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Do Serine Octamers Exist in Solution? Relevance of this Question in the Context of the Origin of Homochirality on Earth

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Recent MS studies have suggested that serine clusters could have played a role in the origin of homochirality on Earth. Aqueous serine solutions have been probed in order to see if serine clusters, such as those observed by MS, are present in solution. IR measurements as well as NMR chemical shift and diffusion coefficient measurements, as a function of pH and serine concentration, suggest that these clusters do not exist in solution.

Many chiral molecules present in the living world are homochiral, meaning they are only present in one of their enantiomeric forms. This is the case for all proteinic amino acids which are L and for the sugars ribose and deoxyribose, present in RNA and DNA, respectively, which are D. Homochirality seems to be a prerequisite for life and this is illustrated, for example, by the fact that the secondary structures of proteins are generally not observed if the amino acid chain is not homochiral.[1,2] The search for the origin of homochirality on Earth is directly related to the search for the origin of life.[3] Whether an initial enantiomeric excess was the consequence of a symmetry breaking or the consequence of parity violation,[3–8] amplification processes were necessary in order to obtain a first set of homochiral molecules. Starting from this set and through chirality transfers, non racemic mixtures of other molecules could have been obtained.

In the last few years, it has been suggested that amplification of an initial enantiomeric excess could have taken place through the formation of clusters of amino acids and more precisely of serine. Various serine clusters have been observed by MS using different evaporation/ionization techniques:[9–19] electrospray ionization, sonic spray ionization and thermal ionization. The octamer clearly appears as a magic number cluster which furthermore presents a marked preference for homochirality.[10,12] Based on these observations, it has been proposed that an enantiomeric amplification of serine could have occurred prebiotically by evaporation and condensation cycles.[15,18,19] It has also been shown that the homochiral gas phase clusters are able to incorporate enantioselectively glucose and other amino acids[20,21] suggesting that serine clusters could have played a role not only in amplification processes but also in transfer processes.

Even if the evaporation of serine solutions on hot surfaces leading to gas-phase clusters can be considered a plausible prebiotic scenario for enantiomeric amplification,[17] serine is a hydrophilic molecule and solution scenarios should not be neglected. The fact that serine clusters are observed in the gas phase does not in any way suggest that they are present in solution. It is, however, interesting to point out that, even if the authors of the MS papers are aware of this,[13,14,17] all the reported MS studies were undertaken using what has been classified as soft-sampling conditions because they are reported to be able to transfer non-covalently bound complexes from the solution to the gas phase.[22] The aim of this paper is to study the potential clustering of serine in solution, using experimental tools well adapted to the study of intermolecular association in the liquid phase: NMR and IR spectroscopy.

With NMR one can obtain information on the structure and dynamics of systems in solution. NMR chemical shifts are sensitive to the nucleus environment and can be expected to be different if the system under study is in a monomeric form or if it forms clusters. We therefore measured the chemical shifts of the non-exchangeable protons of serine (the α-CH and the two β-CH) as a function of concentration and pH. In all the spectra, a single resonance is observed for each of these protons, indicating that any exchange process that could be taking place in solution, such as clustering, is fast on the NMR chemical shift timescale. Under fast exchange conditions, the observed chemical shift is the weighted average of the chemical shifts in the various environments. The chemical shifts measured in this study are independent of the solution concentration (Figure 1a), which means either that the chemical shifts in the monomeric and clustered environments are near identical, or that

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non-monomeric forms are present in very low concentrations, or even that non-monomeric forms are completely absent in solution for all the studied concentrations. The measured chemical shifts are, however, a function of pH (Figure 1b). The pK_a of the carboxylic group of serine is 2.2 and that of its amino group 9.4, and it is clear that the chemical dependence on pH simply reflects the titration of these groups. It is interesting to point out that the MS studies reported in the literature highlight an influence of solution concentration[10] on the octamer peak intensity (total ion count) and also of the reported "so-called" pH for measurements made in 50:50 (v/v) methanol/water.[14]

