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Ion/molecule reactions of the protonated serine octamer†

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The protonated homochiral octamer of serine exchanges all 33 of its labile hydrogens with CH_3OD and undergoes ligand switching reactions with amines in a quadrupole ion trap mass spectrometer.

Since Cooks *et al.* discovered that the protonated, octameric cluster of serine, $(Ser)_8H^+$, is unusually stable,[1,2] there has been intense interest in the structural features that lead to its enhanced stability.[3–13] Several structures have been suggested for the serine octamer, based on tandem mass spectrometry, ion mobility measurements, and theoretical calculations. In addition, the octamer also exhibits a high preference for homochirality. For example, solutions containing a mixture of isotopically labeled D and L-serine have a strong tendency to form octameric clusters that are either all D or all L.[1,12] This result has critical implications in crystal growth processes and the emergence of homochirality in biological systems. Here we report the gas phase ion-molecule reactions of mass selected homochiral $(Ser)_8H^+$ using quadrupole ion trap mass spectrometry.[14–16]

H/D Exchange with CH_3OD

The first reaction we studied was H/D exchange with CH_3OD. Previously, Cooks *et al.* have examined the reaction of CH_3OD with protonated serine clusters under a variety of conditions.[9] They have observed two populations of ions, one that undergoes rapid exchanges and one that undergoes slow exchanges and argued that slow exchange could be used as a diagnostic for the ionic structure that was generally formed from enantiomerically pure solutions (*i.e.*, all D or L-serine).

Our system differs from some of the previous work in that the protonated serine octamers are mass-selected before the reaction with CH_3OD. From the data presented in Fig. 1, it appears that only a single ion population exists under our conditions since there is a relatively smooth shift in the label distribution as a function of the reaction time. The process is relatively slow, with a measured rate constant of 1.4×10^{-11} cm^3 s^{-1} molecule^{-1} for the first H/D exchange. As noted by Cooks *et al.*, it is possible to exchange up to 33 hydrogens for deuterium (*i.e.*, every O–H and N–H bond and the additional proton).[9] This is a remarkable result and offers insight into the structure of the serine octamer complex. The ability to exchange all the labile protons, including the ones on the side chains, suggests a flexible structure where intra-complex proton exchange (*i.e.*, from one serine to another) occurs readily and CH_3OD can access the majority of sites in the complex without causing its disintegration. Moreover, the accepted models for gas phase H/D exchange would require that the side chain OH groups be able to interact with the charged centers in the complex.[17] The degree of flexibility that the H/D exchange results suggest is incompatible with two distinct ion populations possessing different, non-interchanging gas phase structures. If the complex can undergo intramolecular proton swapping and bring all of the side chain groups to a charge center, it is likely that it can also readily rearrange and adopt its most energetically favorable structure (or ensemble of structures).

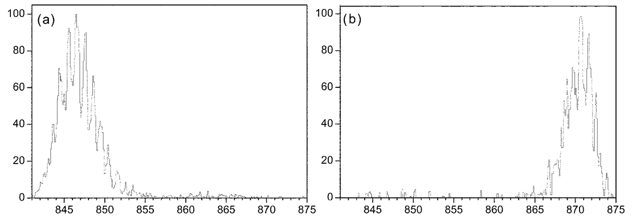

Fig. 1 Ion populations for reaction of $(Ser)_8H^+$ with CH_3OD (4×10^{-6} Torr): (a) 1500, (b) 10000 ms.

