Homochiral Preference of Serine Octamer in Solution and Formed by Dissociation of Large Gaseous Clusters

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Complete List of Authors:	Jordan, Jacob; University of California Berkeley, Department of Chemistry Williams, Evan; University of California, Berkeley,

Homochiral Preference of Serine Octamer in Solution and Formed by Dissociation of Large Gaseous Clusters

Running Title: Homochiral Preference of Protonated Serine Octamer

Jacob S. Jordan and Evan R. Williams*

Department of Chemistry, University of California, Berkeley, CA 94720

*To whom correspondence should be addressed

e-mail: erw@berkeley.edu

Abstract

The ability of electrospray emitters with submicron tip diameters to significantly reduce and even eliminate aggregation of analyte molecules that can occur inside evaporating droplets was recently demonstrated to show that serine octamer exists in bulk solution, albeit in low abundance. Results using 222 nm emitter tips for D-serine and deuterium labeled L-serine show that the serine octamer that exists in 100 μM solution has a strong homochiral preference. Dissociation of large multiply protonated clusters results in formation of protonated octamer through a doubly protonated decamer intermediate. Remarkably, dissociation of the doubly protonated decamer from solution, which has a *heterochiral* preference, results in protonated octamer with strong *homochiral* preference. This homochiral preference is higher when protonated octamer is formed from larger clusters and approaches the chiral preference of the octamer in solution. These results show that the doubly protonated decamer has a different structure when formed from solution than when formed by dissociation of larger clusters. These results indicate that the unusually high abundance of protonated homochiral octamer that has been reported previously can be largely attributed to aggregation of serine that occurs in rapidly evaporating electrospray droplets and from dissociation of large clusters that form abundant protonated octamer at an optimized effective temperature.

Introduction

Protonated serine octamer has been widely studied since the initial discoveries of its strong homochiral preference and unusually high abundance in electrospray ionization (ESI) mass spectra.[1–5] These remarkable characteristics have been observed from a variety of different spray ionization methods,[6–10] from vaporization of serine during rapid solvent evaporation[11] and by pyrolysis,[12] leading to the suggestion that this complex may have played a role in homochirogenesis.[1,3,4,7,11–16] The structures of the protonated octamer and of related complexes have been extensively investigated.[1–4,7,9,10,15–27] The homochiral preference was reported to be the result of 3-point hydrogen bonding interactions between six of the constituent serine molecules.[26] The side-chain hydroxyl groups of the remaining two molecules are not involved in hydrogen bonding within the octamer, making them exchangeable with serine molecules of different chirality.[26] Neutral serine octamer is also formed by spray ionization[28] and by condensation of sublimated serine in the gas phase.[29] Both the protonated and neutral forms of the octamer have similar homochiral preferences, suggesting that their structures may be related.[28,29]

The homochiral preference of protonated serine octamer formed from racemic solutions has been investigated using deuterium labeled L-serine in order to distinguish the two enantiomeric forms of the amino acid by mass.[3–5,8–10,13–15,21,22,27–31] Homochiral 8D:0L and 0D:8L cluster compositions are much more abundant than expected from a binomial distribution whereas the more heterochiral 5D:3L, 4D:4L, and 3D:5L compositions are significantly lower in abundance.[1,3,5,9,11,29–31] This indicates that these clusters are not formed by statistical aggregation of serine molecules. The chiral preference of other serine clusters has also been investigated.[9,29,31] Protonated trimer (3^+), 6^+, and doubly protonated 8^{2+} - 11^{2+} serine clusters have a heterochiral preference, where mixed compositions, such as 3D:3L for the hexamer, are more abundant than expected from a binomial distribution.[29,31]

Beauchamp and coworkers characterized the homochiral preference of serine clusters using the ratio of the ion abundance to the abundance expected from a binomial distribution.[3] These data indicate that the pure homochiral forms of the protonated octamer were 15x more abundant than expected. Similarly, Nanita *et al.* introduced the "magnitude of chiral preference" (M_{cp}) as a measure of the chiral preference of a cluster.[8,29] The M_{cp} is defined as the observed probability of a cluster composition divided by the theoretical probability of a cluster composition predicted by a binomial distribution and normalized to one. Thus, the M_{cp} is similar to the ratio used by Beauchamp and coworkers but normalized such that all compositions sum to one. A plot of the M_{cp} or the ratio of the observed to statistical intensities versus the cluster composition results in a "V"-shaped curve for protonated octamer, characteristic of a homochiral preference.[3,29,31] In contrast, heterochiral species result in an inverted "V"-shaped plot and clusters with no chiral preference are characterized by a flat plot.[31]

Whether serine octamer exists *in solution* has been extensively debated. Results from NMR and IR spectroscopy experiments showed no evidence for any serine clusters in solution indicating that its high abundance in spray ionization mass spectra is likely a result of aggregation inside evaporating electrospray droplets.[32] However, recent data using small emitter tips indicate that protonated octamer does exist in solution, albeit in low abundance.[33] In these experiments, small emitter tips and low serine concentrations were used such that there is, on average, one or zero analyte molecules present in electrospray droplets that are initially formed. Under these conditions, aggregation inside an electrospray droplet should not occur to a significant extent, yet a variety of serine clusters were observed, indicating that the octamer and other clusters exist in solution. These data also indicate that the unusually high abundance of protonated octamer observed in many prior studies, in which significantly larger emitters were used, results from aggregation within ESI droplets. Solvent evaporation leads to smaller droplets and more concentrated serine in the remaining solution, enhancing aggregation during the ESI process. However, the chiral preference of the octamer that exists in solution prior to electrospray ionization has not been reported.

