Atmospheric Pressure Gas-Phase H/D Exchange of Serine Octamers

Zoltan Takats,[†,‡] Sergio C. Nanita,[†] Gitta Schlosser,[‡] Karoly Vekey,*[,‡] and R. Graham Cooks*[,†]

Department of Chemistry, Purdue University, West Lafayette, Indiana 47907, and Institute of Chemistry, Hungarian Academy of Sciences, Pusztaszeri ut 59-67, Budapest, H-1025 Hungary

The recently discovered homochiral serine octamer has been a focus of interest because of its possible implications for the origin of homochirality in living systems. Electrospray ionization (ESI) and sonic spray ionization (SSI) tandem mass spectrometry have been used to generate this unusually stable magic number cluster. Several structures have been suggested for the serine octamer, based on tandem mass spectrometry, ion mobility measurements, and quantum mechanical calculations. We now report experimental hydrogen/deuterium (H/D) exchange data, which demonstrate the existence of two different structures for the serine octamer. These forms undergo exchange at significantly different rates. One form may correspond to solution-phase assembled clusters and the other to octamers formed during the ionization process. Experiments done at higher resolution confirm that the experimental observations made here apply to the serine octamer without interference from metaclusters, namely, higher order clusters $(Ser_{16} + 2H)^{+2}$, etc., the ^{12}C isotopes of which have mass-to-charge ratios identical to the protonated octamers. H/D exchange of racemic serine shows predominantly the extensively exchanged ion population, as well as providing evidence that racemic serine generates abundant metaclusters. The evidence presented here shows that one type of serine octamer is responsible for the strong chiral effects associated with the formation of these magic number clusters. These slowly exchanging more fragile clusters are the octamers that might have played a role in homochirogenesis.

Homochirogenesis, the process by which particular chiral forms of some molecules came to be favored in the course of chemical and biological evolution remains poorly understood,[1] yet it is a key aspect of the origin of life. Chiral signatures are potentially significant tools in modern searches for past or contemporary occurrences of life in the solar system.[2] The chirality of the common α-amino acids has been a focus of interest in considerations of homochirogenesis and in meteoritic analysis and other experiments relevant to searches for life. Serine, one of the

simpler and putatively most primitive α-amino acids,[3] has become a subject of particular interest since it was shown to undergo chirally selective clustering,[4−7] a reaction that only threonine among the common α-amino acids shares to a limited extent.[8] When sprayed from concentrated solutions, serine octamers of unusual stability ("magic numbers") are formed and can be identified by mass spectrometry. In these experiments, which employ electrospray ionization (ESI)[9,10] or the milder method of sonic spray ionization (SSI),[11,12] mass spectrometry is used to identify molecular clusters present in the solution phase, although the exact timing of cluster formation (e.g., in evaporating microdroplets vs nascent solution) is generally not known. What is known is that the octamer is accompanied by larger clusters (metaclusters of the type $[Ser_{8n} + nH]^{+n}$, namely, multiply protonated oligomers of the protonated octamer with exactly its mass/charge ratio),[13,14] and that it dissociates mainly by loss of serine dimers. It has been reported that ion mobility measurements show a single form of protonated octamer together with accompanying metaclusters, which have different collision cross-sections but identical mass/charge ratios.[7,14] Isotopic labeling experiments show that the formation of the octamer is strongly favored for chirally pure serine solutions. On the basis of tandem mass spectrometry data, ion mobility data, and quantum mechanical calculations, two types of structures have been suggested for the octamer: (i) irregular clusters made up of zwitterionic monomers[7,14,15] and (ii) regular 4×2 clusters of neutral serine.[6] The structural difficulties are indicated by the fact that several

---

* Corresponding authors. Tel: (765) 494-5262. Fax: (765) 494-9421. E-mail: cooks@purdue.edu.
† Purdue University.
‡ Hungarian Academy of Sciences.

(1) Quack, M. *Angew. Chem., Int. Ed. Engl.* **1989**, *28*, 571−586.
(2) Lunine, J. I. *Proc. Natl. Acad. Sci.* **2001**, *98*, 809−814.

(3) Jukes, T. H. *Biochem. Biophys. Res. Commun.* **1967**, *27*, 573−578.
(4) Zhang, D.; Koch, K. J.; Tao, W. A.; Cooks, R. G. *Proc. Am. Soc. Mass. Spectrom.,* Long Beach, CA, 2000.
(5) Hodyss, R.; Julian, R. R.; Beauchamp, J. L. *Chirality* **2001**, *13*, 703−706.
(6) Cooks, R. G.; Zhang, D.; Koch, K. J.; Gozzo, F. C.; Eberlin, M. N. *Anal. Chem.* **2001**, *73*, 3646−3655.
(7) Counterman, A. E.; Clemmer, D. E. *J. Phys. Chem. B* **2001**, *105*, 8092−8096.
(8) Nanita, S. C.; Takats, Z.; Meiser, A.; Cooks, R. G. *J. Mass Spectrom.* Unpublished results.
(9) Fenn, J. B.; Mann, M.; Meng, C. K.; Wong, S. F.; Whitehouse, C. M. *Science* **1989**, *246*, 64−71.
(10) Cole, R. B. *Electrospray Ionization Mass Spectrometry: Fundamentals, Instrumentation, and Applications*; Wiley: New York, 1997.
(11) Hirabayashi, A.; Sakairi, M.; Koizumi, H. *Anal. Chem.* **1994**, *66*, 4557−4559.
(12) Hirabayashi, A.; Sakairi, M.; Koizumi, H. *Anal. Chem.* **1995**, *67*, 2878−2882.
(13) Koch, K. J.; Gozzo, F. C.; Zhang, D.; Eberlin, M. N.; Cooks, R. G. S *Chem. Commun.* **2001**, 1854−1855.
(14) Julian, R. R.; Hodyss, R.; Kinnear, B.; Jarrold, M.; Beauchamp, J. L. *J. Phys. Chem. B* **2002**, *106*, 1219−1228.
(15) Schalley, C. A.; Weis, P. *Int. J. Mass Spectrom.* **2002**, *221*, 9−19.

stable cluster structures have been calculated and proposed, but so far none of them can be considered to be fully established as the structure of the serine octamer.

