Serine sublimes with spontaneous chiral amplification†

Richard H. Perry, Chunping Wu, Marcela Nefliu and R. Graham Cooks*

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Sublimation of near-racemic samples of serine yields a sublimate which is highly enriched in the major enantiomer; this simple one-step process occurs under relatively mild conditions, and represents a possible mechanism for the chiral amplification step in homochirogenesis.

Non-racemic samples of solid serine (Ser), with enantiomeric excess (ee) values of 3% to 75%, when heated to moderate temperatures (175–230 °C) in an inert atmosphere, sublime to give serine that is enriched in the major enantiomer. For example, a serine sample 3% enriched in the L-enantiomer, heated at 190 °C for 2 h, gives a sublimate shown to be up to 98% ee L-serine. Sublimation has been used previously to isolate amino acids from natural samples[1] but changes in enantiomeric composition upon sublimation have not been observed except in an earlier qualitative study of serine.[2]

This non-mass spectrometric investigation has its basis in previous mass spectrometric studies of the homochiral preference of serine cluster ions. Positive and negative ion mass spectra of serine solutions recorded using spray ionization techniques,[3–6] or the corresponding spectra of solid samples recorded using corona discharge ionization of sublimed material,[2,7] show magic number octameric ions with a strong homochiral preference. There is evidence that the neutral octamer (Ser_8) is responsible for homochiral clustering.[2,7,8] Such clustering is a feature not shared by any of the other DNA-coding amino acids except (to a small extent) by threonine[2,9] and proline.[10] These earlier mass spectrometric studies also demonstrated that the chirality of the ionized serine octamer can be transferred to other biologically relevant molecules in ion–molecule reactions.[5]

Chiral amplification has been shown in various chemical systems.[11] For example, when monomeric building blocks with small ee's are used to form polyisocyanates, the helicity of the polymer is governed by the major enantiomer.[12] Other examples of note include the Soai asymmetric autocatalysis,[13] chiroselective adsorption of amino acids to enantiomorphous crystal faces of calcite,[14] and the asymmetric amplification of amino acids in equilibrium solid–liquid phase systems.[15] A concurrent study by Hayashi *et al.*[16] demonstrated that dissolution of near-racemic samples of proline (1.0% ee L-Pro) in $CHCl_3$–1% EtOH yields a solution that has a high ee of L-Pro due to preferential precipitation of the heterochiral species, which are energetically more favorable than their homochiral counterparts. In addition, it was recently demonstrated that some ammonium picrates form homochiral dimeric ion pair aggregates from racemic solutions in preference to the heterochiral species, and that their dimerization equilibrium constants determine whether a conglomerate or racemate is formed upon crystallization.[17] As these examples indicate, there is growing evidence for a link between chiroselective aggregation and enantiomeric amplification during a change in physical state. In this report, we investigate quantitatively the chiral amplification of serine by simple sublimation by studying the bulk neutral materials, not the gas phase ions.

An experiment was designed based on the purification technique of zone refining. By allowing multiple cycles of sublimation, mass transfer, and condensation before collection of the sublimate, it was hoped that high chiral purities could be achieved. This was indeed seen in experiments in which a heated zone was moved slowly (18 h) down a tube containing solid serine (coated on the inner surface) while sublimate was collected downstream. The sublimate was derivatized to form the *N*-(*O*)-pentafluoropropionyl-2-propyl esters and analyzed using chiral gas chromatography to separate the enantiomers.[18] Starting with ~1 g 5% ee L-serine (physical mixture of pure L- and D-crystals obtained directly from the manufacturer), the sublimate (15 mg) collected at 205 °C was found to have an ee value of 65%. The percent excess of the major enantiomer was calculated using the equation $[(\boldsymbol{L} - \boldsymbol{D})/(\boldsymbol{L} + \boldsymbol{D})] \times 100\%$, where $\boldsymbol{L}$ and $\boldsymbol{D}$ denote the areas under the peaks in ion chromatograms for the L- and D-enantiomers, respectively. When the same 5% ee L-Ser mixture was heated at temperatures other than 205 °C, a lower amplification was observed. For example, when heated at 195 °C, 210 °C and 220 °C, the ee values of L-Ser in the sublimates were 35%, 49% and 4%, respectively. These initial results demonstrate that there is a temperature regime below the melting point (mp) of serine (222 °C) in which the sublimates of non-racemic serine mixtures are enriched in the major enantiomer. Both the sublimate and the residual solid in the tube consisted largely of serine, although thermolysis products[19] were also observed in amounts depending on the temperature profile and other sublimation conditions. These products include D,L-alanine (Ala) and ethanolamine (EA) (Fig. 1).

These initial results justified systematic experiments using optimized apparatus and experimental parameters. Physical mixtures of enantiomerically pure crystals of L- and D-serine were slurried with acetone to make a paste which was coated on the inner surface of a glass tube and sublimed using the apparatus shown in Fig. 2. In these simple experiments the heating tape was held stationary.

