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Thermal formation of serine octamer ions

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Vigorous evaporation of aqueous serine solutions yields abundant protonated serine octamer ions. So does pyrolysis of L-serine crystals in a corona discharge.

Serine is unique among the amino acids in forming chirally selective, high-stability octamers in spray ionization experiments.[1–5] Electrospray ionization (ESI)[6,7] produces the octamer and its dimers (Ser_{16}) and trimers (Ser_{24}) as the protonated or sodiated species, besides showing the expected simple monomeric and dimeric (Ser_2) ions. Sonic spray ionization (SSI)[8,9] of serine solutions produces almost exclusively the octameric species, independently of the solvent or the pH of the solution.[10]

Serine octamer has received attention as a molecular cluster that possibly played a key role in the early stages of biochemical evolution, especially with regard to homochirogenesis (the preference in biological systems for a particular chirality) and chiral transmission.[5,11] The octamer undergoes a number of different chiroselective reactions with other building blocks of living systems, including other α-amino acids and various carbohydrates.[5,11] Besides organic molecules, phosphate anion also can be incorporated into the octamer structure, and the proton or sodium cation can be substituted by other metal cations, including alkali-earth metals and transition metals.

In spite of these unique chemical features, the association of serine octamers with prebiotic biochemical evolution has been subject to criticism, mainly due to its formation by spray ionization. Serine octamer could be thought a possible artifact of these ionization methods, not necessarily present in the solution phase but only formed in the nanodroplets produced by ESI or SSI.

The structure of the serine octamer ions has also been a subject of debate.[1–4] The proposed structures can be divided into two groups, distinguished by whether the octamer is built up of zwitterionic or neutral serine molecules. Zwitterionic structures have been associated with solid-phase serine and it has been suggested that the octamer is simply two unit cells from the crystal.[3] Ion mobility[2,3] and gas-phase H/D exchange[12] experiments have been performed to elucidate the structure of the octamer. Ion mobility data showed one single species, which was associated with one of the zwitterionic structures based on the measured average collisional cross section of the ion. Results of gas-phase H/D exchange experiments, however, clearly showed the presence of two structures one of which could be associated tentatively with the non-zwitterionic structure. The apparent contradiction between the two sets of results might be due to different ion sources and different environments experienced by the ions prior to detection.

Ionization of non-volatile species can be accomplished by a wide selection of "energy sudden"[6] or desorption methods[13] in addition to spray ionization methods. The thermal techniques derive from an idea of Friedman *et al.*[14] based on unimolecular dissociation rate theory. They sugggested that sufficiently rapid heating could transfer large molecules into the gas phase before decomposition would occur. Most of the desorption ionization methods, including MALDI, plasma desorption, SIMS and less well-known methods like K^+IDS, are consistent with these ideas.[15–18] These methods are generally assumed to directly transfer ions from the condensed phase into the gas phase; the high-surface area colloidal transition state which apparently is always involved in the case of spray techniques is avoided.

Droplets of aqueous solution of L-serine were evaporated vigorously from a hot surface and the vapor was sampled using the experimental setup depicted in Fig. 1a. A typical result is shown in Fig. 2a. The optimal temperature for observation of the signal at 841 Th, corresponding to the protonated octamer (and to its higher oligomers) was in the range 200–250 °C. Changes in temperature in either direction shifted the ion intensity ratios towards the formation of simple monomeric and dimeric ions. Ions were formed after each droplet (~2–5 μL) fell onto the hot surface but only while it levitated above it. Note that no voltage was applied to any parts of the experimental setup. The optimal temperature range is close to the Leidenfrost temperature of water (~220 °C),[19] which strongly suggests that film evaporation produces the serine octamer ions. No cavitation inside the droplet was apparent, the water only being evaporated from the surface. These considerations emphasize the similarity of this method to those thermal ionization[20] experiments in which liquid-phase containing ions are evaporated into free gaseous ions.

Racemic solutions were used to test for the homochirality of the clusters. Considerably weaker cluster formation occurred from the

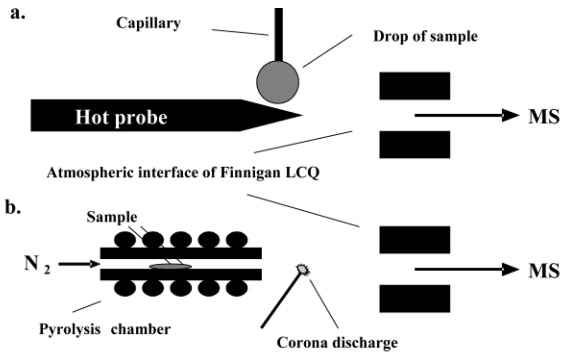

Fig. 1 Two types of thermal ionization experiments implemented using a Finnigan LCQ Classic ion trap mass spectrometer (Thermo Finnigan, San Jose, CA).