Protonated octamer can also be formed by dissociation of larger serine clusters in the gas phase.[23,24,34] Dissociation of multiply protonated serine clusters with between 10 and 37 serine molecules produces protonated octamer as the most abundant cluster consisting of three or more serine molecules at an optimum dissociation energy through charge loss and neutral evaporation processes.[34] Thus, protonated octamer can also be a significant magic number cluster in mass spectra where larger clusters are dissociated in the gas phase. Doubly protonated decamer was found to be the critical precursor to formation of protonated octamer in the gas phase through charge separation to form the corresponding protonated dimer. Formation of clusters containing up to 600 serine molecules was reported with sonic spray ionization[9] and these clusters are expected to dissociate under source conditions optimized to produce abundant protonated octamer.[34] Spencer *et al*. reported that the protonated octamer formed by dissociation of large clusters from racemic solutions does not display a homochiral preference, but the precursor identities and collision energies were not reported.[24] The dimer to octamer abundance ratio was used to deduce the extent of homochiral preference in these experiments instead of isotopically labeled enantiomers.

The heterochiral preference of the doubly protonated decamer, the critical intermediate for formation of protonated octamer by gaseous dissociation of larger clusters, seems to be consistent with the conclusion that protonated octamer formed by gaseous dissociation of larger clusters does not have a significant homochiral preference. Yet the dominant abundance and homochiral preference of protonated octamer in many previous experiments where large clusters are likely formed due to aggregation and dissociated warrants further investigation into the chiral preference of protonated octamer formed both in solution prior to droplet formation and by dissociation of large clusters.

Materials and Methods

Nanoelectrospray ionization (nESI) emitters with diameters of 222 ± 8 nm and 2.4 ± 0.04 μm were pulled from borosilicate capillaries (1.0 mm outer diameter, 0.78 mm inner diameter, Sutter Instruments, Novato, CA) using a Flaming/Brown P-87 micropipette puller (Sutter Instruments). The tip puller parameters used to produce these emitters are given in Table S1. The inner diameters of the emitters were measured using a Hitachi TM-1000 scanning electron microscope (Schaumberg, IL) in the Electron Microscopy Lab at the University of California, Berkeley. Four replicate tips were pulled in order to measure the standard deviation of the tip diameters.

Serine cluster ions were formed from a 50% D-serine (Sigma Aldrich, St. Louis, MI, 98% purity) and 50% 2,3,3-d_3-L-serine (Cambridge Isotopes Labs, Andover, MA, 98% purity) solution at a total serine concentration of 10 mM or 100 μM in 49.95:49.95:0.1 water:methanol:acetic acid (Sigma Aldrich). Mass spectra were acquired between 50 – 2000 *m*/*z* using a Waters Q-TOF Premier quadrupole time-of-flight mass spectrometer (Waters Corporation, Milford, MA). Electrospray was initiated by applying a voltage of 0.4 – 1.0 kV to a 0.127 mm platinum wire inserted into the capillary and in contact with the solution. The spray voltage was increased until stable spray was achieved and the spray was allowed to reach a steady state for one minute prior to data acquisition. Instrument conditions were optimized to produce abundant protonated serine octamer. The sampling cone, extraction cone, and ion guide voltages were 20 V, 2.0 V, and 2.0 V, respectively. Unless otherwise noted, the collision cell entrance and exit potentials were 0 V and -10 V, respectively, with an argon gas flow rate of 0.35 mL/min, resulting in a pressure of ~4.2×10^{-3} mbar in the collision cell. Data were analyzed using MassLynx V4.1.

Mass selection for collision induced dissociation experiments was done with a ~30 *m/z* window tuned to include all constituents of a given cluster size while preventing transmission of neighboring cluster ions. The collision voltage was varied between 0 and 60 V and spectra were averaged for 1 min. The charges and abundances of clusters were determined from the isotopic distributions.

Mass isolation of the individual isotopically labeled forms of the protonated decamer was performed under conditions that minimized ion activation with a *m/z* = 3 isolation window. The gas flow rate was 0.01 mL/min, resulting in a pressure of 5.4×10^{-5} mbar in the collision cell region. Data was averaged for 15 min for each cluster composition. To measure the change in the chiral preference of clusters with increasing collision voltage, the collision voltage was increased until the precursor abundance was reduced by over 70%. The gas flow rate was tuned between 0.01 mL/min and 0.1 mL/min to produce minimal activation at 0 V collision potential while still being able to acquire sufficient signal for the dissociation products at higher collision energies.

Results and Discussion

Chiral Preference of Protonated Serine Clusters in Solution

A nanoESI mass spectrum of a solution containing D-serine and 2,3,3-d_3-L-serine at 50 μM each in 49.95:49.95:0.1 water:methanol:acetic acid is shown in Figure 1a. Numerous protonated clusters, including an octamer, are observed. The highest mass cluster with an abundance >0.01% relative to the protonated monomer is 30^{3+}. An electrospray emitter with a 222 nm diameter tip was used in order to minimize any cluster formation due to aggregation inside a rapidly evaporating electrospray droplet. The initial droplet size produced from these emitters is estimated to be roughly 1/17 of the emitter tip diameter[35,36] corresponding to droplet diameters of ~13 nm. At a 100 μM total analyte concentration, only one out of every 14 initially formed ESI droplets is expected to contain an analyte molecule. When the average number of analyte molecules per droplet is significantly less than one, clusters observed in a mass spectrum should reflect their existence in the bulk solution.[33,37] Thus, these results indicate that clusters with up to ~30 serine molecules exist *in solution* and are not produced by aggregation within the droplet.