Interest in the chemistry of the serine octamer is driven by the hypothesis[5,6] that serine might have played an important role in homochirogenesis. One indication of this possibility is the fact that individual serine molecules in the octamer can be substituted by other amino acids, provided they have like chirality.[16] In addition, the octamer reacts with a number of key biological compounds in ways that are unusual or unique compared to other amino acids. Thus, the octamer binds phosphoric acid, forms complexes with transition metals, and incorporates hexoses in a chirally dependent fashion.[17] Glucose shows a strong chiral effect, favoring the natural L-amino acid/D-sugar configuration.[17] These reactions of serine and its clusters indicate that it allows *chiral enrichment* and *chiral transmission*, two key steps in the overall process of homochirogenesis.[17] The single most important aspect of homochirogenesis is *symmetry breaking.* This is variously proposed to be the result of external chiral factors (e.g., circularly polarized light,[18] vortex motion,[19] magnetic forces[20]) operating on earth or in extraterrestrially, or to be a result of statistical effects in small number events or the result of kinetic and thermodynamic effects operating on the small intrinsic energy differences between enantiomers, including those associated with avoided parity violation.[1] This is a fascinating issue, but it is not addressed further in this paper.

Instead, in this study, we apply an established method of ion structure elucidation, gas-phase hydrogen/deuterium (H/D) exchange, to the problem of the structure and mode of formation of the protonated serine octamer. The method has been applied successfully to obtain structural information on various types of molecules, from singly charged small species to large multiply charged protein ions.[21−28] It has been particularly useful in distinguishing between isomeric ions and establishing connections between the gas and liquid-phase conformations of ionized proteins and peptides.[23,29−31]

In general, H/D exchange rate constants[30,32] are controlled by energetic and structural factors and, hence, by the types of exchangeable protons (amine, amide, hydroxyl, carboxyl, guanidyl, etc.); the presence of intramolecular hydrogen bonds (or inter-molecular hydrogen bonds in the case of molecular complexes); the nature of the isotopic exchange agent; the number of collisions; and steric factors, including the accessibility of exchangeable hydrogens. These features make the technique capable of providing insights into the roles of various noncovalent interactions in stabilizing the appropriate 3-D structure of gaseous ions, including information on the conformations of proteins ("folded or unfolded")[33,34] and cluster ions. They also make detailed interpretation of the data difficult, especially in larger ions.

H/D exchange mechanisms in amino acids, primary alkanols, oligopeptides, and oligonucleotides have been studied in detail.[21,22] These investigations were focused on the structure and energetics of the deuterating reagent-ion complex, including careful investigations of steric effects and thermodynamic parameters in particular cases. Various mechanisms have been proposed for H/D exchange with ND_3, D_2O and DI, among others, and a picture of several processes that operate in H/D exchange of peptides, in particular, has emerged.[30,31]

Experimentally, most gas-phase H/D exchange reactions are carried out on trapped ions[35−37] although exchange of ions in low-energy ion beams is also well-known. Different trapping techniques allow the use of various reagent pressures and reaction times and, hence, numbers of collisions. A method of carrying out gas-phase H/D exchange in the atmospheric interface of an ESI source was described recently.[38] In this experiment, the exchange reaction was carried out by introducing a deuterating agent (CH_3OD) into the curtain gas stream of a commercial triple quadrupole mass spectrometer. In contrast to other H/D exchange techniques, the ions are not trapped in this case. This feature allows H/D exchange to be studied in a population of metastable ions beginning immediately after their formation. This population is not the same as that encountered in trapped ion experiments or experiments on ion beams within the mass spectrometer, neither is it well-characterized.

Hydrogen/deuterium exchange reactions undergone by protonated serine and its dimer have been reported by Lifshitz and co-workers,[39] who compared the isotopic exchange reactions of protonated serine to those of protonated glycine, revealing similar site-specific rate constants. However, there have been no previous reports on isotopic exchange reactions involving serine octamers. In the current work, we detail electrospray-H/D exchange experiments that give new insights in the structures of serine octamer ions. Homochiral solutions of serine show bimodal ion distributions, indicating that the protonated serine octamers sampled in

(16) Koch, K. J.; Gozzo, F. C.; Nanita, S. C.; Takats, Z.; Eberlin, M. N.; Cooks, R. G. *Angew. Chem., Int. Ed.* **2002**, *41*, 1721−1724.

(17) Takats, Z.; Nanita, S. C.; Cooks, R. G. *Angew. Chem., Int. Ed.* **2003**, *42*, 3521−3523; *Angew. Chem.* **2003**, *115*, 3645−3647.

(18) Burnham, K. S.; Schuster, G. B. *J. Am. Chem. Soc.* **1999**, *121*, 10245−10246.

(19) Ribo, R. M.; Crusats, J.; Sagues, F.; Claret, J.; Rubires, R. *Science* **2001**, *292*, 2063.

(20) Rikken, G. L. J. A.; Raupach, E. *Nature* **2000**, *405*, 932−935.

(21) Dookeran, N. N.; Harrison, A. G. *J. Mass Spectrom.* **1995**, *30*, 666−674.

(22) Dookeran, N. N.; Harrison, A. G. *J. Am. Soc. Mass Spectrom.* **1995**, *6*, 19−26.

(23) Wood, T. D.; Chorush, R. A.; Wampler, F. M.; Little, D. P.; O'Connor, P. B.; McLafferty, F. W. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 2451−2454.

(24) Green, M. K.; Lebrilla, C. B. *Mass Spectrom. Rev.* **1997**, *16*, 53.

