An astrophysically-relevant mechanism for amino acid enantiomer enrichment

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The sublimation of low ee amino acids was examined while exploring simple mechanisms by which high ee amino acids can be generated under conditions that exist in space; significant enantioenrichment of a variety of amino acids by sublimation was achieved.

There are longstanding theories[1] that homochirality on Earth is derived from extraterrestrial organic material. Natural amino acids occur exclusively in the levorotatory (L) or left-handed form. The origins of this phenomenon are unknown,[2] but homochirality may reduce entropic barriers to the formation of large organized structures,[3] which supports the notion that it is a prerequisite for life.[4] Recent attempts to understand the origin of life on Earth have been shaped by the possibility that most of the organic material on Earth was brought in by comets, asteroids, or the Earth passing through interstellar dust clouds.[5] Only a fraction of the enantiomeric excess (ee) of amino acids known to exist in space by their observation on meteorites[6] can be accounted for experimentally,[7] and no suitable explanation for the observed ee has been suggested.[8]

Amino acids exist in space and it has been demonstrated[9] that, under space-like conditions, many amino acids are produced from a mixture of water, ammonia, methanol, carbon monoxide, and carbon dioxide. The Earth likely encounters several organic rich molecular clouds as it revolves around the galaxy,[5] and 60000 million tons of amino acids are estimated to have arrived on Earth during the period when it was cool enough for amino acids to survive.[5d] The observation that nonracemic amino acids of extraterrestrial origin have been found on meteorites with up to 15% ee supports an extraterrestrial mechanism for the origin of homochirality.[6] Theories on the origin of homochirality must be able to account for these observations.[8] Amino acids in space are subjected to circularly polarized radiation or other processes that could cause partial symmetry breaking.[1b] Astronomical sources of circularly polarized light (CPL) have been observed in the IR range[1c] and include electromagnetic radiation from white-dwarf binaries that can be circularly polarized up to 50%.[8] Ultraviolet light plays an important role in the organic photochemistry of dust particles[5d] and there are a number of mechanisms by which UV irradiation can become circularly polarized in star forming regions.[8] The destructive asymmetric photochemistry of biologically relevant molecules has been verified experimentally,[1] although only small ee's have been produced in amino acids. The highest value reported is 2.6% for leucine in solution,[7a] a value recently reproduced under space-like conditions.[7b] It is unclear how the relatively high ee's observed for meteorite amino acids could be produced using this mechanism as it would require destruction of virtually all the material.[8] The observed ee for these compounds is in fact larger than would be theoretically allowed if the photolysis by CPL in the UV range had been the only source of their asymmetry.[10] If we accept the observations that symmetry breaking can occur by CPL, the problem of homochirality becomes one of explaining how amplification could take place to enhance the initial asymmetry.[10]

The discrepancy between the ee's of amino acids present on meteorites and those of amino acids produced using CPL to achieve asymmetric photolysis led us to explore simple mechanisms for asymmetric amplification under relevant conditions. Presumably astrophysical environments involve solid amino acids, a vacuum, and extreme differences in temperature. Temperature gradients may occur by an object blocking light or heat, which may result in sublimation of solid volatiles. During sublimation a solid can physically move from a warmer location to a colder one *via* the gas phase (Fig. 1). Partial separation of enantiomorphs from racemates under preparative[11] and analytical (mass spectrometry vaporization) conditions[12] has been described in isolated cases. While enantioenrichment by sublimation is much less well known than enantioenrichment by crystallization, it is understood in terms of the vapour pressures of solid racemates and enantiomers differing just as their melting points and solubilities differ.[13] Enantiomeric ratios of amino acids that are subjected to sublimation are, however, believed to be preserved,[14] except in the case of "magic-number" serine clusters.[15] These clusters can be detected in the gas phase when generated through sublimation[15b] or electrospray ionization.[15a] A recent report[15c] by Cooks and coworkers prompted us to disclose our results on enantioenrichment of amino acids by sublimation. They illustrate that a serine

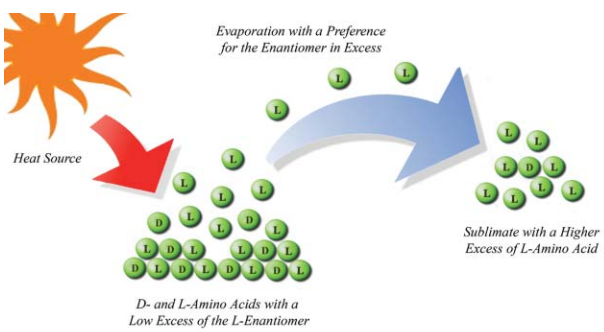

Fig. 1 Enantioenrichment of a low ee amino acid by sublimation.

