Supporting Information

Effects of Electrospray Droplet Size on Analyte Aggregation: Evidence for Serine Octamer in Solution

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Contents

Table S1. Cluster Assignments for Each Peak in ESI Mass Spectra of 10 mM L-Serine

m/z	Cluster Number	Charge
106	1 + H	1
128	1 + Na	1
211	2 + H	1
233	2 + Na	1
316	3 + H	1
421	4 + H, 8 + 2H	1, 2
474	9 + 2H	2
526	5 + H	1
579	11 + 2H	2
631	6 + H, 12 + 2H	1, 2
684	13 + 2H	2
736	7 + H, 14 + 2H	1, 2
772	22 + 3H	3
789	15 + 2H	2
807	23 + 3H	3
841	8 + H, 16 + 2H, 24 + 3H	1, 2, 3
877	25 + 3H	3
894	17 + 2H	2
912	26 + 3H	3
946	9 + H, 18 + 2H, 27 + 3H	1, 2, 3
982	28 + 3H	3

999	19 + 2H	2
1016	29 + 3H	3
1051	20 + 2H, 30 + 3H, 40 + 4H	2, 3, 4
1077	41 + 4H	4
1086	31 + 3H	3
1104	21 + 2H, 42 + 4H	2, 4
1121	32 + 3H	3
1130	43 + 4H	4
1156	22 + 2H, 33 + 3H, 44 + 4H	2, 3, 4
1182	45 + 4H	4
1191	34 + 3H	3
1209	23 + 2H, 46 + 4H	2, 4
1226	35 + 3H	3
1235	47 + 4H	4
1261	24 + 2H, 36 + 3H, 48 + 4H	2, 3, 4
1287	49 + 4H	4
1296	37 + 3H	3
1314	25 + 2H, 50 + 4H	2, 4
1331	38 + 3H	3
1340	51 + 4H	4
1366	26 + 2H, 39 + 3H, 52 + 4H	2, 3, 4
1392	53 + 4H	4
1401	40 + 3H	3

1419	27 + 2H, 54 + 4H	2, 4
1436	41 + 3H	3
1445	55 + 4H	4
1471	28 + 2H, 42 + 3H, 56 + 4H	2, 3, 4
1497	57 + 4H	4

Derivation of Equation One

Equation one is based on several approximations: the initial ESI droplet is a sphere with a diameter that is 1/17th the diameter of the ESI emitter tip, the distribution of analyte molecules within the initial droplet and the solution prior to droplet formation is homogeneous, fusion of the highly charged droplets after their initial formation does not occur, and that all droplets produced by ESI are the same diameter. The volume of a droplet is modeled as the volume of a sphere from the approximations listed above. To convert the value from nm^3 to L when the ESI emitter diameter is expressed in units of nm, a unit conversion of 10^{-24} is applied. Multiplying the volume by the molarity of the solution and then Avogadro's number gives the estimated number of molecules in each droplet produced under the stated approximations.

$$m = \left(\frac{4}{3}\right)\pi\left(\frac{D_E\left(\frac{1}{17}\right)}{2}\right)^3 * 10^{-24} * M * N_A$$

Ion Transfer Capillary Temperature

      The ion transfer capillary temperature was optimized by tuning the instrument ion optics for protonated octamer signal using the automated tuning feature of the instrument control software, then changing the transfer capillary temperature to the following values: 60 °C, 100 °C, 140 °C, 180 °C and 220 °C. A 100 μM serine solution was sprayed using 210 nm emitter tips in order to determine the effect of transfer capillary temperature on cluster abundances. There were no significant trends in the abundances of clusters over this temperature range (Figure S1). The large variability in the abundance of the octamer is likely due to these experiments being performed over several hours in order to allow the ion transfer capillary to equilibrate at each temperature. The average for the octamer abundance across all temperatures was calculated to be $0.56 \pm 0.1\%$, closely matching the value found in the initial experiments at 100 °C. Similarly, the average population abundances of the 15^{2+} and 23^{3+} clusters across the temperature range are $0.11 \pm 0.02\%$ and $0.13 \pm 0.03\%$ respectively, which agree with the reported value at 100 °C in the tip size experiment, $0.11 \pm 0.02\%$ and $0.12 \pm 0.02\%$, respectively. These results indicate that gas-phase dissociation of higher order clusters that may be induced by higher transfer capillary temperatures is not a significant contribution to the protonated octamer abundance observed under these conditions.