(25) Valentine, S. J.; Anderson, J. G.; Ellington, A. D.; Clemmer, D. E. *J. Phys. Chem. B* **1997**, *101*, 3891−3900.

(26) Valentine, S. J.; Clemmer, D. E. *J. Am. Chem. Soc.* **1997**, *119*, 3558−3566.

(27) Levy-Seri, E.; Koster, G.; Kogan, A.; Gutman, K.; Reuben, B. G.; Lifshitz, C. *J. Phys. Chem. A* **2001**, *105*, 5552−5559.

(28) Valentine, S. J.; Clemmer, D. E. *J. Am. Soc. Mass Spectrom.* **2002**, *13*, 506−517.

(29) Suckau, D.; Shi, Y.; Beu, S. C.; Senko, M. W.; Quinn, J. P.; Wampler, F. M.; McLafferty, F. W. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 790−793.

(30) Campbell, S.; Rodgers, M. T.; Marzluff, E. M.; Beauchamp, J. L. *J. Am. Chem. Soc.* **1994**, *116*, 9765−9766.

(31) Campbell, S.; Rodgers, M. T.; Marzluff, E. M.; Beauchamp, J. L. *J. Am. Chem. Soc.* **1995**, *117*, 12840−12854.

(32) Evans, S. E.; Lueck, N.; Marzluff, E. M. *Int. J. Mass Spectrom.* **2003**, *222*, 175−187.

(33) Freitas, M. A.; Marshall, A. G. *Int. J. Mass Spectrom.* **1999**, *182*, 221−231.

(34) Engen, J. R.; Smith, D. L. *Anal. Chem.* **2001**, *73*, 256A−265A.

(35) Schaaff, T. G.; Stephenson, J. L.; McLuckey, S. A. *J. Am. Soc. Mass Spectrom.* **1999**, *121*, 8907−8919.

(36) Schaaff, T. G.; Stephenson, J. L.; McLuckey, S. A. *J. Am. Soc. Mass Spectrom.* **2000**, *11*, 167−171.

(37) Mao, D.; Ding, C.; Douglas, D. J. *Rapid Commun. Mass Spectrom.* **2002**, *16*, 1941−1945.

(38) Takats, Z.; Vekey, K. *Int. J. Mass Spectrom.* In press.

(39) Ustyuzhanin, P.; Ustyuzhanin, J.; Lifshitz, C. *Int. J. Mass Spectrom.* **2003**, *223−224*, 491−498.
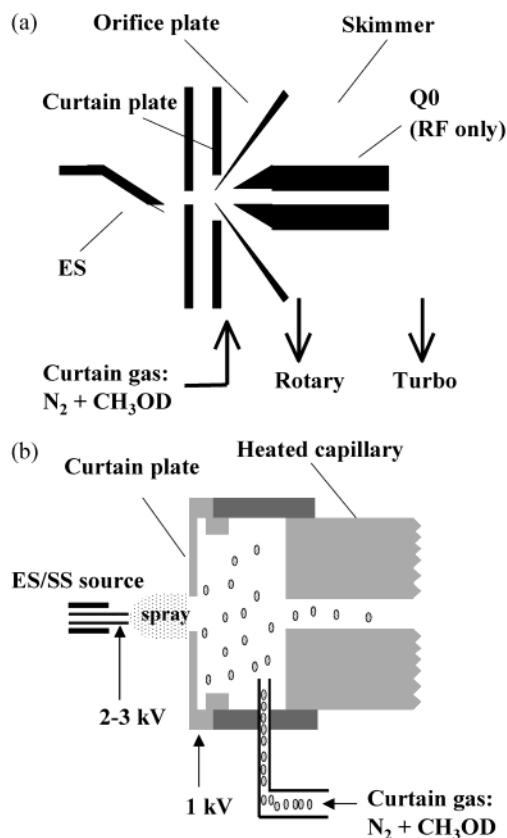
Figure 1. Schematic diagrams of the atmospheric interfaces of the modified (a) Sciex API 2000 and (b) Finnigan LCQ mass spectrometers, which were employed to record H/D exchange mass spectral data.

these experiments have at least two different structures in the gas phase. Examination of mixtures of L- and D-serine reveals that the abundance of one of the ion populations shows a strong chiral dependence, whereas the intensity of the signal due to the other is much less dependent or not at all dependent on the chiral composition of the serine sample. Multiply charged $Ser_{8n}{}^{+n}$ metaclusters are often associated with the latter ion population, although conditions can be found in which they are also associated with the former population too.

EXPERIMENTAL SECTION

Gas-phase H/D exchange spectra of protonated serine octamer ions were recorded using a modified Sciex API 2000 triple quadrupole mass spectrometer and a modified Finnigan LCQ quadrupole ion trap mass spectrometer. Schematics of the atmospheric interfaces of the instruments are shown in Figure 1. Instrumental parameters and the operating conditions used are given in Table 1. A home-built spray ion source,[40] operable in both the sonic spray ionization (SSI) and electrospray ionization (ESI) modes, was used instead of the standard ESI source of the Finnigan LCQ instrument. Detailed information on the home-built ion source was recently provided.[40]

d_1-Methanol (Aldrich, Milwaukee, WI) was used as the deuterating reagent in all experiments; it was directly injected and

(40) Takats, Z.; Nanita, S. C.; Schlosser, G.; Vekey, K.; Cooks, R. G. *Anal. Chem.* **2003**, *75*, 1514−1523.