At 10 mM, where on average there are more than one analyte molecules per droplet, the largest clusters observed at >0.01% relative abundance from 222 nm and 2.4 μm diameter emitters were 75^{5+} and 85^{5+}, respectively. The larger clusters formed with larger emitter diameters is consistent with some aggregation occurring within the ESI droplets. The large clusters formed with the smaller emitters at this higher concentration could be due to a shift in the solution-phase equilibrium that favors formation of higher order clusters at higher serine concentrations, although aggregation inside the droplets may also occur.[33] The similar maximum cluster size using the 222 nm and 2.4 μm emitter tips, where droplets are expected to contain approximately 7 and ~9200 analyte molecules on average, indicates that most of these larger clusters are likely present in solution because of a shift in equilibrium owing to higher serine concentration.

The minimum cluster sizes for the doubly and triply protonated clusters are 8 and 21, respectively, the same as those from enantiopure solutions, suggesting that the presence of an enantiomer and mixed clusters does not significantly affect cluster charging.[33] The abundances of clusters formed from 100 μM solutions are significantly lower than those formed from 10 mM, similar to trends from enantiopure solutions.[33] This result is consistent with a shift in the equilibrium that makes clusters less favorable at lower serine concentrations. The protonated octamer is a magic number cluster, but the relative abundance of any single form of the octamer is significantly lower in spectra acquired from racemic solutions compared to enantiopure solutions consistent with previous reports.[1,22,23] This is primarily due to splitting of the octamer signal into nine different compositions. The population abundance of the protonated octamer from enantiopure solutions is ~1.2 ± 0.6 % compared to ~0.8 ± 0.2 % (all protonated octameric forms) from racemic solutions. These numbers are approximate because effects of m/z dependent ion transmission and detection efficiency are not taken into account. The similar population abundances indicate that the presence of another enantiomer in solution does not significantly affect the formation of the protonated octamer. The population abundance of all other clusters is higher from racemic solutions than from enantiopure solutions, similar to results reported by Julian *et al*.[30]

The extent of chiral preference of a cluster is determined by modeling the cluster composition as a binomial distribution and comparing the simulated and experimental data.[3,8,29] The ratio of the observed abundance to that expected from a statistical distribution, or enhancement factor (EF), and the magnitude of chiral preference (M_{cp}) can be used to measure the degree of chiral preference. An EF of greater than one indicates that a particular cluster composition is preferred, a value of less than one indicates a disfavored composition, and a value of one indicates no preference, i.e., a statistical distribution of constituents.[3] Chiral preferences are reported in both EF and M_{cp} in order to compare results to prior literature. The protonated octamer is the only cluster with resolvable isotope peaks that has a homochiral preference. The 3^+, 6^+ and 8^{2+} - 11^{2+} clusters have resolvable isotope peaks and these clusters have heterochiral preference. All other clusters, including the 2^+, 4^+, 5^+, and 7^+ have no chiral preference. These results are in excellent agreement with those reported previously.[3,29,31]

There is a strong preference for homochirality of the octamer in solution. The EF value for the 8D form of the octamer is ~16.7 ± 0.9 (M_{cp} = 0.37 ± 0.03; Figure 1b). This is slightly higher than the value of ~15 (calculated M_{cp} = 0.39) reported by Beauchamp and co-workers. Cooks and co-workers have reported M_{cp} values between 0.33 and ~0.4 from which we compute an average value of 0.36 ± 0.03 from these prior reports.[8,10,28,29] This value does not take into account any differences in measurement uncertainty in the individual studies. Thus, the value of the homochiral preference of serine octamer in solution is among the highest values previously reported in mass spectral data. In contrast, the doubly protonated decamer has EF values slightly greater than one for 4D:6L, 5D:5L and 6D:4L forms and values as low as 0.3 for more homochiral forms (Figure 1c). This pattern indicates a structure or structures where heterochirality is significantly preferred. The heterochiral preference of 8^{2+} could indicate a different form of protonated octamer in solution, or it could be formed by loss of serine molecules from higher order doubly protonated clusters, such as the 10^{2+} that have a heterochiral preference.

Different extents of sodium adduction to clusters formed with small and large emitters provide support for our conclusion that aggregation does not occur to a significant extent with the smaller emitters at 100 μM concentration. With 2.4 μm emitters and 10 mM solution, ~12%

of the dimer population is sodiated (Figure S1a). A similar result is obtained with 222 nm emitters, where initial droplets contain multiple analyte molecules (Figure S1b). However, less than ~0.9% of the dimer population that is formed from 100 μM serine solution is sodiated with 222 nm emitters (Figure S1c). These data are consistent with a reduction in aggregation within electrospray droplets with smaller tip sizes and concentrations and provide further evidence that the clusters observed in mass spectra under these conditions are not formed during the electrospray process.

