

Racemic amino acids from the ultraviolet photolysis of interstellar ice analogues

Max P. Bernstein^{*†}, Jason P. Dworkin^{*†}, Scott A. Sandford[†], George W. Cooper[†] & Louis J. Allamandola[†]

^{*} The Center for the Study of Life in the Universe, SETI Institute, 2035 Landings Drive, Mountain View, California 94043, USA

[†] NASA-Ames Research Center, Mail Stop 245-6, Moffett Field, California 94035-1000, USA

The delivery of extraterrestrial organic molecules to Earth by meteorites may have been important for the origin and early evolution of life¹. Indigenous amino acids have been found in meteorites²—over 70 in the Murchison meteorite alone³. Although it has been generally accepted that the meteoritic amino acids formed in liquid water⁴ on a parent body, the water in the Murchison meteorite is depleted in deuterium⁵ relative to the indigenous organic acids^{6,7}. Moreover, the meteoritical evidence⁸ for an excess of laevo-rotatory amino acids is hard to understand in the context of liquid-water reactions on meteorite parent bodies. Here we report a laboratory demonstration that glycine, alanine and serine naturally form from ultraviolet photolysis of the analogues of icy interstellar grains. Such amino acids would naturally have a deuterium excess similar to that seen in interstellar molecular clouds, and the formation process could also result in enantiomeric excesses if the incident radiation is circularly polarized. These results suggest that at least some meteoritic amino acids are the result of interstellar photochemistry, rather than formation in liquid water on an early Solar System body.

As the most ancient and pristine bulk material studied in the laboratory, primitive meteorites are the clearest windows to the birth of the Solar System. Planetary systems such as our own are believed to form from the collapse of an interstellar dense molecular cloud composed of gas and sub-micrometre sized grains. In such 'dark' clouds the temperatures are low ($T < 50$ K), and all but the

most volatile species (that is, H₂, He, Ne) condense onto grains, coating them with a thin layer of ice⁹. This ice is composed primarily of amorphous H₂O, but usually also contains a variety of other simple molecules^{9,10}, such as CO₂, CO, CH₃OH, and NH₃. Laboratory studies¹¹ and astronomical observations^{10,12} indicate that radiation processing of such ices can create complex organic compounds¹³. Many of the organic molecules that are present in carbonaceous chondrites (primitive carbon-rich meteorites) and comet and asteroid dust are thought to come, at least in part, from the ice and complex compounds constructed in the interstellar medium (ISM).

Perhaps the most convincing molecular evidence for the interstellar heritage of meteoritic molecules is their high deuterium (D) enrichment^{3,14}. At the low temperatures in dense molecular clouds deuterium fractionation is efficient and elevated D/H ratios are seen in grain mantles¹⁵; such increased ratios are also found in several gas-phase interstellar molecules, including amino acid precursors, such as formaldehyde and ammonia¹⁶. Although it had been accepted that the deuterium in meteoritic organics indicated that their precursors formed in the ISM, the actual hydroxy and amino acids are still commonly believed to have formed on the asteroid or comet parent body from reactions in liquid water⁴ that, at least in Murchison, was apparently deuterium poor⁵. It is difficult to explain how these compounds retain relatively high amounts of deuterium, let alone how it is distributed. For example, it seems contradictory that the hydroxy acids in the Murchison meteorite have one-third as much deuterium as the amino acids, and yet have a lower rate of deuterium exchange in water than amino acids^{3,7}. If, however, the hydroxy and amino acids had formed in the ISM their deuterium enrichment would be a logical consequence of the photochemistry of already deuterium-enriched pre-solar ices.