Table 1. Instrumental Conditions Used to Record Spectra in the Sciex and LCQ Mass Spectrometers

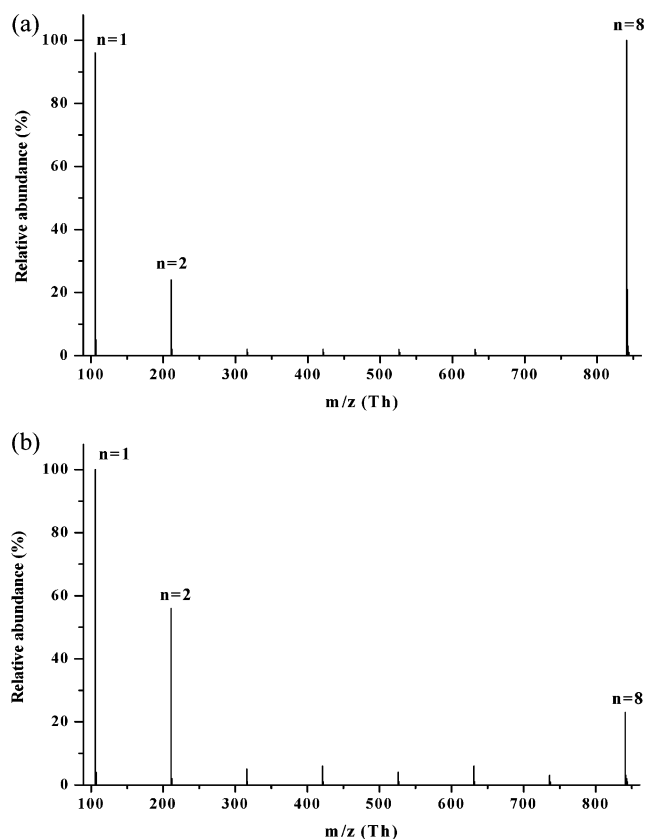| instrumental parameter	Finnigan LCQ	Sciex API 2000
spray voltage	2500 kV	4800 V
nebulizer gas pressure, Pa	2×10^5	2×10^5
auxiliary gas pressure, Pa		3×10^5
curtain gas pressure, Pa	0.7×10^5	2×10^5
source temperature	ambient	ambient
heated capillary temperature	$30-120$ °C	
heated capillary voltage	3 V	
axial spray position, mm	$2-3$	3
tube lens offset/orifice voltage, V	40	30

Figure 2. ESI-MS of 0.01 M L-serine in aqueous methanol recorded in (a) the Finnigan LCQ quadrupole ion trap and (b) the Sciex API 2000 triple quadrupole mass spectrometers. Both spectra show strong serine octamer magic numbers. The difference in the serine octamer relative abundance suggests that more fragmentation occurred in b as a result of higher internal energy deposition in the octameric ions.

evaporated into the nitrogen nebulizing gas/curtain gas stream, as shown in Figure 1. The partial pressure of *d*-methanol in nitrogen was varied roughly in the range of $5-90$ Torr, as estimated by using the curtain gas mass flow rate and deuterating agent infusion rate and assuming 100% evaporation. Serine (Sigma-Aldrich, St. Louis) solutions in methanol (Mallinckrodt)/water 1:1 were used in all experiments unless otherwise indicated.

RESULTS AND DISCUSSION

ESI−H/D Exchange Experiments. The ESI-MS spectra of L-serine recorded using both the Finnigan LCQ and the Sciex API 2000 are shown in Figure 2. Note the unusually abundant magic number octamers (especially in the case of Figure 2a), which are
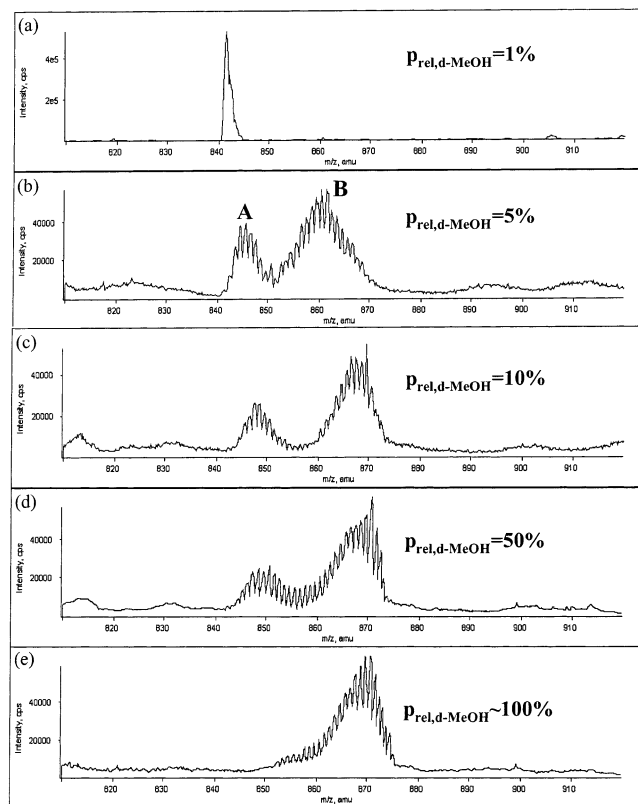
Figure 3. Gas-phase H/D exchange data of L-serine octamer ions obtained using the Sciex API 2000 triple quadrupole instrument and different relative pressures of MeOD: (a) P_{MeOD} = 1%, (b) P_{MeOD} = 5%, (c) P_{MeOD} = 10%, (d) P_{MeOD} = 50%, and (e) P_{MeOD} = 100%

Figure 4. Gas-phase H/D exchange of serine octamer ions from racemic serine solutions obtained using the Sciex API 2000 triple quadrupole instrument and different relative pressures of MeOD: (a) P_{MeOD} = 1%, (b) P_{MeOD} = 5%, (c) P_{MeOD} = 10%, (d) P_{MeOD} = 50%, and (e) P_{MeOD} = 100%

the type of ions of interest here. The extent of gas-phase H/D exchange of L-serine octamer ions using the Sciex API 2000 triple quadrupole instrument showed, as expected, a dependence on the partial pressure of d-methanol (see Figure 3). The number of observed exchanges could be increased from zero and made to approach the expected maximum of 33 (four in each of the eight serine units plus one) by increasing the partial pressure of methanol from 0 to 90 Torr. Remarkably, two distinct ion populations (named A and B, as shown in Figure 3b) appear in the ESI spectra, with different average exchange numbers. For example, under the particular conditions shown in Figure 3b, these numbers are 6 and 20, respectively. Note that neither the average mass/charge ratio nor the difference in m/z between the two ion populations is constant: both vary with the pressure of the deuterating reagent, excluding the possibility of population B being trivially related to population A.