Homochirality Emerges from Dissociation of Heterochiral Precursors

Racemic serine clusters consisting of between 8 and 37 serine molecules with between one and three protons were collisionally activated to investigate the dissociation products, pathways, and minimum cluster sizes for each charge state as a function of precursor cluster size. Loss of neutral serine molecules and/or charge separation to produce predominantly protonated dimer and the corresponding fragment ion occurs to various extents depending on cluster size and charge. Charge separation is increasingly favored as the cluster size approaches the minimum cluster size for each charge series. The minimum cluster size observed for the doubly and triply charged series was 8^{2+} and 21^{3+} respectively. The dissociation pathways for clusters produced from a racemic solution are the same as those for clusters from enantiopure solutions and are described as a function of cluster size in detail elsewhere.[34]

The protonated octamer is produced by CID of serine clusters consisting of 10 – 37 serine molecules at sufficient collision energies. Activation of 8^{2+} and 9^{2+} results in no protonated octamer. There are no singly protonated ions larger than the octamer. This indicates that the doubly protonated decamer is the smallest precursor to form protonated octamer by charge separation of a protonated dimer, in agreement with results from enantiopure cluster dissociation.[34] Results for protonated octamer formed by CID of 10^{2+} are shown in Figure 2a,b. The EF/M_{cp} values do not depend on collision voltage within a range of 0 – 10 V. As can be inferred from the “V” shape of the EF/M_{cp} plot, the protonated octamer has a significant *homochiral* preference (Figure 2b) despite being formed from a precursor with a *heterochiral* preference (Figure 1c). The magnitude of homochiral preference of the protonated octamer formed by dissociation is lower than that of the protonated octamer formed from solution (Figures 1b and 2b for protonated octamer formed in solution and by CID of 10^{2+}, respectively). The maximum EF value (3.94, M_{cp} value = 0.25) occurs for the 7D:1L composition of the protonated octamer formed by CID of 10^{2+} compared to the stronger preference for a purely homochiral 8D octamer in solution (EF = 16.7, M_{cp} = 0.37).

Homochiral Enrichment of Protonated Octamer From Dissociation of 10^{2+}

To gain insight into how dissociation of a *heterochiral* precursor leads to protonated octamer with a strong *homochiral* preference, constituents of the doubly protonated decamer were isolated and dissociated individually. Dissociation of the 5D:5L form of 10^{2+} produces protonated octamer that is composed of 3D:5L (19%), 4D:4L (32%), and 5D:3L (20%) and protonated dimers in roughly corresponding abundances (Figure 3a). These data are consistent with statistical formation of protonated dimer and protonated octamer that does not show a significant homochiral preference. The presence of 6D:2L and 2D:6L in the protonated octamer distribution reflects the low resolution used for precursor selection in order to acquire adequate signal under very gentle dissociation conditions and the high abundances of adjacent 6D:4L and 4D:6L forms of 10^{2+}.

Dissociation of 4D:6L results in preferential loss of protonated 2D:0L to form a protonated 2D:6L octamer with an EF of 3.3 (Figure 3b). The corresponding 2D:0L dimer has an EF of ~2. These results indicate that there is a strong homochiral enrichment when a slightly homochiral form of the doubly protonated decamer complex dissociates.

Dissociation of 2D:8L results in the formation of 0D:8L (5%), 1D:7L (52%) and 2D:6L (43%). Both 1D:7L and 2D:6L have an EF of ~1.5 but 0D:8L has an EF ~0.3 indicating that loss of a protonated D homodimer is unfavored. The protonated heterodimer is significantly more abundant than expected. This is likely due to interference as a result of sequential serine loss from protonated pentamer, which overlaps in *m*/*z* with the precursor. Differences in binding energies for the different enantiomeric forms of the protonated dimer that can dissociate to protonated monomer could contribute to this as well. The abundances of the protonated dimer are roughly consistent with the trends in the corresponding protonated octamer. These data indicate that the strong homochiral preference of the protonated octamer formed from dissociation of 10^{2+} from racemic solutions is primarily due to dissociation of clusters with a disproportionate number of D and L forms of serine. Even though 10^{2+} has a heterochiral preference when formed from solution, the majority of this cluster composition has differing numbers of D and L serine. It is these clusters that have an intrinsic bias that results in the homochiral enhancement of the protonated octamer upon dissociation. For larger clusters, the proportion of clusters that have the same number of the two forms of serine decreases relative to the cluster population, suggesting that protonated octamers formed by dissociation of even larger clusters may lead to greater homochiral enhancement.

Homochiral Enrichment of Protonated Octamer Formed by CID of Large Clusters

Dissociation of large serine clusters to produce protonated octamer occurs primarily through a 10^{2+} intermediate. In order to investigate the extent to which dissociation of larger clusters leads to a chiral preference of product ions, clusters up to 37^{3+} were dissociated at sufficient collision energies to produce protonated octamer. A wide *m*/*z* window was used for precursor isolation to ensure that the majority of cluster compositions for a given cluster size were activated. Precursor ions were chosen to avoid interferences from other ions. Dissociation of 17^{2+}, 19^{2+}, 23^{3+}, 29^{3+}, and 37^{3+} all lead to protonated octamer with significant homochirality despite the fact that these initial precursor clusters do not have a homochiral preference (Figure S2). For example, protonated octamer produced by dissociation of 37^{3+} (Figure 2c,d) has a strong homochiral preference with an EF value of ~10.1 ($M_{cp} = 0.30$) for the pure enantiomeric 8D form. This extent of homochiral preference is significantly closer to that of the protonated octamer formed directly from solution (Figure 1b) than when protonated octamer is formed by dissociation of 10^{2+} formed directly from solution (Figure 2b). The chiral preference of the 10^{2+} that is formed from solution and that formed by dissociation of larger clusters is remarkably similar (Figures 1c and S3). Because the octamer formed by dissociation passes through a decamer intermediate, this suggests that 10^{2+} formed from solution has a different structure than this same ion formed by dissociation of larger clusters.