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sublimate with enhanced ee can be isolated, but two other amino acids examined (including alanine, *vide infra*) did not undergo enantioenrichment, and it is hypothesized that the "magic-number" serine octamer, which is serine specific, is responsible for the effect.[15c]

In our initial experiments a 10% ee sample of leucine (the amino acid of choice for CPL studies) was prepared by mechanical stirring (Table 1, entry 1), added to a standard sublimation apparatus, and subjected to slow, incomplete, sublimation (0.2 mmHg, 90 °C, water cooled cold finger). Under these conditions only a small fraction of the sample undergoes sublimation. Surprisingly, the enantiomeric excess of the sublimate was found to be 82%.

Concerned that mixing of the racemate and the enantiomorph was incomplete at the molecular level, we examined the sublimation of low ee leucine prepared with different mixing methods. Mixtures of the appropriate amounts of D,L-leucine and L-leucine were obtained by vigorous shaking for 40 min (Table 1, entries 2 and 3), extensive grinding with a mortar and pestle (entries 4 and 5), or fully dissolving the compound in boiling water followed by *in vacuo* removal of the solvent (entries 7, 10, 12). After partial sublimation, some samples were subjected to sublimation a second time (entries 6 and 11). In all cases uniformity of the starting amino acid samples was examined by taking multiple random small samples ($\sim$1 mg) and measuring the enantiomeric excess. Partially sublimed L-enriched leucine always gives highly enantioenriched sublimates regardless of the mixing procedure. It was also established that the use of a vacuum is not essential for ee enrichment by rapid and vigorous (1 min, entry 8), or slower sublimation (heating in a sublimation apparatus from 179–191 °C over a period of 25 min, entry 9). A mixture of 2.6% ee L-leucine (the highest value achieved by selective photodestruction of an amino acid with CPL),[7] sublimed under vacuum at 100 °C for 4 h (entry 10), gave a thin film of sublimate which had an ee of 27%. L-Leucine of 1% ee was partially sublimed over 21 h to provide the sublimate with an ee of 39% (entry 12).

Table 1 Enantioenrichment by partial sublimation of leucine with low enantiomeric excess

Entry	Starting ee[a,b]	ee sublimate[b]	T (°C)	mmHg	Time
1	10%	82%	90	0.2	14 h
2	9%	89%	120	0.005	72 h
3	9%	84%	120	0.1	96 h
4	9%	75%	125	0.01	22 h
5	9%	72%	125	0.1	18 h
6[c]	<9%	80%	125	0.01	96 h
7[d]	6%	37%	120	0.02	2 h
8	10%	25%	270	760	1 min
9	10%	39%	179–191	760	25 min
10	2.6%	27%	100	0.2	4 h
11[c]	<2.6%	17%	95	0.2	38 h
12	1%	39%	100	0.1	21 h

[a] Leucine with low ee was prepared by mixing the appropriate amounts of racemic leucine with its L-enantiomer. This mixture was homogenized by extensive mechanical stirring (entries 1, 8, 9), shaking (entries 2 and 3), grinding with a mortar and pestle (entries 4, 5, 6) or dissolving in water followed by *in vacuo* removal of the water (entries 7, 10, 11, 12). [b] Enantiomeric excess was determined by chiral GC after derivatization with ethyl chloroformate. [c] A depleted sample was submitted a second time to sublimation. [d] Crystalline leucine that contains water undergoes "bumping" which causes amino acid to splatter over the sublimation vessel. While this splatter contaminates the sublimate, substantial enantioenrichment is still observed.

We examined enantioenrichment with other α-amino acids. It is worth noting that we were interested in the generality of the effect and so the examples were not optimized to provide the highest ee values possible. As in the case of leucine, only a very small amount of the sublimate relative to the starting material was collected (typically a few mg out of a g of amino acid). A 7% ee sample of phenylalanine at 170 °C under a vacuum of 0.005 mmHg provided a sublimate with 23% ee (Table 2, entry 1). The residue from this material was subjected to sublimation a second time at a slightly higher temperature of 210 °C under a vacuum of 0.01 mmHg to provide a sublimate with an ee of 26% (entry 2). Alanine was also found to undergo enantioenrichment, although we arbitrarily used slightly lower temperatures (170 °C *vs.* 190 °C) than in previous work,[15c] and a standard sublimation apparatus. Starting from 5% ee, after one hour at 170 °C at 0.01 mmHg, the sublimate had an ee of 19% (entry 3). Lower temperature (entry 4), and atmospheric pressure (entry 5) also allow enantioenrichment of alanine. Valine (entries 7 and 8) exhibits similar behaviour. The relatively fragile heteroatom substituted amino acids methionine and serine also readily undergo enantioenrichment on partial sublimation under vacuum (entries 9 and 10).