In a typical result, when 3% ee L-Ser (100 mg) was heated at 205 °C for 2 h, the collected sublimate (1 mg) had an ee value of 69% L-Ser (Fig. 3). To determine the effect of temperature on the enantiomeric composition of the sublimate, the same 3% ee L-Ser sample was heated at different temperatures in the range

Department of Chemistry, Purdue University, West Lafayette, IN 47907, USA. E-mail: cooks@purdue.edu; Fax: +1 765 494-9421; Tel: +1 765 494-5263

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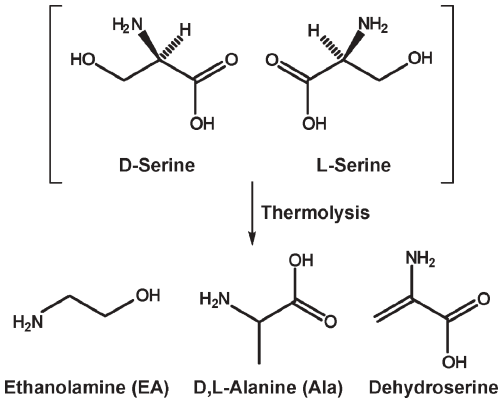

Fig. 1 High-temperature thermolysis products of serine.

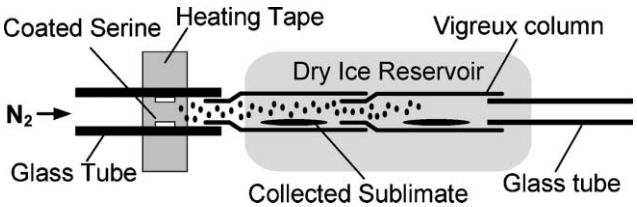

Fig. 2 Apparatus (not to scale) used to sublime serine and collect the sublimate.

175–250 °C (Fig. 4). The chiral purity of the sublimate was found to range from 68% to 92% excess of L-Ser in the optimum temperature range of 190–205 °C (◆'s in Fig. 4, where the error bars indicate the standard deviation of the ee values of the sublimate in three or more trials), with the total amount of collected sublimate ranging from 1–3 mg. The low chiral purity observed below 190 °C is consistent with earlier MS data suggesting that serine sublimes readily as monomers and non-octameric clusters.[2] The chiral purity maximizes in the temperature range 190–210 °C then falls as thermolysis (▲'s in Fig. 4) becomes favorable.

Irrespective of initial chiral compositions (varied in the range 3–99% ee L-Ser), the product composition was consistently

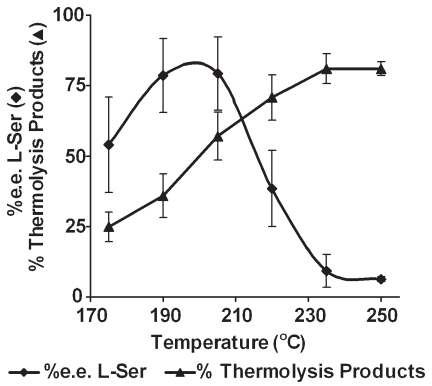

Fig. 4 Changes in chiral purity and chemical composition of the sublimate from a 3% ee L-Ser sample heated for 2 h, shown as a function of temperature. The sublimate (◆) has its highest chiral purity between 190 and 205 °C, decreasing above and below this range, whereas thermolysis (▲) shows a steady increase to a maximum at 230 °C.

68–99% ee of L-Ser using the conditions described above. When starting with a very high initial ee value, such as 99% ee of L-Ser, the sublimate had a *decreased* chiral purity (74% ee). This is interpreted to be the result of competition from racemization,[20] which presumably proceeds *via* the thermolysis product dehydroserine (Fig. 1).[19]

The thermolysis products Ala and EA make up 18% and 34%, respectively, of the signal in the ion chromatograms for the sublimate at 205 °C (Fig. 3). With increasing sublimation temperature, the proportion of thermolysis products in the collected sublimate increases. A plot of the relative amount of thermolysis products as a function of temperature (▲'s in Fig. 4), shows that the chiral purity of the sublimate decreases as the thermolysis products increase. This suggests that chiral accumulation upon sublimation and thermolysis are competitive processes that have maximum rates at different temperatures.

When 3% ee serine mixtures are sublimed, the chiral composition of the residue is typically indistinguishable from that of the starting material. However, changes in the residue due to heating