Because larger clusters require higher collision energies to produce the octamer, the effect of collision energy on the chiral selectivity of the protonated octamer was investigated. To investigate effects of collision energy, dissociation spectra for all of the larger clusters were measured as a function of increasing collision voltage until no protonated octamer remained. There was no significant change in the chiral preference of the protonated octamer with collision voltage. The absence of a significant change in the chiral preference of the octamer formed by dissociation of the doubly protonated decamer at different collision energies indicates that the chiral enhancement of the protonated octamer is not due to differences in stabilities of the different forms of the doubly protonated decamer.

The chiral preference of the doubly protonated decamer from dissociation of larger clusters does not change significantly with cluster size (Figure S3) and is similar to that from solution. For example, dissociation of 37^{3+} shows that the 5D:5L decamer formed in the gas phase has nearly the same magnitude of heterochiral preference as the 5D:5L decamer from solution (EF = 1.07; $M_{cp} = 0.16$ and EF = 1.07; $M_{cp} = 0.15$, respectively) (Figures 1c and S3). Similarly, a comparison of the protonated octamer chiral preference with increasing precursor cluster size reveals no obvious trend for clusters larger than 10^{2+} (Figure S2). Interestingly, the EF of the homochiral octamers produced by dissociation of the 37^{3+} (10.1, $M_{cp} = 0.30$) is ~60% of the magnitude of the homochiral octamers produced directly from solution (16.7, $M_{cp} = 0.37$). Although the homochiral preference of protonated octamer formed in solution and by dissociation of 10^{2+} is significantly different, this difference is much smaller for protonated octamer produced by dissociation of larger multiply protonated serine clusters. Larger clusters of serine dissociate into the octamer under a wide range of experimental conditions often used in studies of amino acid clustering and our results indicate that both clusters formed by aggregation in droplets and by gas-phase dissociation of larger clusters likely contribute to the large abundance of homochiral protonated octamer observed in prior reports.

Our findings that dissociation of larger serine clusters produces protonated octamer that has a strong homochiral preference is in striking contrast to a prior report by Spencer *et al.* who concluded that the octamer generated by successive dissociation of serine clusters in the gas-phase has no preference for homochirality.[24] In this study, homochiral preference was inferred from the relative abundances of the protonated octamer and the protonated dimer formed from enantiopure and racemic mixtures without a deuterium label. No change was observed in the octamer/dimer ratio when the octamer was produced by gas-phase dissociation from solutions of enantiopure and racemic mixtures. This is likely a result of similar dissociation energies for the enantiomeric forms of the clusters, consistent with our findings that there is no significant energy dependence to the enantiomeric preference of the protonated octamer formed by dissociation of larger multiply protonated clusters.

Conclusions

Prior results using electrospray emitters that have submicron diameter tips showed that protonated octamer exists in solution, albeit in low abundance.[33] Extending these measurements here, we show that the octamer that exists *in solution has a strong homochiral preference*. Thus, the strong homochiral preference of the octamer is an intrinsic property of serine in solution and is not solely the result of rapid aggregation in droplets or a reflection of differences in gas-phase stabilities. Protonated octamer can also be formed by aggregation that occurs inside an electrospray droplet and by gaseous dissociation of larger serine clusters.[33,34] Dissociation of larger clusters consisting of mixed L and D forms of serine to form protonated octamer follows the same mechanisms observed for enantiopure clusters and results in abundant protonated octamer that has a strong homochiral preference.

A surprising result of this study is that the doubly protonated decamer formed from solution has a heterochiral preference, yet dissociation of this cluster results in a protonated octamer with a homochiral preference. This homochiral selectivity upon dissociation is a result of the decamer population that has a greater abundance of one chiral form of serine. Dissociation of larger clusters leads to an even higher chiral selectivity in the protonated octamer that is formed. The homochiral preference of the protonated octamer formed by larger clusters is close to that of serine octamer that exists in solution.

These results support the hypothesis that the high abundance of protonated octamer observed in many prior experiments using spray ionization methods with large emitter tips is due to aggregation that occurs inside the electrospray droplets. The presence of protonated octamer as a magic number cluster is further enhanced by gas-phase dissociation of large clusters, which produce protonated octamer as the most abundant cluster (except protonated dimer) at a normalized collision energy, i.e., temperature.[34]

The chiral preference of protonated octamer is different in solution than it is when the protonated octamer is formed by dissociation of the doubly protonated decamer that is also formed from solution. Dissociation of larger multiply protonated clusters leads to a protonated octamer with a homochiral preference that is only slightly less than that of the octamer in solution. These results indicate that the decamer in solution and that formed by dissociation have different structures, providing further evidence for the existence of clusters larger than the octamer in solution. Structural differences in these ions may be identified using ion mobility or ion spectroscopy, which may provide insights into how homochiral enhancement of the protonated octamer formed by dissociation occurs. These results also indicate that the magnitude of chiral preference of the protonated octamer may be used to distinguish octamer that exists or is formed in solution from that formed in the gas-phase by dissociation of larger clusters.

Conflicts of Interest

There are no conflicts to declare.