The laboratory experiments described here were designed to elucidate potential pathways from interstellar chemistry to the organic molecules extracted from meteorites. We have conducted laboratory experiments at temperatures, pressures and radiation conditions that are representative of the interstellar clouds from which planetary systems form. In a series of experiments, gases were vapour deposited onto a substrate at 15 K forming an ice film consisting primarily of amorphous H₂O with other compounds over a range of concentrations (0.5–5% NH₃, 5–10% CH₃OH and 0.5–5% HCN, relative to H₂O). These solid mixtures are

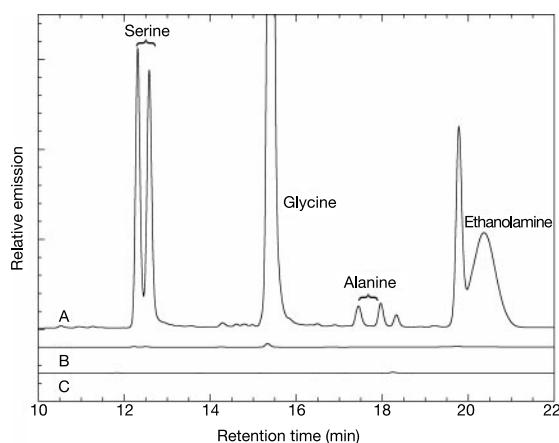


Figure 1 Amino acids are formed by the UV photolysis of a realistic interstellar ice analogue. This is demonstrated by the comparison of HPLC traces of derivatized amines resulting from: trace A, the UV photolysis of an H₂O:CH₃OH:NH₃:HCN = 20:2:1:1 ice showing that the amino acids serine, glycine and alanine, as well as other molecules, are produced; trace B, a control of the same ice with no UV photolysis; and trace C, a procedural blank. In trace A, a single peak indicates the presence of the amino acid

glycine but the chiral fluorescent tag, which separates enantiomeric amines, causes the racemic serine and alanine to appear as pairs of peaks. Differing molar absorptivities of the labelled D,L serine and alanine diastereomers account for asymmetry in the peak pairs. The unlabelled peaks are unidentified amines. The racemic nature of the serine and alanine and the absence of prominent peaks in traces B and C indicate that contamination is not significant.

representative of the composition of interstellar ice mantles in dense clouds and towards forming stars (protostars). For example, relative to H₂O, NH₃ has been observed at 10–30% in the ice around the massive protostars NGC7538¹⁷ and GCS3¹⁸, and CH₃OH has been commonly observed in clouds around forming stars¹⁹. Thus, H₂O, CH₃OH and NH₃ are reasonable starting materials because they are among the most abundant molecules frozen onto grains in the dense ISM. In addition, it is reasonable to include HCN because it is abundant in cometary coma and the dense ISM²⁰, where the majority of HCN should be frozen onto grains. Other simple molecules present in interstellar ices at comparable or greater abundances (CO, CO₂) rapidly form *in situ* in our experiments as a result of ultraviolet (UV) photolysis^{13,21}.

The interstellar ice simulations were performed in a cryogenic sample chamber, and great care was taken to exclude contamination and ensure that the starting materials did not react prior to deposition. Amorphous H₂O ices containing CH₃OH, HCN and NH₃ were vapour-deposited at temperatures and pressures representative of dense molecular clouds (15 K and 10⁻⁸ torr, respectively) while simultaneously exposed to UV radiation characteristic of the ISM. On warming under dynamic vacuum, the ice sublimates, leaving behind an organic residue that was analysed by gas chromatography–mass spectrometry (GC-MS) and high-precision liquid chromatography (HPLC). In GC-MS, the identity of molecules are ascertained from both their retention time and mass fragmentation patterns. The HPLC allows us to identify amines bearing a fluorescent label by the retention time with the co-injection of authentic standards.

N-formyl glycine, cycloserine (4-amino-3-isoxazolidinone) and glycerol were detected in the organic residue before hydrolysis, and glycine, alanine, serine, glycerol, ethanolamine and glyceric acid were observed by GC-MS after hydrolysis. Amino malonic acid and hydantoin were also tentatively identified. After hydrolysis, glycine, alanine, serine urea and ethanolamine were detected by HPLC (Fig. 1). The carbon yield of glycine, the most abundant amino acid when starting from methanol, was typically about 0.5%. Most biological amino acids are chiral, meaning that they exist in two mirror image (enantiomeric) forms—dextro-rotatory (D) and laevo-rotatory (L). Living systems employ L-amino acids almost exclusively, while the amino acid products of (abiotic) laboratory syntheses are usually a 50:50 mixture of D and L (a racemic mixture). The chiral amino acids produced in our experiments—serine and alanine—were determined to be racemic, (100.1 ± 1.6)%, within the integration error, providing evidence that they were not contaminants. This is also demonstrated by controls showing that if the UV was omitted the amino acids detected by HPLC (Fig. 1, trace B) are 1,000 times less abundant than in otherwise identical experiments where UV light was included during deposition. In addition, GC-MS of organics produced in experiments consisting of fully ¹³C labelled starting material showed fully labelled products, further confirming that contamination is minimal.