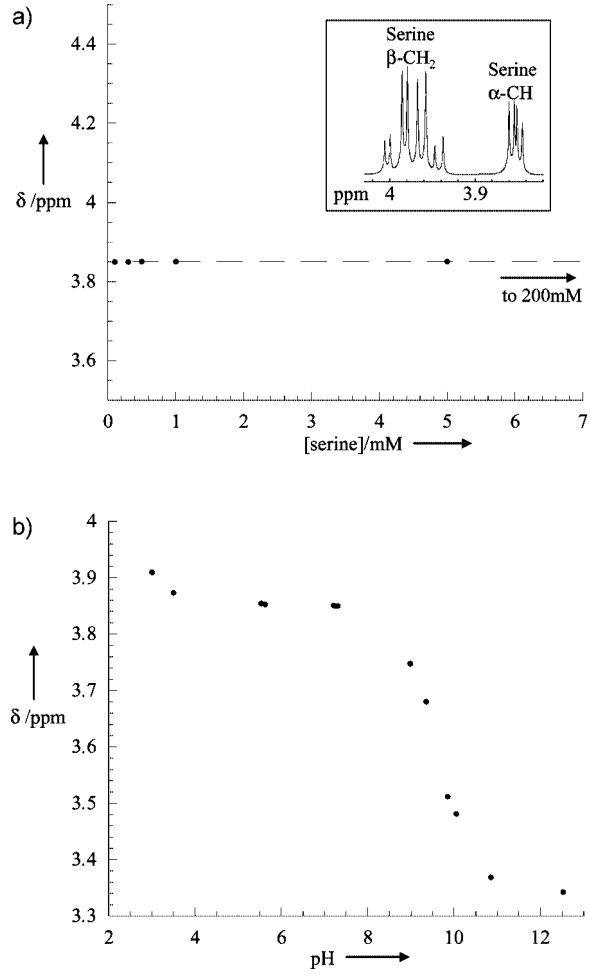

Figure 1. Chemical shift of the α-CH of L-serine in aqueous solutions at (a) different concentrations, pH = 6 (the dotted line represents the best fit, which also takes into account the data at 20 mM and 200 mM) and at (b) different pH values, 200 mM concentration. Error bars are smaller than symbols used. The inset shows the 600 MHz ^{1}H NMR spectrum of the serine non-exchangeable protons.

We measured by Diffusion Ordered Spectroscopy NMR (DOSY[23–26]) the diffusion coefficient of serine at various concentrations and at different pH. The DOSY timescale is longer than the chemical shift timescale and a mono-exponential decrease of signal intensity as a function of gradient intensity is observed. The diffusion coefficient is independent of concentration and pH (Figure 2) and is equal to $7.5 \pm 0.1 \cdot 10^{-10}$ m^{2}s^{-1}. This value is identical, within experimental error, to the value reported in the literature for serine in human seminal plasma.[27] Using Equation (1) it is possible to calculate from the diffusion coefficient the hydrodynamic radius (R_H) of a molecule in a solution of dynamic viscosity η.