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Figures:

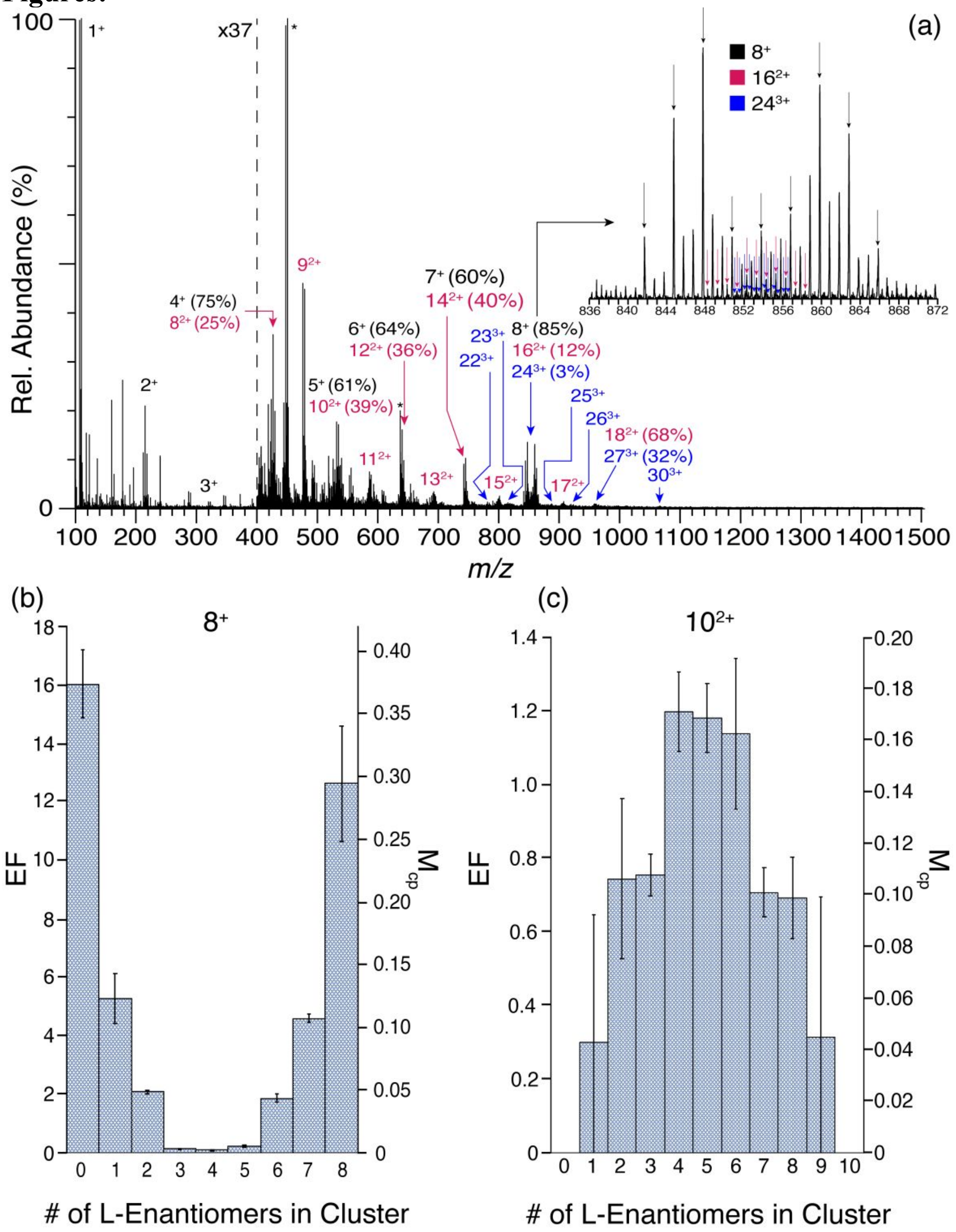

Figure 1. Electrospray mass spectrum of a solution consisting of a) an equimolar concentration of D-serine and 2,3,3-d_3-L-serine at 50 µM each in 49.95:49.95:0.1 water:methanol:acetic acid with an expansion of the various forms of protonated octamer inset, and chiral enhancement factors and magnitude of chiral preference for b) protonated octamer showing strong homochiral preference and c) doubly protonated decamer showing heterochiral preference. Signal for 16^{2+} and 24^{3+} overlaps that of the protonated octamer but can be deconvolved based on their isotopic signatures. The 30^{3+} ion is the largest cluster observed (S/N ~5) that has both a resolved isotope distribution and an abundance greater than 0.01% of the monomer abundance. Asterisks denote PDMS contamination peaks.