Although our experiments point to a single population, it appears that under other conditions, two populations can be observed. It is possible that two populations of $(Ser)_8H^+$ ions coexist during the ionization process and were probed in Cooks *et al.*'s ion source H/D exchange experiments. To reconcile this situation with our H/D exchange data, the two populations must coalesce to one by the time they reach the mass analyzer of the LCQ. Alternatively, the source H/D exchange experiments could involve a wide range of clusters of varying sizes, some of which exchange faster than others. Larger clusters that breakdown to the $(Ser)_8H^+$ cluster while they are being transferred to the mass analyzer could then represent the 'second' ion population. Once in the mass analyzer, a single population of $(Ser)_8H^+$ ions exists, but the differing levels of deuterium incorporation are a reflection of their parent ions' differing rates of H/D exchange (*i.e.*, octamer clusters *vs.* higher clusters). However, the formation of a second population in other experiments involving mass-selected ions suggests that the second population is very sensitive to the experimental setup and is only observable under a limited set of conditions that we were not able to access with the source settings used in our LCQ.

Ligand switching reactions with $(Ser)_8H^+$

In addition, we have compared the ion-molecule reactions of neutral ligands with $(Ser)_8H^+$, $(Ser)_2H^+$ and $(Ser)H^+$. Recent studies have suggested ligand-switching reactions can provide insights into the structures of proton bound clusters.[18] In principle there are three main types of reactions that can be observed for these protonated serine clusters: deprotonation [eqn. (1)], adduct formation [eqn. (2)] and ligand switching [eqn. (3)].

$$(Ser)_nH^+ + L \longrightarrow (Ser)_n + LH^+ \tag{1}$$

$$(Ser)_nH^+ + L \longrightarrow (Ser)_n(L)H^+ \tag{2}$$

$$(Ser)_nH^+ + L \longrightarrow (Ser)_{n-1}(L)H^+ + Ser \tag{3}$$

We first turned our attention to the reactions of dimethylsulfoxide (DMSO), a species with modest gas phase basicity (GB = 854 kJ mol^{-1})[19] but a high dipole moment (μ = 2.99 D). While DMSO reacts with $(Ser)H^+$ *via* eqn. (2) and with $(Ser)_2H$ *via* eqn. (2) and (3), it is totally unreactive towards $(Ser)_8H^+$. In contrast, amines, which have high gas phase basicities, but small dipole moments, react with $(Ser)_8H^+$, $(Ser)_2H^+$ and $(Ser)H^+$ to give a range of products. $(Ser)H^+$ typically reacts *via* proton transfer [eqn. (1)] and adduct formation [eqn. (2)], while $(Ser)_2H^+$ tends to undergo all three classes of reactions [eqn. (1)–(3)]. $(Ser)_8H^+$ exhibits very

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selective reactivity, tending to mainly switch out one of the serines [eqn. (3)] although some adduct formation [eqn. (2)] is observed.

For the (Ser)$_8$H$^+$ cluster, the rates of the ligand-switching reactions with the amines are very dependent on the substitution pattern of the amine. Secondary and tertiary amines react at near the collision limit whereas primary amines give very low rates (less than one successful reaction in every 10000 collisions). Data for diethylmethylamine are given in Table 1. The basicity of amines is linked to their substitution pattern and the observed variation in reaction rates strongly suggests that the proton affinity of the amine is the key factor in determining the rate, with a GB of at least 919 kJ mol^{-1} (diethylamine) being needed to initiate the reaction.

It is initially surprising to note that with the tertiary and secondary amines, the first ligand-switching reaction occurs at near the collision-controlled limit, but the second one (replacement of a second serine with the amine) is too slow to observe. However, this result can be rationalized by considering the expected ionic structure of the (Ser)$_8$H$^+$ cluster. It has been accepted that it is composed of serine in its zwitterionic form ($^+H_3NCHRCO_2^-$).[10] In the cationic cluster, one of the serines is protonated and bears a neutral carboxylic acid group. It appears that it is this unique serine that is able to undergo the exchange process with the amine to give the (Ser)$_7$(amine)H$^+$ cluster, in which the charge is now effectively localized on the introduced amine. The remaining serines in the cluster should maintain their zwitterionic form, rendering them unreactive towards exchange with the amine.