$$D = k_b T/6\pi\eta R_H \quad (1)$$

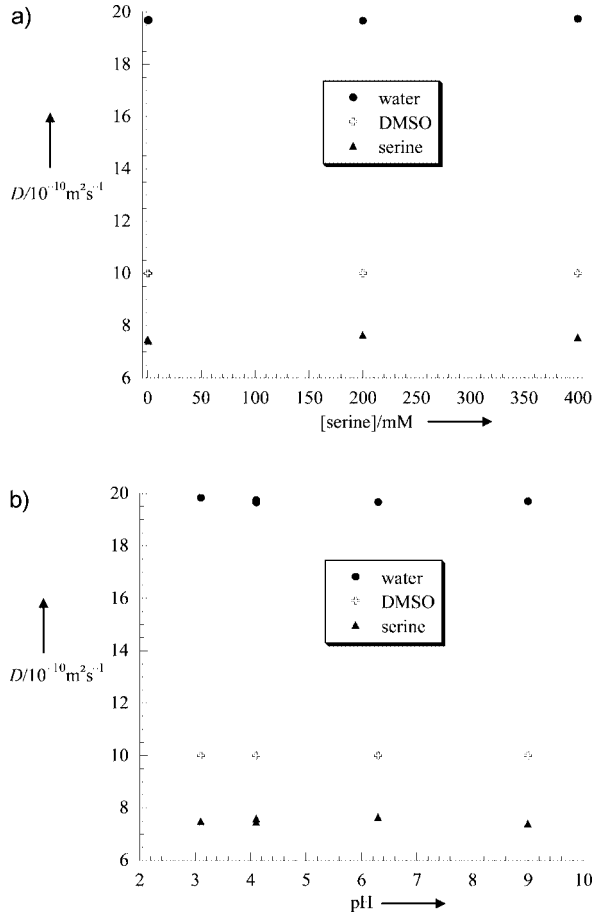

Figure 2. Diffusion coefficient of water, DMSO (used as reference) and L-serine (a) at different concentrations, pH = 7 and (b) at different pH values, 200 mM concentration. Error bars are smaller than symbols used.

For a diffusion coefficient of $7.5 \cdot 10^{-10}$ m^{2}s^{-1}, R_H is 2.9 Å, which corresponds to a section of 8.4 Å^2. This is the value that would be expected for a molecule the size of serine which is present in a monomeric form. The serine octamer, according to reported gas phase measurements[9,12] has a section of about 190 Å^2 which would correspond to a diffusion coefficient of approximately $2.3 \cdot 10^{-10}$ m^{2}s^{-1}. A $0.2 \cdot 10^{-10}$ m^{2}s^{-1} difference in the diffusion coefficient would in our case be experimentally significant which means that, if the only cluster present in solution is the octamer, the presence of 4% of octamer should be detectable. In the mass spectra reported in the literature the octamer is a dominant species but many other clusters are observed,[16]

most of which are larger in size. This suggests that the diffusion coefficient should be affected for much lower concentrations of clusters than the one estimated.

In IR spectroscopy, exchange is always slow on the spectroscopy time-scale and, if clusters are present in solution, it would be expected that bands characteristics of these species be present in the spectra. The C=O stretching should in the case of serine be sensitive to clustering. Indeed, in the models proposed for the clusters, this functional group is involved in non-covalent interactions with other units of the cluster.[10,12,13] We acquired IR spectra of serine solutions in D_2O at different concentrations (1–200 mM, pH = 4.5). No differences are observed between the spectra and the C=O stretching band is perfectly symmetrical, which is the signature of the presence of only one species in solution. Furthermore, our spectra are identical to those reported by Ramirez et al.[28] for a 500 mM serine solution in D_2O. The authors were able to assign all the bands to the monomeric form of serine and used their data to validate a DFT model of serine which forms intramolecular hydrogen bonds. In this reported study the concentration used is near to the solubility of serine in water,[29] and no clusters are reported.

Our NMR and IR results suggest that serine does not form clusters in solution or if it does, the cluster concentration is extremely low, much lower than what would be expected from the MS studies. It is therefore possible that the clusters observed by MS are generated during the evaporation step and are thus not present in solution. In order to test this hypothesis, we acquired ESI-MS spectra of 10 mM serine solutions in different solvents [water, 70:30 (v/v) water/methanol, 50:50 (v/v) water/methanol, 50:50 (v/v) water/acetonitrile and 50:50 (v/v) water/acetone]. A saturated methanol solution was also analysed. All the spectra exhibit essentially the same characteristics: the octamer is a magic number cluster and many other clusters, which exhibit up to 3 charges and are composed of at least up to 32 serine units, are observed. The only observable differences between the spectra are the relative intensity of the different peaks and this may be attributed to the difference of the volatility of the solvents. The mass spectra of serine in water and in 50:50 (v/v) water/methanol are shown in Figure 3. The fact that for all these solvents the MS spectra are essentially identical clearly suggests that the clusters are formed during the evaporation process.