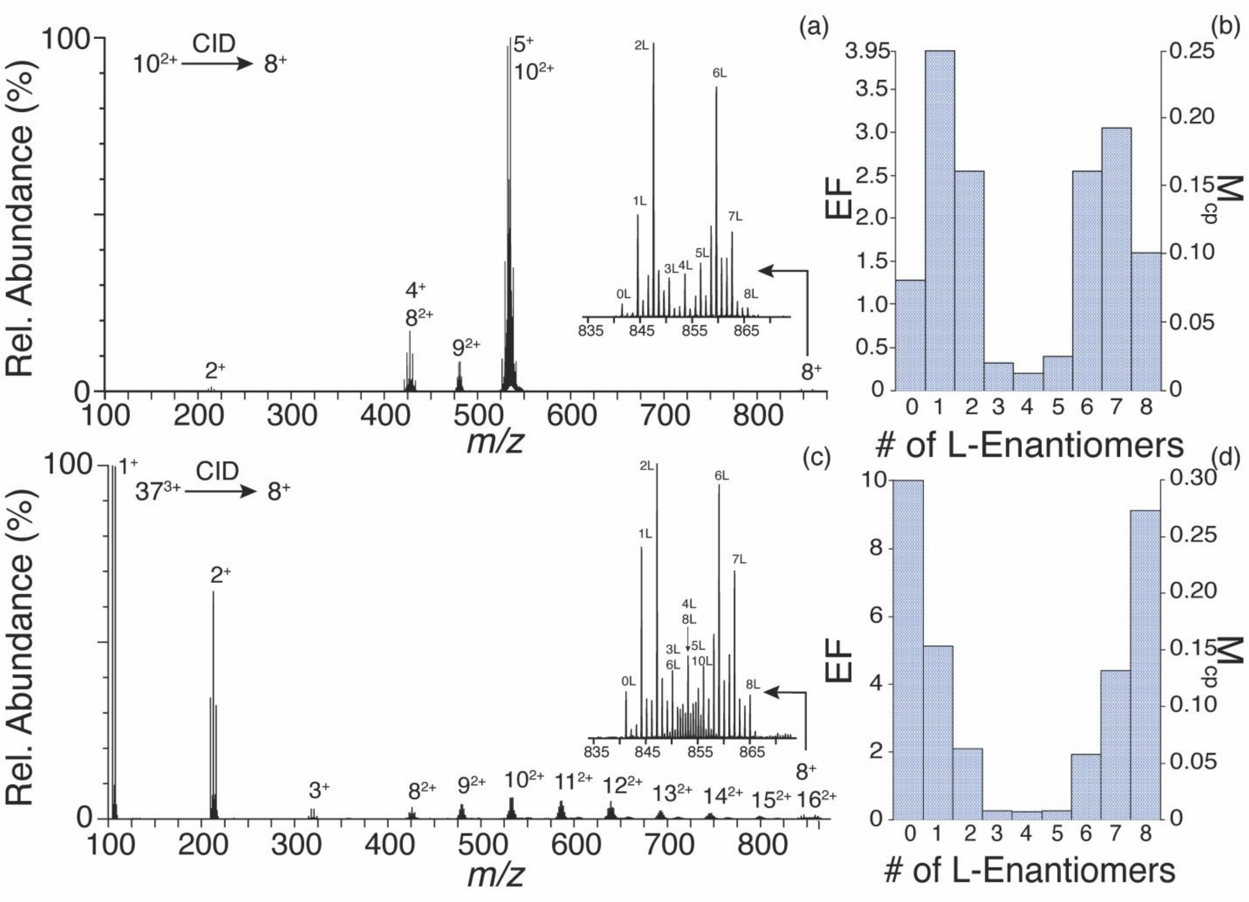

Figure 2. Dissociation of a) serine 10^{2+} and c) serine 37^{3+} produces protonated octamer with distinct homochiral chiral preferences (b and d, respectively), with that formed by dissociation of the heterochiral 10^{2+} (b) having lower homochiral preference than that formed from the 37^{3+} (d).

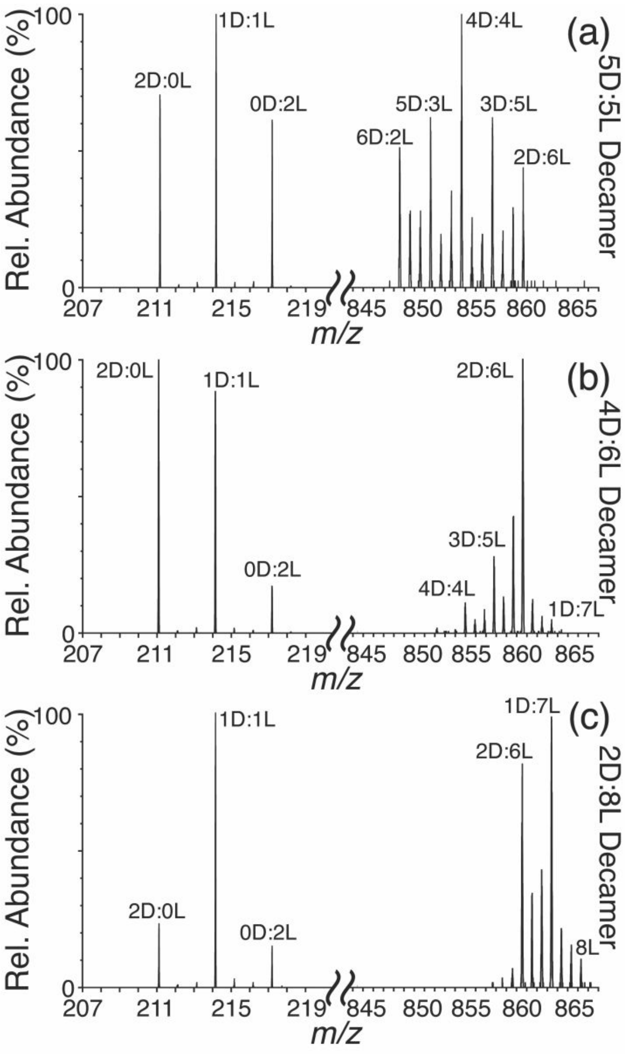

Figure 3. Dissociation of different chiral forms of doubly protonated decamer consisting of a) 5D:5L, b) 4D:6L, and c) 2D:8L; regions around the protonated dimer and protonated octamer are shown.