The ions in population A undergo less H/D exchange than ions in population B (under similar conditions), giving a distribution of exchange peaks with lower m/z values than that of B-type octamers. Population B shows a more rapid increase in degree of exchange with partial pressure of deuterating agent. The abundance of ion population A continuously decreases along with the increased degree of exchange that accompanies increased deuterating agent partial pressure. For population B, statistical H/D exchange assuming 33 equivalent protons fits the experimental mass spectral data well. Note that the reaction time is roughly the same for all data shown in Figure 3. Theoretically, the degree of H/D exchange depends on the number of collisions with the
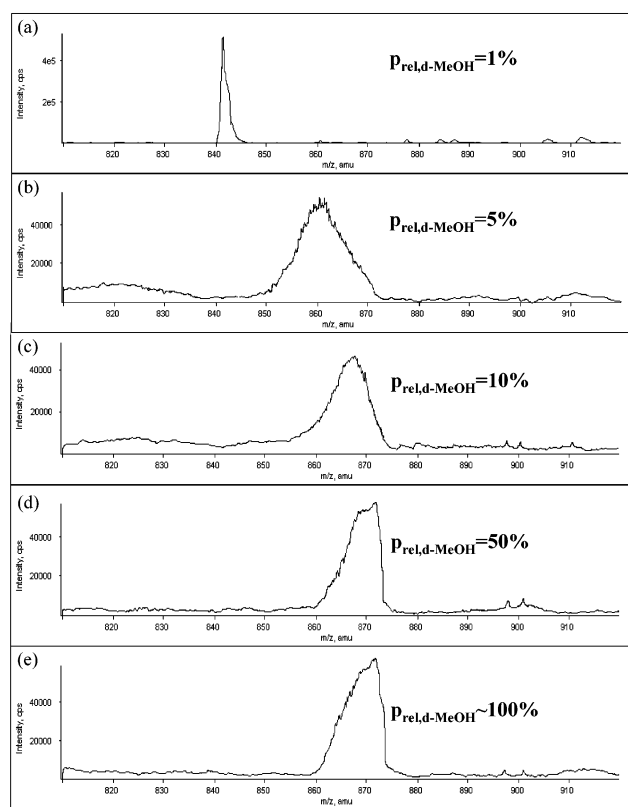
deuterating reagent (CH_3OD), and experimentally this can be controlled by changing the partial pressure of CH_3OD and the reaction time.

Serine octameric clusters (including $[Ser_{8n} + nH]^{+n}$ metaclusters) generated by ESI of racemic serine do not show the behavior just described for the protonated octamers generated from chirally pure serine. The average number of exchanges still increases with the partial pressure of d_1-methanol; however, mainly one ion population is observed (Figure 4). The most probable exchange number for this ion population matches that of ion population B in the exchange spectra of pure L-serine, within the error limit of the experiment, using the same partial pressure of deuterating reagent. In this case, all the ions rapidly undergo a large number of exchanges approaching the maximum number of 33 exchanges. (Under these conditions, A is either not formed from racemic serine, or is fragile to dissociation, or it converts to B.)

In summary, the data suggest that, under specific conditions (those of Figure 3), two different types of serine octamers coexist in the gas phase after the ionization process. H/D exchange of mobile protons in serine octamers of type A appears to be hindered by either steric or thermodynamic factors, as compared to structure B. It is important to note that H/D exchange reactions are influenced not only by the accessibility of certain functional groups, but by their nature, as well.

To further characterize the effects of chirality on the structure of protonated serine octamers, a series of D-/L-serine mixtures (100:0, 75:25, 50:50, 25:75, 0:100) was examined. The deuterating
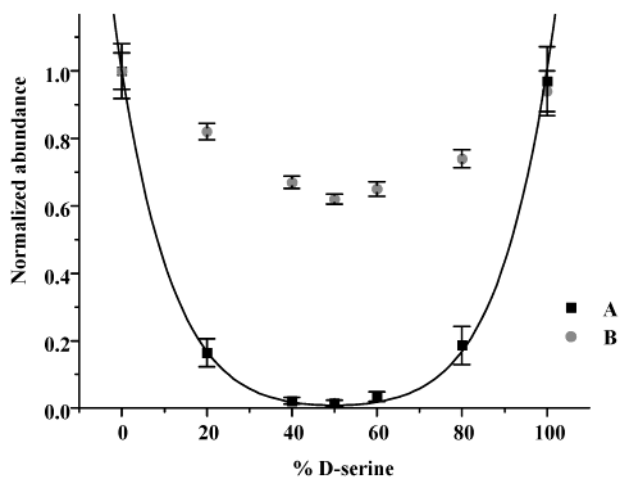
Figure 5. Relative abundances (Sciex instrument) for ion populations A and B as a function of enantiomeric composition of serine. The chiral effect is much stronger for ions in population A.

reagent partial pressure was set to achieve the highest degree of separation between the two ion populations, although separation was not complete. Normalized abundances of each ion population are shown as a function of enantiomeric composition in Figure 5. The behavior of ion population A is in excellent agreement with the statistical binomial function, indicating a very strong chiral preference for formation of ions of this structure. Another possibility, that the chiral preference is a manifestation of the H/D exchange kinetics rather than the formation of two different types of octamers, cannot be discounted but is considered unlikely.