In-Source Activation of Larger Clusters at Various Electrospray Source Conditions

In order to examine the effect of voltage on the transfer capillary and tube lens on gas-phase dissociation of large clusters, a 100 µM solution of L-serine was electrosprayed using 210 nm emitters and the transfer capillary voltage and the tube lens voltage were varied. The transfer capillary voltage was varied between 5 V and 120 V. The abundance of the protonated octamer was similar at 5 V and 23 V, but decreased significantly at larger voltages, consistent with collisional dissociation of both the octamer and higher order clusters (Figure S2a).

The tube lens voltage was varied between 50 V and 250 V to determine the effect on the protonated octamer population abundance. The octamer abundance varies with tube lens voltage, from a minimum value of 0.4% at 91 V to a maximum of 1.2% at 250 V (Figure S2b). The reason for the variation in abundance with increasing tube lens voltage is unclear, but may be related to relative ion transmission efficiencies of clusters at different *m/z,* which would affect the calculated population abundances of clusters. This data indicates that the tube lens voltage used in the tip size experiments does not result in breakdown of larger clusters into the protonated octamer in the gas phase.

In sum, these data indicate that the protonated octamer observed with the 210 nm tip size under the conditions of these experiments is not a result of gas-phase dissociation of larger clusters, consistent with these ions originating from solution.

Waters Q-TOF Premier Mass Spectrometer Instrument Parameters and Protonated Octamer Data

A Q-TOF Premier quadrupole time-of-flight mass spectrometer (Waters Corporation, Milford, MA) was also used in these experiments in order to investigate the extent to which source design and ion transmission in different instruments affect our reported results. Instrument parameters were manually tuned to maximize the protonated octamer abundance with ions formed from a 10 mM L-serine solution using an ESI emitter with a 1.2 µm tip. Experiments were then performed using ESI emitters with 210 nm tips to measure the population abundances of the protonated octamer at both 10 mM and 100 µM. The source temperature was 80 °C, the sampling cone and extraction cone voltages were 20 V and 2.0 V respectively. The ion guide voltage was 2.0 V. The collision cell parameters were optimized to a cell entrance potential of 0 V and a cell exit potential of -10 V with a collision gas flow rate of 0.35 mL/min, resulting in a pressure of ~4.4 x 10^{-3} mbar in the collision cell. Stable ion formation was typically obtained at a spray voltage of 0.6 – 0.7 kV.

The population abundance of the protonated octamer was 5.8 ± 0.2% and 2.8 ± 0.3% at 10 mM and 100 µM, respectively. Abundant high mass clusters up to 57^{4+} were observed with a 10 mM solution. The largest clusters were not observed with the LTQ instrument. In contrast, the same distribution of clusters were observed with the LTQ instrument and the Q-TOF instrument at 100 µM albeit at higher abundance with the Q-TOF instrument. These data indicate that the observed abundance of the protonated octamer does have some instrument dependent variability.

Thermo Fisher Velos Pro Mass Spectrometer Instrument Parameters and Protonated Octamer Data