References

1 R. G. Cooks, D. Zhang, K. J. Koch, F. C. Gozzo and M. N. Eberlin, *Anal. Chem.*, 2001, **73**, 3646–3655.

2 A. E. Counterman and D. E. Clemmer, *J. Phys. Chem. B*, 2001, **105**, 8092–8096.

3 R. R. Julian, R. Hodyss, B. Kinnear, M. F. Jarrold and J. L. Beauchamp, *J. Phys. Chem. B*, 2002, **106**, 1219–1228.

4 C. A. Schalley and P. Weis, *Int. J. Mass Spectrom.*, 2002, **221**, 9–19.

5 R. Hodyss, R. R. Julian and J. L. Beauchamp, *Chirality*, 2001, **13**, 703–706.

6 P. Nemes, G. Schlosser and K. Vékey, *J. Mass Spectrom.*, 2005, **40**, 43–49.

7 Z. Takats, S. C. Nanita, R. G. Cooks, G. Schlosser and K. Vekey, *Anal. Chem.*, 2003, **75**, 1514–1523.

8 S. C. Nanita, Z. Takats, R. G. Cooks, S. Myung and D. E. Clemmer, *J. Am. Soc. Mass Spectrom.*, 2004, **15**, 1360–1365.

9 S. Myung, R. R. Julian, S. C. Nanita, R. G. Cooks and D. E. Clemmer, *J. Phys. Chem. B*, 2004, **108**, 6105–6111.

10 S. C. Nanita, E. Sokol and R. G. Cooks, *J. Am. Soc. Mass Spectrom.*, 2007, **18**, 856–868.

11 P. Yang, R. Xu, S. C. Nanita and R. G. Cooks, *J. Am. Chem. Soc.*, 2006, **128**, 17074–17086.

12 Z. Takáts and R. G. Cooks, *Chem. Commun.*, 2004, 444–445.

13 S. C. Nanita and R. G. Cooks, *Angew. Chemie Int. Ed.*, 2006, **45**, 554–569.

14 Z. Takats, S. C. Nanita and R. G. Cooks, *Angew. Chemie Int. Ed.*, 2003, **42**, 3521–3523.

15 F. X. Sunahori, G. Yang, E. N. Kitova, J. S. Klassen and Y. Xu, *Phys. Chem. Chem. Phys.*, 2013, **15**, 1873–1886.

16 K. J. Koch, D. Zhang, R. G. Cooks, F. C. Gozzo and M. N. Eberlin, *Chem. Commun.*, 2001, **1**, 1854–1855.

17 S. Gronert, R. A. J. O'Hair and A. E. Fagin, *Chem. Commun.*, 2004, 1944–1945.

18 U. Mazurek, O. Geller, C. Lifshitz, M. A. McFarland, A. G. Marshall and B. G. Reuben, *J. Phys. Chem. A*, 2005, **109**, 2107–2112.

19 J. Ren, Y.-Y. Wang, R.-X. Feng and X.-L. Kong, *Chinese Chem. Lett.*, 2017, **28**, 537–540.

20 G. Liao, Y. Yang and X. Kong, *Phys. Chem. Chem. Phys.*, 2014, **16**, 1554–1558.

21 A. B. Costa and R. G. Cooks, *Phys. Chem. Chem. Phys.*, 2011, **13**, 877–885.

22 U. Mazurek, *Eur. J. Mass Spectrom.*, 2006, **12**, 63–69.

23 B. Concina, P. Hvelplund, A. B. Nielsen, S. B. Nielsen, J. Rangama, B. Liu and S. Tomita, *J. Am. Soc. Mass Spectrom.*, 2006, **17**, 275–279.

24 E. A. C. Spencer, T. Ly and R. R. Julian, *Int. J. Mass Spectrom.*, 2008, **270**, 166–172.

25 K. J. Koch, F. C. Gozzo, S. C. Nanita, Z. Takats, M. N. Eberlin and R. G. Cooks, *Angew. Chemie Int. Ed.*, 2002, **41**, 1721–1724.

26 V. Scutelnic, M. A. S. Perez, M. Marianski, S. Warnke, A. Gregor, U. Rothlisberger, M. T. Bowers, C. Baldauf, G. von Helden, T. R. Rizzo and J. Seo, *J. Am. Chem. Soc.*, 2018, **140**, 7554–7560.

27 J. Seo, S. Warnke, K. Pagel, M. T. Bowers and G. von Helden, *Nat. Chem.*, 2017, **9**, 1263–1268.

28 H. Zhang, Z. Wei, J. Jiang and R. G. Cooks, *Angew. Chemie*, 2018, **130**, 17387–17391.

29 R. Chen, Z. Wei and R. G. Cooks, *Anal. Chem.*, 2021, **93**, 1092–1099.

30 R. R. Julian, S. Myung and D. E. Clemmer, *J. Phys. Chem. B*, 2005, **109**, 440–444.

31 R. R. Julian, S. Myung and D. E. Clemmer, *J. Am. Chem. Soc.*, 2004, **126**, 4110–4111.

32 S. Vandenbussche, G. Vandenbussche, J. Reisse and K. Bartik, *European J. Org. Chem.*, 2006, **2006**, 3069–3073.

33 J. S. Jordan and E. R. Williams, *Anal. Chem.*, 2021, **93**, 1725–1731.

34 J. S. Jordan and E. R. Williams, *Analyst*, 2021, **146**, 2617–2625.

35 K. L. Davidson, D. R. Oberreit, C. J. B. Hogan and M. F. Bush, *Int. J. Mass Spectrom.*, 2017, **420**, 35–42.

36 A. C. Susa, Z. Xia and E. R. Williams, *Anal. Chem.*, 2017, **89**, 3116–3122.

37 Z. Xia and E. R. Williams, *Analyst*, 2019, **144**, 237–248.