The normalized abundance of ion population B is also dependent on the chiral composition of the serine solution; however, in this case, the dependence is much weaker, and it is likely to be due to unresolved contributions from population A. There is also a substantial difference with regard to the dependence of the charge state distribution on chiral composition. Population A, when the H/D exchange spectrum of L-serine is examined at unit mass/charge resolution, clearly displays only singly charged octameric ions, whereas population B, in the case of the racemic serine sample, contains multiply charged $[Ser_{8n} + nH]^{+n}$ metaclusters, as well. When pure enantiomers are examined, this phenomenon is less apparent; however, a distinct loss of resolution of ion population B is observable (Figure 4), indicating the presence of the multiply charged metaclusters. These data show the two ion populations and their change in relative abundance with reaction conditions. They also show that the presence of multiply charged metaclusters under these conditions is mainly associated with population B, and this causes the loss of resolution in the Sciex API 2000 spectra.

A similar set of experiments was carried out using a Thermo Finnigan LCQ ion trap mass spectrometer equipped with a curtain plate−curtain gas system (Figure 1b). As shown in Figure 6, one major ion population appears in the ESI H/D exchange spectra. The similarities between this population and ion population A observed in the Sciex API 2000 instrument suggest that both correspond to the same type of ions. At 1 order of magnitude lower intensity, another ion population with a higher average number of exchanges, corresponding roughly to the earlier population B, appears.

The LCQ data (Figure 6) with their apparent maximum exchange number of nine or fewer need to be treated cautiously. Large amounts of water can be adsorbed onto the surface of the heated capillary in the ESI source of this instrument, causing efficient back-exchange of the examined species. In a series of experiments aimed at complete exchange, this could not be achieved; water seems to be too strongly bound to the metal heated capillary surface to allow complete gas-phase H/D exchange to be performed.

An important difference between the two mass spectrometers employed to record the data is the internal energies deposited in the electrosprayed ions. Electrospray ion sources with heated capillaries, such as is used in the Thermo Finnigan LCQ, are generally assumed to produce ions with lower internal energies, an effect that favors clustering.[41] This phenomenon is illustrated by the comparison of L-serine mass spectra (Figure 2) recorded on the two instruments. In the spectra acquired using the Finnigan LCQ, the protonated octamer corresponds to one of the most intense peaks in the mass spectrum, whereas in the Sciex instrument, the maximum relative abundance of the serine octamer that could be recorded was 26%. This spectrum also shows intense dimeric, tetrameric, and hexameric peaks, which have been previously ascribed to dissociation of octameric ions.[6] These results lead to the conclusion that population A is preferred under mild conditions. Conversely, ion population B in the ESI spectrum can be identified as one that is formed (or stable) under higher-energy conditions.

In an effort to provide more information on the two ion populations, the temperature of the heated capillary atmospheric interface in the Finnigan LCQ was varied. The temperature is expected to influence the internal energy of ions in the capillary and, hence, to affect the distribution of ions between the two populations in the ESI spectra. At the lowest temperature that can be used without unacceptable loss in ion intensity (60 °C), population A is seen exclusively (exchanging up to five protons); the low intensity signals corresponding to population B completely disappear from the spectra. This observation is consistent with population B originating in the gas phase, possibly but not necessarily from ions in population A rearranging during the electrospray process, yielding presumably an "opened" structure which is therefore able to exchange more protons.

Sonic Spray-H/D Exchange Experiments. Serine octamers can be formed in high yield using a recent modification[40] of the gentle technique of sonic spray ionization. This ionization technique is even softer than electrospray, and SSI of L-serine gives almost exclusively the octameric ion. It has been argued[40] that ionization in SSI occurs by the charge residue mechanism (CRM), whereas in ESI, ions generated by ion evaporation make a larger contribution. In the CRM, formation of charged droplets containing the analyte ion is followed by simple evaporation of the excess solvent. In this experiment, ions are more likely than in ESI to preserve their solution-phase structures, since there are no accompanying processes that increase their internal energy. In ESI, small (nonmacromolecular) ions are generally assumed[42] to be formed by the ion-evaporation mechanism,[43] a process in which

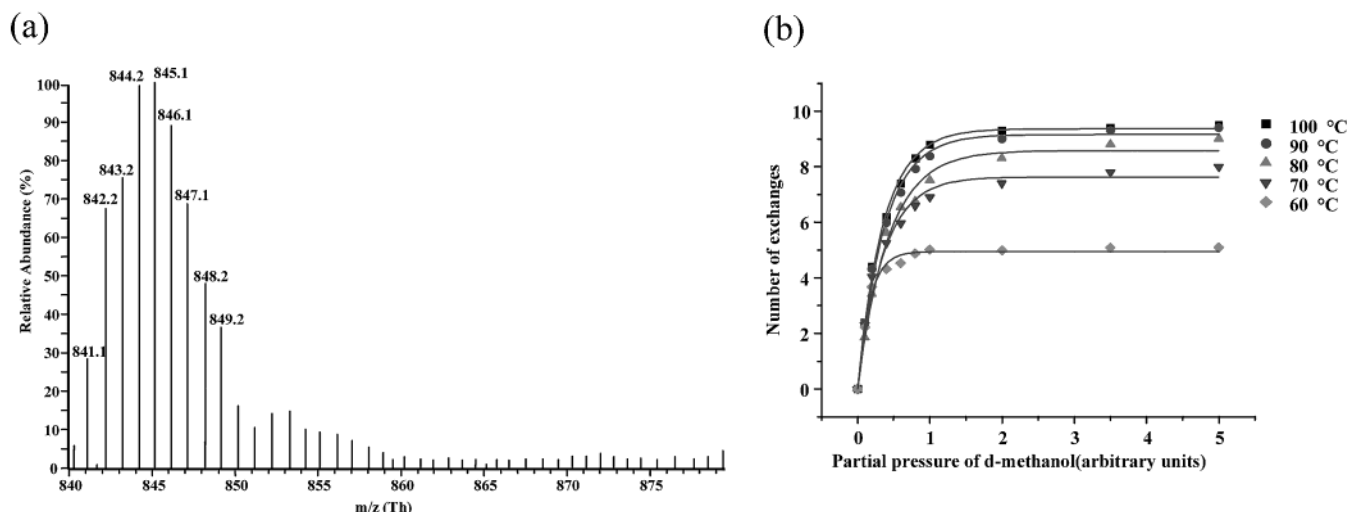(41) Takats, Z.; Drahos, L.; Schlosser, G.; Vekey, K. *Anal. Chem.* **2002**, *74*, 6427−6429.