Ligand switching reactions with derivatives of (Ser)$_8$H$^+$

Although it is not possible to exchange a second serine for an amine, clusters with the general structure (Ser)$_7$(amine)H$^+$ will exchange the amine component for another amine (Table 1). This process allows us to probe the stabilities of the (Ser)$_8$H$^+$ derivatives. Although they can be formed by gas phase reactions with (Ser)$_8$H$^+$, it is more convenient to generate them in the ion source by introducing additives into the electrospray solution. This approach also allows for the introduction of compounds that are not volatile enough for gas phase reactions.

The addition of simple amines to the solution leads to the formation of (Ser)$_7$(amine)H$^+$ clusters that behave in the same way as those generated in gas phase reactions (*e.g.*, give similar CAD spectra). For example, when subjected to CAD, the (Ser)$_7$(Et$_3$N)H$^+$ clusters give a mixture of (Ser)$_6$H$^+$ and (Ser)$_5$(Et$_3$N)H$^+$ (the former is favored). It is also possible to generate mixed clusters containing chiral amines. Clusters incorporating *R* and *S* *N*,*N*-dimethyl-1-phenylethylamine (DMPA) react with simple amines to give ligand-switching reactions where DMPA exclusively is replaced with the added amine. The rates are somewhat sensitive to the chirality of the DMPA and the cluster containing the *S*-enantiomer reacts more quickly, suggesting that it is less stable (Table 1).

Previous studies have shown that clusters containing cysteine can be formed[7,11] and we have investigated the reactions of (L-Ser)$_7$(L-Cys)H$^+$ and (L-Ser)$_7$(D-Cys)H$^+$ with amines. In these reactions, either serine or cysteine can be lost during the exchange process. Despite the statistical advantage for serine loss (7 : 1), cysteine loss is favored (Table 1) indicating an inherent preference of over 20 : 1 for retaining the serine in the reaction, presumably because its side chain offers more stabilizing interactions with the other partners in cluster. There is essentially no difference between the branching ratios for reactions of the L-Cys and D-Cys containing clusters, but the clusters incorporating D-Cys react somewhat faster (Table 1). The higher rate constant suggests lower stability, which is consistent with the greater ease of forming octamer clusters that contain L-Cys.[7,11] With diethylmethylamine as the reagent, the derivative clusters have higher rate constants than the parent, protonated serine octamer; however, all the rates are close to the collision-controlled limit.

Table 1 Rate constants for the gas phase reactions of clusters with diethylmethylamine (DEMA)

Species	k[a]	% (L-Ser)$_7$(DEMA)H$^+$
(L-Ser)$_8$H$^+$	62	100
(L-Ser)$_7$(L-Cys)H$^+$[b]	89	72[c]
(L-Ser)$_7$(D-Cys)H$^+$[b]	130	72[c]
(L-Ser)$_7$(*R*-DMPA)H$^+$[b]	81	100
(L-Ser)$_7$(*S*-DMPA)H$^+$[b]	150	100

[a] Units are 10^{-11} cm^3 s^{-1} molecule^{-1} and are the average of 6 or more measurements carried out as described previously.[14,15] [b] Generated *via* ESI by adding an amine to the solution. [c] The other product is (L-Ser)$_6$(L or D-Cys)(DEMA)H$^+$.

Overall, the results of this study show that the (Ser)$_8$H$^+$ clusters have surprising flexibility and readily undergo exchange reactions in the gas phase with amines. The reactions of the derivatives tend to be faster than the parent (Ser)$_8$H$^+$ cluster and suggest that substitution probably reduces the stability of the cluster. The fact that (Ser)$_7$(Cys)H$^+$clusters are the only derivatives which exchange a serine for an amine suggests that the amino acids exists as zwitterions in these clusters. In the mixed clusters, the chirality of the unique component appears to have a limited impact on the cluster's reactivity and therefore factors beyond homochirality are also important in these clusters. The results of a wider range of reactions and extensive computational modeling studies will be reported shortly.

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