The L-serine solutions were also analyzed using a Thermo Fisher Velos Pro mass spectrometer. Instrument parameters were optimized using the automated tuning feature of the control software while spraying a 10 mM L-serine solution using a 1.2 μm emitter tip. The ion transfer capillary temperature was set to 100 °C to mimic conditions in the LTQ instrument and the S-lens RF percentage was optimized to 67.5% by the automatic tuning process. The data was recorded using the same protocol described in the methods section. Using 210 nm emitter tips, the population abundance of the octamer was found to be $5.6 \pm 0.1\%$ and $0.8 \pm 0.2\%$ for the 10 mM and 100 μM concentrations respectively.
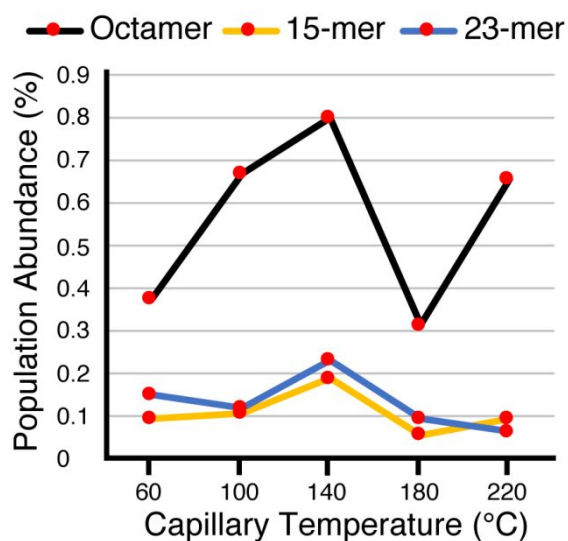
Figure S1. Abundances of Several Serine Clusters as a Function of Ion Transfer Capillary Temperature. This figure shows the population abundance of the protonated octamer, 15^{2+}, and 23^{3+} clusters with increasing transfer capillary temperature. There is no significant trend in any of the cluster abundances within this temperature range, indicating that the capillary temperature does not lead to significant dissociation of gas-phase serine clusters under these conditions.
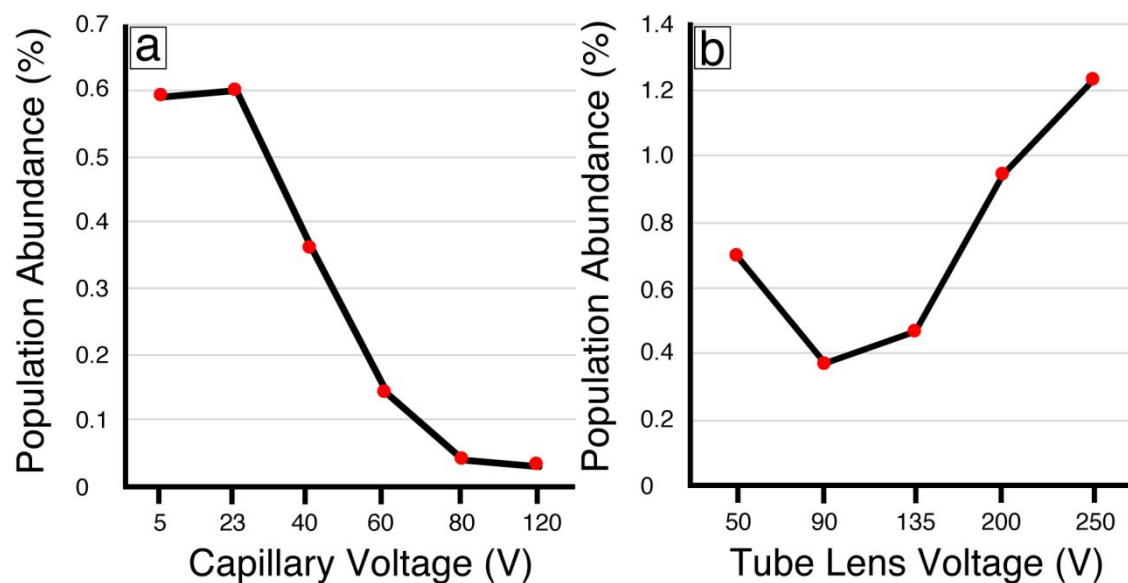
Figure S2. Protonated Octamer Abundance as a Function of MS Operating Conditions.
The effect of the MS operating conditions on the population abundance of the octamer is shown as a function of (a) transfer capillary voltage and (b) tube lens voltage. The abundance of the octamer is similar at 5 V and 23 V (used in the tip size experiments) but decreases at higher voltages. Results from varying the tube lens show a minimum value in the population abundance of protonated octamer at ~90 V, possibly due to differences in ion transmission at different *m/z*.