We detect N-formyl glycine and cycloserine before hydrolysis, but observe that the abundances of these molecules diminish with hydrolysis along with a concomitant appearance of glycine and serine. This strongly suggests that at least part of the amino acids we observe by HPLC after hydrolysis had formed as individual molecular species, and are not degradation products of macromolecules, such as HCN polymer²². We recently reported rates of amino acid decomposition on exposure to vacuum UV under interstellar conditions²³. This allowed us to put constraints on the lifetimes of amino acids in the ISM, both in the gas-phase and in ices thin enough to be penetrated by UV light. Those results are consistent with this report suggesting that meteoritic amino acids could have formed in the ISM. However, taken together they suggest that the precursors are generated by UV radiation in the ice at low temperature, but the amino acids themselves probably do not form until the ice is warmed, and photo-products can react with one another. As

our ices contain HCN, NH₃ and H₂CO (formaldehyde, from the photolysis of CH₃OH), it is possible that the formation mechanism is related to the Strecker synthesis—the reaction of these three molecules in liquid water to make glycine⁴. However, mechanisms involving radicals have also been proposed²⁴.

Recent careful measurements have established that certain indigenous, non-biological, meteoritic amino acids favour the L form⁸. This would be very difficult to explain using exclusively aqueous parent-body reactions, but astronomical observations of circularly polarized radiation in OMC-1²⁵ have lent credence to the notion that a radiative process might account for such enantiomeric excesses of amino acids in meteorites²⁶. However, only inefficient mechanisms based on enantio-selective photodestruction of a racemic starting population have been demonstrated in the laboratory²⁷. Thus, it had been generally assumed that a radiative process was synthesizing such chiral molecules⁸, but formation of racemic amino acids under simulated interstellar conditions in the laboratory had not been demonstrated until now.

Thus, ice photochemistry potentially provides a single simple explanation for the presence, deuterium-enrichment, and enantiomeric excess of at least some amino acids and related compounds in meteorites. Furthermore, whereas parent body reactions in liquid water require conditions that are relatively uncommon, dense molecular clouds, being thousands of light years across, are vast, ubiquitous, chemical reactors. As the materials from which all stellar systems are made pass through such clouds, amino acids should have been incorporated into all other planetary systems, and thus been available for the origin of life. □

Methods

Materials

The water was purified with a Millipore Milli-Q water system to 18.2 MΩ. Methanol (Aldrich, HPLC grade, 99.93%) and water were freeze-pump-thawed three times before being transferred under vacuum into the glass bulb holding the matrix gases. Anhydrous NH₃ (Matheson) was transferred under vacuum into the glass bulb holding the sample gases without any purification. HCN was generated from the addition of concentrated H₂SO₄ (Poly Research Corp.) to KCN (Mallinckrodt) under vacuum followed by vacuum distillation of the HCN. A 0.03 mm Ni (99.9%) foil (Goodfellow) was used as a substrate.

Sample preparation

Our interstellar ice simulation chamber is a high-vacuum matrix isolation apparatus described in detail elsewhere²¹. Two gas bulbs with H₂O and CH₃OH—one also containing HCN and the other NH₃—were vapour-deposited simultaneously but separately onto a high purity Ni substrate held at 15 K. Care was taken to ensure that the two different gas mixtures (one with HCN, the other NH₃) did not come into contact with one another until frozen onto the substrate. Infrared spectra of ices deposited onto a CsI substrate demonstrated that they had not reacted during the course of deposition.