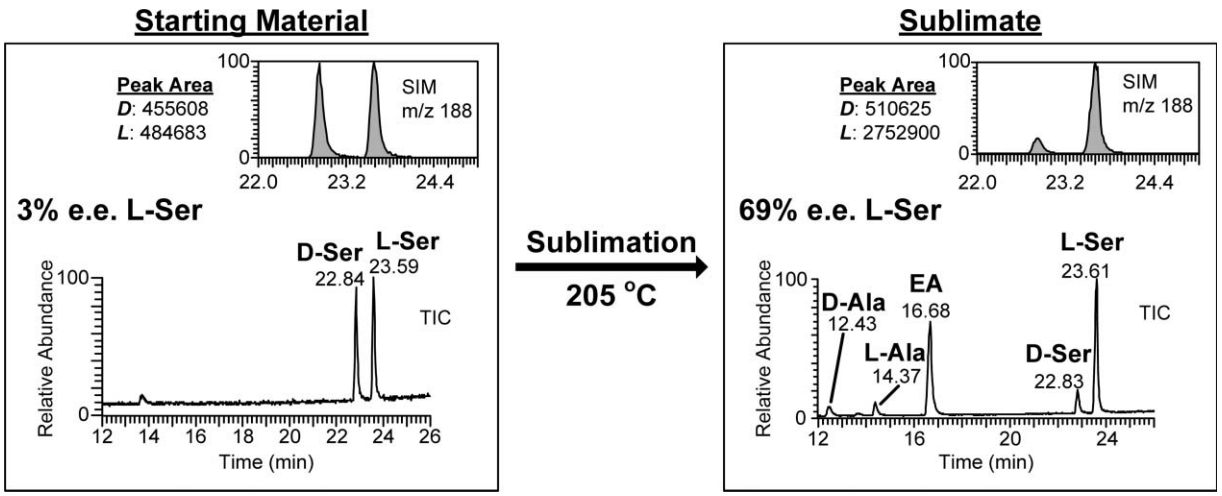

Fig. 3 Total ion chromatograms (signal *vs.* time) showing the composition of the starting material and sublimate. The ion chromatogram for the sublimate shows that it contains the thermolysis products ethanolamine (EA) and alanine (Ala) in addition to serine (Ser). The serine in the sublimate has a much higher chiral purity than the starting material.

do occur and can be inferred from changes in the sublimate. When the residue of a 3% ee L-Ser sample (heated for 2 h) was cooled to room temperature and then reheated for 2 h, the chiral purity of both sublimates (collected separately during each 2 h segment) was 68% ee. However, when heated for another 2 h (for a total of 6 h heating time), the ee value of the sublimate dropped to 53%. We hypothesize that racemization during the long heating period accounts for the lower enrichment. The effect of racemization is even more clearly observed in the residue at higher ee values of L-Ser. When a 98% ee L-Ser sample is heated for 2 h at temperatures ranging from 175–250 °C in 15 °C increments, the chiral purity of the residue decreases to 79% at 205 °C and to 45% at 250 °C.

One possible explanation of the data is that preferential sublimation from the L-crystals in the solid serine mixtures occurs. To investigate this possibility, physical mixtures of the enantiomers were again investigated but, in these new experiments, recrystallized enantiomers (from H_2O) were used. A 2% ee L-Ser mixture sublimed for 2 h was found to yield a sublimate with 61% ee of L-Ser at 205 °C, while a 3% ee D-Ser mixture yielded a sublimate with a chiral purity of 47% ee D-Ser at 200 °C. Although the enrichment values obtained in these experiments were somewhat different and slightly smaller than those seen in the experiments using non-recrystallized material, they clearly demonstrate that preferential sublimation of particular crystal types cannot account for the observed chiral amplification.

To further demonstrate that enantiomeric separation upon sublimation is an inherent feature of serine, solid chemical mixtures of the enantiomers were analyzed. In these experiments, solid serine obtained from recrystallization of a 1% ee L-Ser solution was heated at 205 °C for 2 h. The sublimate had a chiral composition of 27% ee L-Ser. This provides further evidence that the observed phenomenon is not due to preferential sublimation from homochiral L-crystals.

We hypothesize that enantioselective sublimation of serine occurs *via* formation of the magic number homochiral octamer. Therefore, amino acids that do not form magic number clusters, such as Ala, should not exhibit this characteristic. When solid D- and L-Ala (both 99% purity) were physically mixed to produce an 8% ee L-Ala mixture, the sublimate formed at 190 °C had an ee value of 4% of L-Ala. Similarly, when solid D- and L-threonine (Thr) (both 99% purity) were physically mixed to produce a mixture with a 7% ee of L-Thr, the sublimate at 208 °C had an ee value of 1.2% ee of L-Thr. These data indicate that, at least in these cases, chiral amplification was not observable.

The data presented here provide evidence for a large degree of chiral accumulation in the course of single-step sublimation. In an earlier[2] qualitative study from this lab, it was shown that serine sublimes to give serine octamer ions which are enriched in the major enantiomer. Earlier electrospray ionization processes showed the same result and, in one experiment, the gas phase ions were soft-landed and collected and gave an enantiomerically enriched serine sample.[6] Klussmann *et al.*[15] demonstrated that a number of amino acids undergo asymmetric amplification based on their equilibrium behavior at the solid–liquid phase, with serine showing the most marked effect. The present results demonstrate that an almost pure enantiomer separates from near-racemic solid serine during the solid-to-vapor phase transition. The combination of chiral enrichment and physical separation (transport of the purified enantiomer), if it occurs repeatedly in a region with a modest temperature gradient, can readily be imagined to be a source of chirally pure serine. This enriched material could possibly transfer chirality to other amino acids and sugars, processes for which laboratory precedent has been shown.[5] The laboratory data presented in this paper strengthen the indirect evidence based on serine chemistry for its possible involvement in homochirogenesis.

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