaclusters are not excluded.

protonated monomers and other low mass clusters, and this is observed.

Amino Acid Substitution: Mixed Clusters. Incorporation of other amino acids into the serine octamer has been described in recent ESI experiments.[26,27] Incorporation always occurs by substitution, and the maximum number of incorporations in ESI is generally two. There is a preference for homochirality in the cysteine/serine and asparagine/serine octamers.[27] Serine octam-
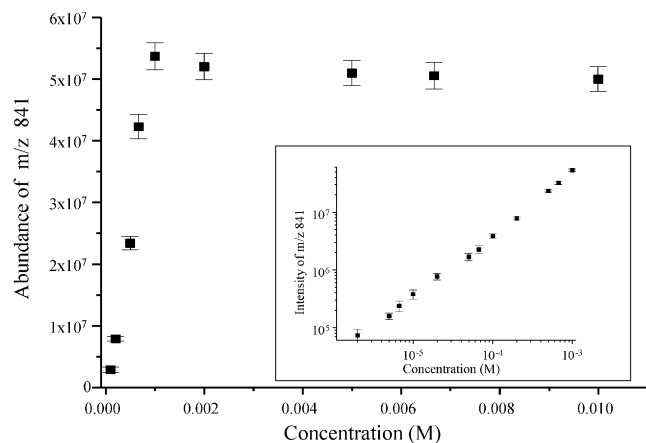
Figure 10. Calibration curves for protonated L-serine octamer. Signal intensity goes into saturation, since the maximum rate of ion formation is strictly determined by the rate of charged droplet formation. The insert shows the linear dynamic range from 10^{-6} to 10^{-3} M.

Table 3. Relative Abundance of Substituted L-serine Octamer Ions[a]

	no. of substitutions							
	1	2	3	4	5	6	7	8
amino acid								
glycine	100	81						
L-alanine	100	74						
L-leucine	89	100						
L-threonine	2	14	55	95	100	88	37	1
L-cysteine	68	100	61	43	18	10	2	1
L-phenylalanine	74	100	22	8				
L-tyrosine	100	84	12					
L-tryptophane	94	100	7	2				
L-asparagine	76	100	8					
L-histidine	100	96						
D-alanine	100	66						
D-leucine	82	100						
D-threonine	64	100	5					
D-cysteine	78	100	4					
D-phenylalanine	97	100						
D-tyrosine	100	68						
D-tryptophane	100	57						
D-asparagine	100	48						
D-histidine	100	86						

[a] 0.01M solutions (MeOH/water 1:1) of L-serine and another amino acid were mixed in a 1:1 ratio. These spectra are strongly dependent on exact spray conditions.

ers containing more than two other amino acids have not been detected by ESI, except in the case of threonine;[26] however, it is not known whether such clusters were simply not formed or formed but destroyed during the ionization process.

The results of sonic spray experiments on binary mixtures containing serine and various other amino acids are summarized in Table 3. In most experiments, two substitutions gave prominent peaks. Clusters with more than two other amino acids are also evident, especially when the amino acid chosen has the same chirality as serine. Although the chiral preferences in herocluster formation are similar to those seen in the ESI data,[27] the chiral effects are not as strong. A 1:1 mixture of L-serine and L-cysteine shows incorporation of two cysteine molecules into the serine octamer, a feature previously described using ESI;[27] however,

further incorporations were also observed in SSI. D-Cysteine showed only two major incorporations, in agreement with ESI data; however, the relative intensity of substituted clusters was considerably higher than in ESI.

A mixture of threonine and serine of the same chirality yields a series of octamer ions (8:0, 7:1, 6:2, 5:3, ..., 0:8 serine/threonine ratios) depicting a continuous transition from the protonated serine to the protonated threonine octamer. In the heterochiral case (L-serine, D-threonine) only two major incorporations were observed. In the case of amino acids with large side chain (Phe, Trp), a similar effect was observed. Incorporation of two L-phenylalanine molecules into both L- and D-serine octamer was detected; however further, minor incorporations were observed only in the homochiral case.

Differences in thermodynamic stability of amino acid clusters are emphasized more in electrospray than in sonic spray, but this is not simply the result of the gentler conditions in SSI; if that were the case, the sonic spray spectrum of serine itself would be expected to show a much greater variety of clusters. Whatever the underlying reason, substitution of multiple serine molecules by other amino acids occurs in SSI for homochiral octamers and, to a much smaller extent, for some heterochiral ones (see Table 3). Under ESI conditions, less thermodynamically stable systems are either destroyed in the ESI process or not formed; hence, the chiral effect in the first two incorporations is enhanced by discrimination against the less stable heterochiral serine/other amino acid octamers.[27]

CONCLUSIONS

A novel ion source utilizing coaxial fused-silica capillaries was constructed on the basis of the sonic spray ionization method of Hirabayashi et al.[31] and used to study fragile noncovalent complexes, specifically the clusters of amino acids. The characteristic features of the ion formation showed good agreement with Dodd's statistical charging model.[35]

The sonic spray ionization technique produces ions with less energy than ESI, a phenomenon presumably associated with the shift of the ion formation mechanism from the ion evaporation[40] to the charged residue[37−39] model. The lower internal energy allows the transmission of delicate noncovalent clusters of various molecules into the gas phase. Applying sonic spray ionization for the investigation of biochemical systems might provide even better insights than does the already powerful ESI technique in regard to macromolecular interactions in solution.

These characteristics of sonic spray ionization make it an attractive method for studying the clustering of serine, a topic with potential implications for the early stages of chemical evolution.[22,23,25−27] Abundant cluster formation was observed, as expected, but the *exclusive* formation of the octameric species (and its metaclusters of the same mass/charge ratio) is a remarkable and incompletely understood result. Certainly this observation provides additional evidence for the remarkable stability of the serine octamer. Despite the fact that clustering is promoted in the sonic spray experiment and that cluster dissociation in the course of ion acceleration in the mass analyzer must be possible, conditions can be found in which pure octameric clusters are generated as gas-phase ions. Although we cannnot exclude the

possibility that octamer formation occurs in the course of ionization, the possibility that octameric serine clusters are present in the *nascent* solution must be entertained and further tested in future experiments.

A general conclusion of this study is that sonic spray provides information about solution-phase clustering behavior of relatively small molecules that is complementary to electrospray. The ions studied in sonic spray do not undergo energetic processes, such as ion evaporation, which is experienced in electrospray. Using sonic spray, threonine shows clustering that is highly similar to, although somewhat less abundant than, that of serine. This result, together with those of amino acid substitution experiments, strongly implies that these two amino acids form similar, homochiral octameric structures.