The mixed molecular ices were UV-photolysed during deposition with a microwave-powered, flowing hydrogen, discharge lamp²⁸. This lamp produces $\sim 2 \times 10^{15}$ photons cm⁻² s⁻¹, the flux being nearly evenly divided between the Lyman α line and a roughly 20-nm-wide molecular transition centred at 160 nm. Under such conditions, the time that elapses between photons arriving in the same molecular neighbourhood is ~ 13 orders of magnitude longer than molecular relaxation times, so multi-photon processes are not relevant. Typically, each sample was photolysed for the equivalent of ~ 30 min $\sim 4 \times 10^{18}$ photons cm⁻² per 0.1 μ m of ice. This is a reasonable interstellar dose, corresponding to ~ 500 yr at the edge of a dense cloud and $\sim 1 \times 10^5$ yr at an optical depth of 5 within a dense molecular cloud²⁸.

After deposition and photolysis were complete, the ices were warmed at ~ 2 K min⁻¹ under dynamic vacuum at $\sim 10^{-8}$ torr to room temperature. Under these experimental conditions the ice sublimates during warm-up, leaving behind an organic residue. At no point during this procedure does the ice melt, nor was the residual organic material exposed to any liquid until the hydrolysis for HPLC analysis. This allowed us to analyse the suite of organic compounds present both before and after the hydrolysis step. The Ni substrate on which the experiment has been performed was then removed from the vacuum system. To guard against contamination of the residue by biological amino acids and other organics, the Ni substrate, all glassware, and tools coming in contact with it, were cleaned of organic material by baking at 550 °C in air for more than 15 h prior to use.

Hydrolysis and residue analysis

The Ni substrate was removed from the vacuum system and cut in two. One half was treated with bis-trimethylsilyltrifluoroacetamide containing 1% trimethyl chlorosilane (Alltech) and anhydrous pyridine in a 1:3 ratio. This solution was sonicated and stirred for 30 min, after which 1 μ l was injected into the GC-MS. Such samples were never exposed to liquid water at any time during formation or analysis.

The other half of the Ni substrate was shaken with 18 MΩ Millipore water, then

removed, and the solution added to an equal volume of 12 M HCl (Ultrex II ultrapure reagent, Baker) and heated for 16 h at 100 °C in an organic-free sealed glass ampoule. This material was dried *in vacuo* to remove water, HCl and any volatile organic acids, then analysed by GC-MS as above. GC-MS was performed on a Thermoquest-Finnigan GCQ with an injector temperature of 240 °C and a DB-17ms-60m (J&W Scientific) column at an initial temperature of 70 °C increasing at 5° per min to 240 °C.

Samples for HPLC were hydrolysed as above, and after drying, 100 µl of 10 mM pH 9.5 sodium borate was added to the tube which, after mixing, was re-dried *in vacuo* to remove volatile amines. The sample was analysed via the OPA/NAC fluorescent labelling and chromatography protocols of ref. 30 on a Hewlett Packard 1100 series HPLC with a Supelco Discovery C-18, 5 µm resin, 4.6 × 250 mm analytical column with a 5 µl sample loop. This chiral label reacts with chiral amines (such as alanine and serine), forming diastereomers which can be separated by HPLC. However, differing absorptivities of these labelled diastereomers results in pairs of peaks where the areas of diastereomers are different even though there are equal amounts of the D and L forms.

After a mild acid hydrolysis with the acid concentration diminished by a factor of 100 (0.06 M HCl), glycine and serine were measured at levels 3–4 times lower than for the standard hydrolysis procedure. Even hot water was adequate to release glycine, albeit an order of magnitude less than the standard hydrolysis.

Received 29 October 2001; accepted 1 February 2002.