Considering that serine clusters most probably do not exist in solution, their role in the origin of homochirality on Earth should be questioned. By saying this we of course do not exclude the possibility that serine clustering could occur in solution under more extreme conditions than those that we used in our study, such as extremely high acid concentrations where the solubility of serine is greatly enhanced,[29] but it is difficult to justify these extreme conditions in a prebiotic context. The role that surfaces of various materials (for example quartz, clay minerals[30] or calcite[31]) could have played in the origin of homochirality on Earth is also not discarded, but to the best of our knowledge, there is no experimental data indicating that serine clusters form on these types of surfaces.

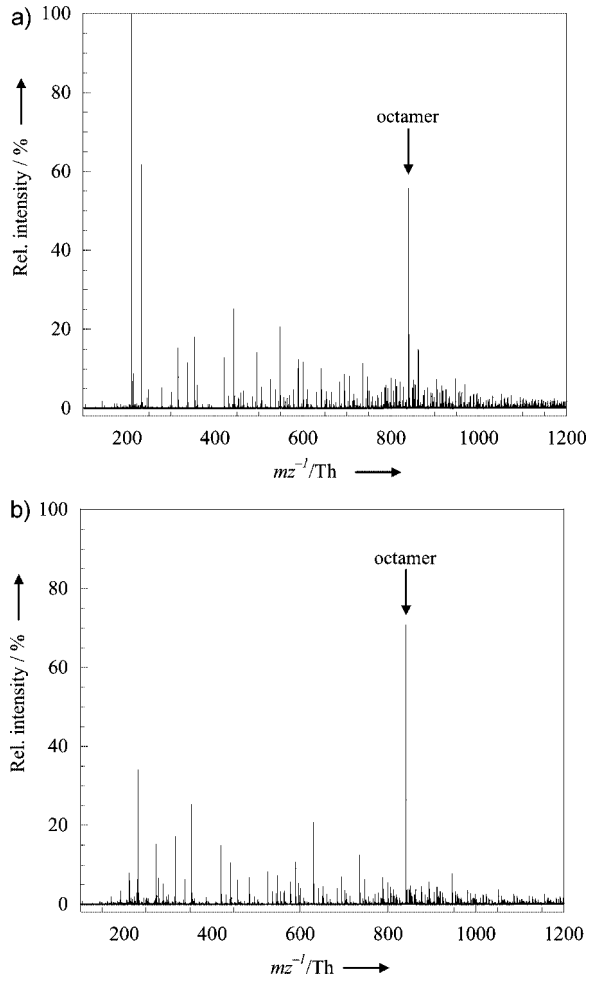

Figure 3. Mass spectra of 10 mM L-serine solutions in (a) 50:50 (v/v) methanol/water and (b) water. The octamer peak is composed of ser_8^{+}, ser_{16}^{2+} and ser_{24}^{3+}.

It is interesting to point out that, in all the papers describing the mass spectrometry study of serine clusters, serine is considered to be a primitive amino acid (see for example J. K. Koch:[20] "We consider here the transfer of chirality from serine, a presumed primitive amino acid, to other biomolecules"). It is of course essential that this be the case in order for any prebiotic model based on the serine clustering hypothesis to be plausible. Arguments which support the fact that serine is a prebiotic amino acid are, however, never presented. On the basis of our survey of the literature, it appears that serine is probably not a prebiotic amino acid and therefore could not have played a dominant role during crucial steps of prebiotic chemistry. Serine has never been detected in chondritic matter[32,33] and it therefore seems highly improbable that serine was brought to the young Earth during the post accretion and prebiotic era. Of course, all prebiotic amino acids do not necessarily have to have an exogenous origin but it is interesting to point out that serine is not detected in the products of the original "Urey-Miller" experiments which are undertaken under reducing conditions (mixture of CH_4, NH_3, H_2O and H_2) and only appears as a minor product of the experiment under-

taken under mildly reducing conditions (mixture of CH_4, N_2, H_2O, with traces of NH_3).[34]

On the basis of our NMR (chemicals shift and DOSY experiments) and IR studies, completed by complementary MS studies, we may conclude by saying that serine clusters are not present in the solutions used for the reported MS studies but are a consequence of the evaporation step. Whether serine cluster formation during evaporation is, or is not, of relevance for the prebiotic emergence of homochirality remains of course, at this stage, an open question.