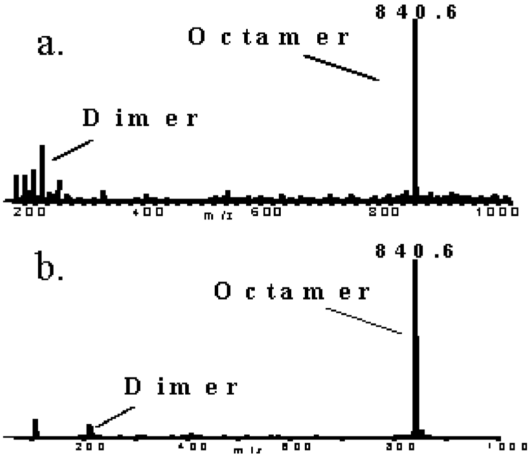

Fig. 2 Spectra of L-serine using (a) droplet evaporation, (b) pyrolysis–corona discharge ionization.

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racemate and this is taken as a preliminary indication of homochiral clustering. Both the positive and the negative ion clusters are homochiral by this test. Previous studies using ESI and SSI, confirmed by isotopic labeling, show the same result. Note that the assignment of the peak at 629 Th to the dodecamer is based on higher resolution data that show the carbon isotopic pattern. Note also that the negatively charged ion is much weaker than the positively charged octamer and the route leading to its formation is more obscure.[1,3,5]

The ionization phenomenon was compared to those occurring in the spray techniques by examining related systems. Mixtures of serine with other amino acids like cysteine or phenylalanine gave similar results to the previously described data,[11] namely substitution into the serine octamer of up to two amino acid molecules. This substitution shows similar chiral effects to those previously described using ESI or SSI. Homochiral (L–L) and heterochiral (L–D) mixtures of serine and threonine were also tested. Again, similarly to the SSI data,[10] statistical mixing of L-Ser with L-Thr was observed in the octamer, while incorporation of only two D-Thr molecules into L-Ser octamer occurred. The results overall show a high degree of similarity to the results previously obtained by spray techniques. However, in contrast to ESI and SSI, there is no charged nanodroplet formation and no analyte concentration in charged nanodroplets in the present case. This fact strongly implies that the observed structures (*i.e.* serine octamer and its substituted derivatves) are present on the surface of the evaporating drop of solution and perhaps in its bulk.

In a second set of experiments, serine crystals were pyrolysed[21] in a nitrogen gas stream, and the gaseous products were exposed to corona discharge ionization and mass analysis. The experiments were carried out using the experimental setup shown in Fig. 1b. The pyrolysis chamber was ballistically heated to 400 °C. Intense ion formation was observed between 200 °C and 300 °C. As is shown in Fig. 2b, more than 99% of the observed ion current corresponded to protonated serine octamer ions. Note that just a single species, not a statistical distribution of clusters, was observed. The resolution in the observed spectra is fairly low, probably because of interference from other, non-ionized pyrolysis products. In the case of racemic serine crystals no octamer was detected but intense monomer and dimer signals were detected; by contrast in this case a mixture of D- and L-serine gave a comparable intensity signal to pure L-serine.

A number of potential scenarios could describe the processes observed in these experiments. In principle the serine crystals might sublime to give monomers or dimers, which re-assemble in the gas phase either before or after the ionization event to give the observed octameric species. Another scenario assumes the direct transfer of octameric units from the condensed to the gas phase. The observed difference between the mixture of L- and D- serine and DL-serine crystals strongly suggests the latter mechanism. It remains a question whether pyrolysis produces serine octamer cations together with counter ions and corona discharge only neutralizes the counter ions, or whether the crystal is evaporated into neutral octamers which are protonated by the corona discharge. Taking the low temperatures involved into account, the latter mechanism seems more likely.

To demonstrate the uniqueness of serine, other amino acids such as L-cysteine and L-phenylalanine were ionized using the two ionization methods described. These amino acids are known to form clusters (cysteine forms a singly-charged hexamer, phenylalanine forms a doubly-charged undecamer) using ESI or SSI, but the magic number character of these clusters is not as pronounced as in the case of serine. These clusters also do not show large chiral effects. In both cases, only monomer and dimer ions were observed using both ionization techniques

The results of the droplet evaporation experiments demonstrate just how stable and easily formed is the serine octamer. They open the way for a wide variety of experiments on this cluster ion in the gas phase at atmosphere pressure. They add to the body of evidence that the serine octamer is an unusual chemical entity, readily generated under conditions that are relevant to homochirogenesis. The present experiments are conducted under conditions much more compatible with those thought to represent early Earth conditions than previous experiments on serine clusters which used conventional electrospray and related ionization conditions. Evaporation of aqueous solutions on hot surfaces was presumably a feasible event on the surface of the young Earth.

The pyrolysis experiments are also significant from a fundamental, structural point of view. Those results bridge the well-characterized crystal structure of serine to the structure of gaseous serine octamer ions.

Ongoing experiments are aimed at collecting macroscopic amounts of evaporated octamer and characterizing it by non-mass spectrometric methods.

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