- Oró, J. Comets and the formation of biochemical compounds on the primitive Earth. *Nature* **190**, 389–390 (1961).
- Cronin, J. R. & Pizzarello, S. Amino acids in meteorites. *Adv. Space Res.* **3**, 5–18 (1983).
- Cronin, J. R. & Chang, S. in *Chemistry of Life's Origins* (NATO ASI) (eds Greenberg, J. M., Pirronello, V. & Mendoza-Gomez, C.) 209–258 (Kluwer, Dordrecht, 1993).
- Peltzer, E. T., Bada, J. L., Schlesinger, G. & Miller, S. L. The chemical conditions on the parent body of the Murchison meteorite: Some conclusions based on amino, hydroxy and dicarboxylic acids. *Adv. Space Res.* **4**, 69–74 (1984).
- Robert, F. & Epstein, S. The concentration and isotopic composition of hydrogen, carbon and nitrogen in carbonaceous meteorites. *Geochim. Cosmochim. Acta* **46**, 81–95 (1982).
- Lerner, N. R. Influence of Murchison or Allende minerals on hydrogen-deuterium exchange of amino acids. *Geochim. Cosmochim. Acta* **59**, 1623–1631 (1995).
- Lerner, N. R. Influence of Allende minerals on deuterium retention of products of the Strecker synthesis. *Geochim. Cosmochim. Acta* **61**, 4885–4893 (1997).
- Cronin, J. R. & Pizzarello, S. Enantiomeric excesses in meteoritic amino acids. *Science* **275**, 951–955 (1997).
- Sandford, S. A. The inventory of interstellar materials available for the formation of the Solar System. *Meteorit. Planet. Sci.* **31**, 449–476 (1996).
- Gibb, E. L. *et al.* An inventory of interstellar ices toward the embedded protostar W33A. *Astrophys. J.* **536**, 347–356 (2000).
- Gerakines, P. A., Schutte, W. A. & Ehrenfreund, P. Ultraviolet processing of interstellar ice analogs. I. Pure ices. *Astron. Astrophys.* **312**, 289–305 (1996).
- Tegler, S. C. *et al.* Detection of the 2165 inverse centimeter (4.619 micron) XCN band in the spectrum of L1551 IRS 5. *Astrophys. J.* **411**, 260–265 (1993).
- Bernstein, M. P., Sandford, S. A., Allamandola, L. J., Chang, S. & Scharberg, M. A. Organic compounds produced by photolysis of realistic interstellar and cometary ice analogs containing methanol. *Astrophys. J.* **454**, 327–344 (1995).
- Kerridge, J. F. Formation and processing of organics in the early Solar System. *Space Sci. Rev.* **90**, 275–288 (1999).
- Teixeira, T. C., Devlin, J. P., Buch, V. & Emerson, J. P. Discovery of solid HDO in grain mantles. *Astron. Astrophys.* **347**, L19–L22 (1999).
- Turner, B. E. Deuterated molecules in translucent and dark clouds. *Astrophys. J. Suppl. Ser.* **136**, 579–629 (2001).
- Lacy, J. H., Faraji, H., Sandford, S. A. & Allamandola, L. J. Unravelling the 10 µm 'Silicate' feature of protostars: the detection of frozen interstellar ammonia. *Astrophys. J.* **501**, L105–L109 (1998).
- Chiar, J. E. *et al.* The composition and distribution of dust along the line of sight towards the Galactic center. *Astrophys. J.* **537**, 749–762 (2000).
- Allamandola, L. J., Sandford, S. A., Tielens, A. G. G. M. & Herbst, T. M. Spectroscopy of dense clouds in the C-H stretching region: methanol and "diamonds". *Astrophys. J.* **399**, 134–146 (1992).
- Irvine, W. M. Spectroscopic evidence for interstellar ices in Comet Hyakutake. *Nature* **383**, 418–420 (1996).
- Allamandola, L. J., Sandford, S. A. & Valero, G. Photochemical and thermal evolution of interstellar/pre-cometary ice analogs. *Icarus* **76**, 225–252 (1988).
- Matthews, C. N. & Moser, R. E. Peptide synthesis from hydrogen cyanide and water. *Nature* **215**, 1230–1234 (1967).
- Ehrenfreund, P., Bernstein, M. P., Dworkin, J. P., Sandford, S. A. & Allamandola, L. J. The photostability of amino acids in space. *Astrophys. J.* **550**, L95–L99 (2001).
- Sorrell, W. H. Origin of amino acids and organic sugars in interstellar clouds. *Astrophys. J.* **555**, L129–L132 (2001).
- Rubenstein, E., Bonner, W. A., Brown, G. S. & Bailey, J. Polarized stellar light. *Science* **283**, 1415 (1999).
- Rubenstein, E., Bonner, W. A., Noyes, H. P. & Brown, G. S. Supernovae and life. *Nature* **306**, 118 (1983).
- Bonner, W. A. & Bean, B. D. Asymmetric photolysis with elliptically polarized light. *Orig. Life Evol. Biosphere* **30**, 513–517 (2000).
- Warneck, P. A microwave-powered hydrogen lamp for vacuum ultraviolet photochemical research. *Appl. Opt.* **1**, 721–726 (1962).
- Prasad, S. S. & Tarafdar, S. P. UV radiation field inside dense clouds—Its possible existence and chemical implications. *Astrophys. J.* **267**, 603–609 (1983).
- Zhao, M. & Bada, J. L. Determination of alpha-dialkylamino acids and their enantiomers in geological samples by high-performance liquid chromatography after derivatization with a chiral adduct of o-phthalaldehyde. *J. Chromatogr. A* **690**, 55–63 (1995).