Figure 6. Gas-phase H/D exchange ESI mass spectrum of L-serine octamer ions recorded using a modified Finnigan LCQ quadrupole ion trap instrument. As in all experiments, CH$_3$OD was used as deuterating reagent. Note that one major and one minor ion population appear. (a) Typical mass spectrum. (b) Number of exchanges as a function of the partial pressure of *d*-methanol for the major ion population.
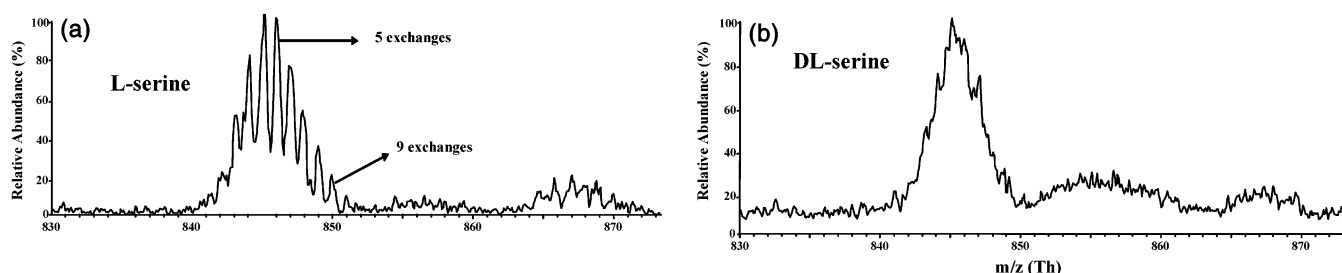

Figure 7. Sonic spray H/D spectra of serine octamers (Finnigan LCQ) using saturated d_1-methanol vapor in N$_2$ as the curtain gas and a 0.01 M L-serine solution; (a) L-serine, (b) DL-serine.

the ions are accelerated after their emission in the coulomb field of the nanodroplet and some conversion of kinetic energy to internal energy occurs. Hence, a large difference in the internal energy acquired by the ions is expected, depending on the ion formation mechanism.

Isotopic exchange experiments were carried out using SSI for both L- and racemic serine (Figure 7). There are two points of contrast between the SSI and ESI data: (i) the degree of exchange in ESI is much greater under similar conditions because of the contribution of population B and (ii) the degree of exchange of population A is measurably greater under ESI than SSI conditions. Since SSI is such a mild ionization method, its H/D exchange behavior is believed to reflect clusters that have not undergone significant secondary processing. Hydrogen/deuterium exchange reactions were also performed for racemic serine. The H/D exchange data on racemic serine solutions ionized by SSI show a decrease in resolution which is consistent with a high abundance of metaclusters (higher-order aggregates of the octamers), associated with population A in this case. Hence, the expected clustering using the mild SSI method is observed.

The number of H/D exchanges is influenced by several factors, including the PA difference between the deuterating reagent and the functional group at which exchange is occurring, the PA difference between the two functional groups involved in the

course of a relay mechanism,[44] and also steric factors. Since other amino acids, including threonine, the higher homologue of serine, readily undergoes complete H/D exchange under the same experimental conditions, PA differences are unlikely to be responsible in themselves for the limited amount of H/D exchange observed in the serine octamer. One must therefore look for structural features of the octamer to produce the exchange patterns. These structural differences between the two forms might be related to the two main types of proposed octamer structures (i.e., zwitterionic vs neutral serine constituents); however, on the basis of the results presented so far, it would be premature to designate one form as zwitterionic and the other as the neutral structure.

Structural Information from Dissociation Data. Tandem mass spectrometry (MS/MS) was used to characterize the two ion populations that arise in the H/D exchange mass spectra of serine. Fragment ion spectra of both ion populations were recorded under a variety of conditions using the Sciex API 2000. The data showed the presence of only one type of serine oligomer, confirming the identification of the exchanged precursor ions as serine octamers (or their metaclusters or both).

A series of MS/MS experiments were performed on unexchanged serine octamers (m/z 841) in the Finnigan LCQ instrument, using an isolation window of 30 mass/charge units and a relative collision energy 14.1%.[45] Conditions were set to generate

(42) Kebarle, P.; Tang, L. *Anal. Chem.* **1993**, *65*, A972−A986.

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

(44) Wyttenbach, T.; Bowers, M. T. *J. Am. Soc. Mass Spectrom.* **1999**, *10*, 9−14.
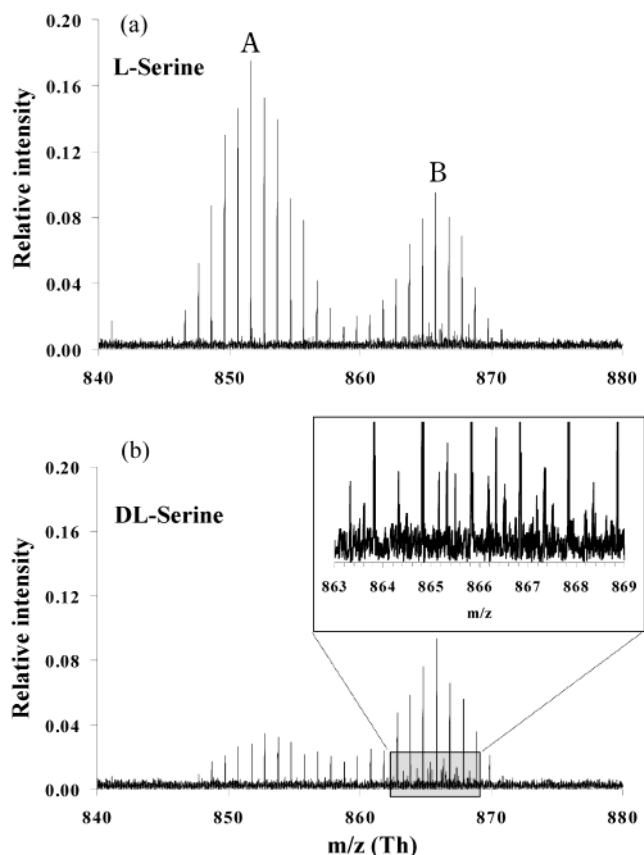
Figure 8. High-resolution H/D exchange data taken using d_1-methanol using an Orbitrap mass spectrometer at a pressure of 2.5×10^{-5} Torr and reaction time of 200 ms, showing populations A and B as well as metaclusters with exchange patterns corresponding to population B.