These results clearly demonstrate that amino acids undergo significant enantioenrichment *via* partial sublimation. While it is tempting to speculate that these observations are caused by "magic-number" clusters of amino acids,[15] the enantioenrichment of amino acids by sublimation is likely caused by intermolecular interactions that cause some amino acid enantiomers and racemates to behave differently in general. The racemates of the amino acids examined here form 1 : 1 mixtures of D and L at the level of the unit cell and likely show stronger intermolecular interactions than their enantiopure counterparts. The different relative solubility of an enantiopure compound and the corresponding racemate forms the basis of enantioenrichment by crystallization.[13] This generally well understood effect has become the subject of recent interest as it has been shown that it provides thermodynamic explanations for non-linear effects in amino acid catalysis.[16]

Table 2 Enantioenrichment of various α-amino acids upon partial sublimation

Entry	Amino acid	Starting ee[a,b]	ee sublimate[b]	T (°C)	mmHg	Time
1	phenylalanine	7%	23%	170	0.005	18 h
2[c]	phenylalanine	<7%	26%	210	0.01	6 h
3	alanine	5%	19%	170	0.01	1 h
4	alanine	5%	17%	125	0.01	72 h
5	alanine	5%	24%	170	760	1.5 h
6	alanine	0.5%	6%	155	0.02	18 h
7	valine	8.5%	28%	145	0.03	22 h
8	valine	8.5%	15%	205	760	1.5 h
9	methionine	10%	44%	155	0.02	19 h
10	serine	6%	38%	220	0.01	1.5 h

[a] Enantiopoor amino acids were prepared by mixing the appropriate amounts of racemate and L-enantiomer. The mixtures were homogenized by extensive grinding with a mortar and pestle, except for the mixture in entry 6 which was dissolved in EtOH followed by *in vacuo* removal of the solvent. [b] The enantiomeric excess was determined by chiral GC after derivatization with ethyl chloroformate, except for serine (entry 10) of which the ee was determined directly by reverse-phase chiral HPLC. [c] A depleted sample was resubmitted to sublimation.

The current results have implications regarding the origin of homochirality. Micrometeorites can be heated to very high temperatures during atmospheric entry deceleration and sublimation has been proposed as a mechanism by which volatile organic compounds survive atmospheric entry heating by vaporizing off of the surface of interplanetary dust particles, micrometeorites, *etc*.[5] The idea that organic rich dust evolves from evaporating comets and allows amino acids to reach Earth without being destroyed has geochemical evidence,[5c] where the vaporized amino acids presumably condense in the upper atmosphere and gradually descend to Earth. These processes for seeding Earth would not just allow enantioenriched amino acids to arrive, but serve to amplify the ee in the process. The modest ee's known to be present in meteorites or interplanetary dust particles would be amplified during entry. Furthermore, if the enantiomorph preferentially sublimes due to atmospheric heating not only would higher ee material be separated from racemic material, but continued rapid heating of material that had not sublimed may serve as a mechanism to destroy the racemate. Similarly, if CPL contains the energy to both cause partial enantioselective photodestruction of amino acids *and* simultaneous vaporization of the enantioenriched material away from the energy source, an enantioenriched sample would grow in mass while the enantiopoor source sample shrinks. This scenario allows for much higher ee's of amino acids to be formed than previously envisioned, without almost complete destruction of the initial material.[8]

The current data have experimental implications which might affect the Mars Organic Detector (MOD), a device currently under development for both NASA and the European Space Agency.[17] Accurate accounts of nonracemic extraterrestrial materials and their degree of enantioenrichment may only be possible by space missions, such as the experiments carried out on Mars as part of the 1976 NASA Viking program. However, equipment designed to identify organic molecules failed to detect even those expected from meteorite bombardment, perhaps due to decomposition on the Martian surface,[18] or insensitivity of the apparatus used.[19] It has also been demonstrated that the instrumentation of the Viking lander could not detect *Escherichia coli* at levels of several million cells per gram.[20] Investigating the Viking analysis procedures and what is known about the organic compounds likely to be found on Mars is timely as the search for organics continues to be a key science goal for future missions. The MOD is currently under development for missions to search for evidence of life on Mars.[17] The observation of highly enantiomerically enriched amino acids on other planets would suggest that extraterrestrial life did or does occur there. The device searches for trace levels of amino acids and determines their enantiomeric excess. A key process in its operating procedure is the heating of soil under vacuum to cause volatiles to sublime onto a cold finger. Field tests of the MOD illustrate that not the entire amino acid sample is extracted and only a very small fraction of the amino acids present in the sample sublime.[17] Based on our results this would lead to enantiomer enrichment. Future field tests of the MOD, and related devices, should include samples of variable amino acid ee in order to determine whether there is any enantioenrichment using the detector.

In summary, we have established that sublimation of amino acids with a low ee by heating, in the presence or absence of a vacuum, can be an efficient mechanism for asymmetric amplification. This property is not limited to serine and several amino acids, such as alanine, readily undergo amplification, pointing to a simple mechanism. To fully elucidate this mechanism detailed studies will be necessary. These results support the idea that homochirality may have its origins in space. It may also be necessary to redesign some of the current analytical strategies used in the search for extraterrestrial life.

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