Experimental Section

L-Serine was purchased from Aldrich (*ee*: 97%) and used without any further purification. D_2O and methanol were purchased from Aldrich, acetonitrile from Acros, acetone from LabScan and DMSO from Merck. For the NMR experiments, L-serine was dissolved in D_2O, and DMSO (1 μL /mL) was added as an internal reference for the chemical shift and the diffusion coefficient measurements (δ = 2.735 ppm; $D = 1{\cdot}10^{-9}$ m^2s^{-1}). The pH values of the solutions were adjusted by addition of small amounts (less than 5 μL in 700 μL) of concentrated DCl or NaOD and measured in the NMR tube. No corrections were made for isotopic effects. 1D ^1H NMR spectra were acquired at 25 °C with a 600 MHz Varian spectrometer using a 90° pulse, a 3.5 s acquisition time, a 40 s relaxation delay, a spectral width of 6000 Hz and a digital resolution 0.09 Hz/point. The diffusion coefficients were measured at 25 °C with a 300 MHz Bruker spectrometer using a Pulsed Field Gradient Bipolar Stimulated Echo sequence[35] with one homospoil gradient. The gradient intensity was varied from 2% to 95% in 16 steps. A minimum of 16 scans were recorded for each spectrum. The spectra were acquired with a 3 s acquisition time, a 2 s relaxation delay, a spectral width of 2700 Hz and a digital resolution of 0.08 Hz/point; 16 dummy scans were performed at the beginning of each experiment. Equation (2) was adjusted to the integrals of the HDO, DMSO, serine signals (S) measured as a function of the gradient value (G). Three DOSY experiments were performed on a sample and the average D value is reported.

$$S = S_0\exp(-D\gamma^2s^2G^2\delta^2\Delta_r) \quad (2)$$

where for a half-sine gradient shape: $\Delta_r = \Delta-(9/8-\pi^2/12){\cdot}\delta-(d_{16}+p_2)/2$, S_0 is the peak area in the absence of a gradient, s the gradient shape factor (for a half-sine: $2/\pi$), δ the length of the gradient pulses, Δ the diffusion delay, d_{16} the homospoil length and p_2 the length of a 180° pulse. For the IR experiments at different concentrations, the samples were prepared by diluting a 200 mM D_2O solution of L-serine. The pH values of the different solutions were adjusted to approximately 4.5 by addition of small amounts (less than 3 μL/400 μL) of concentrated DCl. Attenuated total reflection infrared (ATR-FTIR) spectra (4000–800 cm^{-1}) were acquired, in the double-sided forward-backward mode, with a Bruker IFS55 FTIR spectrophotometer equipped with an MCT detector with a resolution of 1 cm^{-1} (after 2 levels of zero-filling) and using an aperture of 3.5 mm. The spectrometer was continuously purged with dry air. For the mass spectrometry experiments, 10 mM solutions of L-serine were prepared by dissolving L-serine in the different solvents: water, 70:30 (v/v) water/methanol, 50:50 (v/v) water/methanol, 50:50 (v/v) water/acetonitrile and 50:50 (v/v) water/acetone. A saturated methanol solution was also prepared. The samples were loaded into a nanoflow capillary (Proxeon). ESI mass spectra were acquired with a quadrupole time-of-flight instrument (Q-Tof Ultima – Micromass/Waters) operating in the positive ion mode, equipped with a Z-spray nanoelectrospray source. Capillary voltages of 1–2 kV and a cone voltage of 50 V were typically used. The source temperature was maintained at 80 °C. The spectra represent the combination of 1 s scans. Data acquisition was performed with a MassLynx 4.0 system.

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