each octamer population (A and B) separately, followed by isolation and collision-induced dissociation of the octamers (and metaclusters). Under conditions known to generate mainly population A (SSI using a homochiral serine solution), the main fragment ions in MS/MS spectrum are m/z 630 (100%), 841 (precursor ion, 89%), 525 (14%), 421 (4%), and 735 (4%). On the other hand, the ESI-MS/MS of racemic serine, which is expected to mainly produce ions of type B, yielded the following signals: m/z 735 (100%), 630 (95%), 788 (79%), 841 (precursor ion, 49%), 683 (35); 893 (20), 946 (18), and 998 (15). These data indicate that the characteristic loss[4-7] of serine dimer is associated with population A, the slowly exchanging population.

High-Resolution Data. The results shown in Figures 3 and 4 were reproduced in a high-resolution mass spectrometer[46,47] (Figure 8 shows representative data.) The H/D exchange was carried out on ions trapped in a linear quadrupole trap of the ion guide, and the reaction time was varied.[48] This experiment showed behavior that is almost identical to that seen when the partial pressure of CH_3OD is varied in the Sciex instrument. The high-resolution data confirm the presence of metaclusters, including

(45) Manufacturer's nominal relative collision energy (%), where the range from 0 to 100% corresponds to a resonance excitation AC signal of 0−2.5V (zero-to-peak) at the secular frequency of the ion of interest.
(46) Makarov, A. A. *Anal. Chem.* **2000**, *72*, 1156−1162.
(47) Hardman, M.; Makarov, A. A. *Anal. Chem.* **2003**, *75*, 1699−1705.
(48) Li, H.; Makarov, A. A.; Noll, R.; Hardman, J. M.; Wu, G.; Plass, W.; Cooks, R. G. *Proc. Am. Soc. Mass. Spectrom.*, Montréal, QC, Canada 2003.

the abundant ions in the population B. It also shows, as expected, a significant drop in the signal intensity of population A for the racemic serine sample. This confirms the strong chiral effects associated with the formation of octamers of type A. On the other hand, the signal intensity of population B is independent of chiral composition, in agreement with results obtained in the Sciex API 2000.

CONCLUSIONS

This study has provided evidence that the serine octamer is present in two forms, denoted as populations A and B, and distinguished on the basis of the degree of H/D exchange with gas-phase d_1-methanol. Under mild conditions (low-temperature ESI and SSI) mainly population A is formed, and it undergoes H/D exchange more slowly than population B. Formation of octamers belonging to this population is strongly dependent on the chirality of the serine sample. It is possible that the B-type octamers are formed at some stage in the spray process by assembly of smaller serine clusters or serine molecules or by rearrangement of the initially formed serine octamers, yielding a structure capable of undergoing fast H/D exchange. The population formed by SSI, or mild ESI, is likely to reflect the undisturbed solution phase form, and it corresponds to a more slowly exchanging octamer (population A). It is not known for sure if population A exchanges slowly because it is more tightly bound or because its functional groups inherently undergo slower H/D exchange. If the former, A would likely correspond to a compact zwitterionic form; if the latter, to an assembly of covalent serine molecules such as that proposed in ref 6. Form A may be transformed to form B in the course of the H/D exchange, and there is some suggestion of this in the change in relative intensities at different deuterating agent pressures in the Sciex data. The distinct bimodal character of the distributions apparently opposes this assumption; however, if a cyclic structure is assumed, a daisy-chain isomerization could involve considerable activation energy only for the initial step. In this case, the intermediate products would not accumulate, and the expected result is in agreement with the experimental facts.

Racemic serine yields many fewer ions with structure A. Instead, it yields relatively more ions of the second population, B, which exchanges more hydrogens for deuteriums. It also yields racemic metaclusters of the same mass/charge ratio in high abundance. These data explain a puzzling feature of the earlier data on serine clustering, since they account for the generation of octameric ions from racemic serine solutions. The electrospray and sonic spray data strongly imply the existence of $[Ser_{8n} + nH]^{+n}$ clusters in the nanodroplets just prior to gas-phase ion formation. However, the tremendous concentration increase involved in the spray methods cannot be avoided, and therefore, it remains difficult to draw conclusions from the gas-phase data about the existence of preformed clusters in the solution phase.

A feature of the H/D exchange experiments reported here is that the labeling patterns serve as a method of identifying the two ion populations so that their behavior can be readily followed as a function of experimental parameters. This facilitates interpretation of earlier pressure and chiral-dependence results obtained on the mixed population consisting of both structural forms. On the basis of the present data, the existence of at least two

types of serine octamers is demonstrated. One form may, indeed, correspond to solution-phase assembled clusters, and the other to octamers formed during the ionization process. They could very well correspond to clusters comprised of zwitterionic and nonioinic covalent forms of serine. These interpretations need much further investigation, and the formation and dissociation of the metaclusters should provide methods of arriving at more secure assignments. What is clear is that conditions can be selected so that different mixtures of the two structural forms of serine octamers are generated. It also appears possible to generate metaclusters of both types of octamer. In addition, these conditions themselves alter the extent to which particular structures undergo H/D exchange.