Acknowledgements

This work was supported by NASA grants from the Origins of Solar Systems, Exobiology, and Astrobiology programmes, as well as the NASA Ames Director's Discretionary Fund. We thank A. Weber, D. Glavin, O. Botta, J. Chambers and K. Nelson for discussions. We also acknowledge technical support from R. Walker.

Competing interests statement

The authors declare that they have no competing financial interests.

Correspondence and requests for materials should be addressed to M.P.B. (e-mail: mbernstein@mail.arc.nasa.gov).

Amino acids from ultraviolet irradiation of interstellar ice analogues

G. M. Muñoz Caro*, **U. J. Meierhenrich†‡**, **W. A. Schutte***, **B. Barbier†**, **A. Arcones Segovia***, **H. Rosenbauer§**, **W. H.-P. Thiemann‡**, **A. Brack†** & **J. M. Greenberg***

* *Raymond and Beverly Sackler Laboratory for Astrophysics at Leiden Observatory, PO Box 9513, NL-2300 RA Leiden, The Netherlands*
 † *Centre de Biophysique Moléculaire, Rue Charles Sadron, F-45160 Orléans, France*
 ‡ *Bremen University, Department of Physical Chemistry, FB 02, Leobener Straße, D-28359 Bremen, Germany*
 § *Max-Planck-Institut für Aeronomie, Max-Planck-Straße 2, D-37189 Katlenburg-Lindau, Germany*

Amino acids are the essential molecular components of living organisms on Earth, but the proposed mechanisms for their spontaneous generation have been unable to account for their presence in Earth's early history¹. The delivery of extraterrestrial organic compounds has been proposed as an alternative to generation on Earth^{2–5}, and some amino acids have been found in several meteorites^{6–9}. Here we report the detection of amino acids in the room-temperature residue of an interstellar ice analogue that was ultraviolet-irradiated in a high vacuum at 12 K. We identified 16 amino acids; the chiral ones showed enantiomeric separation. Some of the identified amino acids are also found in meteorites. Our results demonstrate that the spontaneous generation of amino acids in the interstellar medium is possible, supporting the suggestion that prebiotic molecules could have been delivered to the early Earth by cometary dust, meteorites or interplanetary dust particles.

Dense interstellar clouds are the birthplaces of stars and planetary systems. Here, dust particles accrete ice layers with H₂O, CO, CO₂, CH₃OH and NH₃ as the main molecular components¹⁰. This ice can undergo considerable processing by stellar ultraviolet (UV) photons and cosmic rays^{11,12}. In star-forming regions it enters circumstellar disks where formation of planetary bodies as well as comets takes place. We simulated in the laboratory the processing going on in such regions.

Our experimental set-up consists of a high-vacuum chamber where a gas mixture is deposited on a cold 'finger' (an aluminium block at 12 K) and irradiated by UV (ref. 13). An ice mixture containing H₂O:CH₃OH:NH₃:CO:CO₂ = 2:1:1:1:1 (molar composition) was selected as representative of the interstellar medium. This composition resembles the ice found close to protostellar sources^{10,14,15}. After warming the system to room temperature a small amount of material remained. The dominant components of