The Formation of Racemic Amino Acids by UV Photolysis of Interstellar Ice Analogs

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Abstract

Small biologically relevant organic molecules including the amino acids glycine, alanine, and serine were formed in the laboratory by the UV photolysis of realistic interstellar ice analogs, composed primarily of H_2O, and including CH_3OH, NH_3, and HCN, under interstellar conditions. N-formyl glycine, cycloserine (4-amino-3-isoxazolidinone), and glycerol were detected before hydrolysis, and glycine, racemic alanine, racemic serine, glycerol, ethanolamine, and glyceric acid were found after hydrolysis. This suggests that some meteoritic amino acids (and other molecules) may be the direct result of interstellar ice photochemistry, expanding the current paradigm that they formed by reactions in liquid water on meteorite parent bodies.

Our solar system is believed to have formed in an interstellar dense molecular cloud composed of gas and sub-micron sized grains.[1] In such "dark" clouds the ambient radiation of the galaxy is screened, so temperatures are low (T < 50 K), and volatile compounds condense onto grains coating them with a thin layer of ice.[1,2] This ice is usually composed primarily of H_2O, but also contains a variety of other molecules.[1,3] Laboratory studies[4] and astronomical observations[3,5] suggest that UV photolysis of these ices modifies them, and creates more complex organic molecules.[6]

The delivery of extraterrestrial organic molecules to the early Earth by meteorites and comet and asteroidal dust, may have played a role in the origin and evolution of life.[7] Among the most important and well-studied prebiotic molecules are amino acids, which have been detected at significant levels in a number of meteorites.[8] The deuterium enrichment of amino acids in carbonaceous meteorites[8,9] is taken as an indication that they or their precursors formed in the interstellar medium (ISM),[1,9] where D/H ratios of molecules are observed to be many times solar system values, as a result of various interstellar processes.[10]

It has been assumed that the amino acids present in meteorites formed in liquid water by a Strecker synthesis[11] on the parent bodies from which the meteorites derived, such as a comet or asteroid. This assumption is reasonable, since there is evidence for aqueous alteration in some

meteorites[12] and laboratory experiments[13] seem consistent with this route. However, the experiments presented here demonstrate that liquid water is *not* necessary for the formation of amino acids. Although glycine was once reported in a case where contamination could not rigorously be excluded,[14] and theorists have suggested that free amino acids should form directly in ice,[15] this is the first report of racemic amino acids formed in the laboratory from the photolysis of simple interstellar molecules, at concentrations and under conditions that are representative of those in dense interstellar clouds.

The ices we studied are comprised primarily of amorphous H_2O ice at 15 K, with 1-10% NH_3, 5-10% CH_3OH, and 1-10% HCN, relative to H_2O. These mixtures are representative of the composition of interstellar ice mantles in dense clouds and towards protostars. For example, relative to H_2O, NH_3 has been observed at the 10% level in NGC 7538,[16] higher towards GCS3,[17] and CH_3OH has been commonly observed at the 5% level in comets,[18] and higher towards protostellar objects.[19] Thus, H_2O, CH_3OH, and NH_3 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 since it is commonly detected in cometary comae,[20] it is one of the most abundant gas-phase interstellar molecules known,[21] and in the dense ISM the majority of HCN should be frozen onto grains.[2]

These interstellar ice simulations were performed in our cryogenic sample chamber, a modified matrix isolation apparatus described elsewhere.[22] Two separate gas mixtures of H_2O and CH_3OH containing one containing HCN and the other NH_3 were vapor deposited together onto a high purity Ni substrate held at 15 K. Care was taken to ensure that these HCN- and NH_3-containing gas mixtures were not allowed to mix until frozen onto the substrate so as to avoid reactions that would occur if they were combined at room temperature. IR spectra of ices deposited onto a CsI substrate indicated that they had not reacted during the course of deposition. To guard against contamination by biological amino acids and other organics the substrate and all

glassware and tools coming in contact with it were heated to 550 °C in air overnight prior to each experiment to insure they are free of all organic matter. The mixed molecular ices were UV-photolysed during deposition with a microwave-powered, flowing hydrogen, discharge lamp.[23] Typically, each layer was photolysed for the equivalent of ~30 minutes (~4×10^{18} photons per cm^2) per 0.1 μm of ice. This represents a reasonable interstellar dose, equivalent to approximately 500 years at the edge of a dense cloud, and ~1×10^5 years at an optical depth of 5, within a dense molecular cloud.[24] After deposition and photolysis were complete, the ices were warmed at ~2 K/minute under dynamic vacuum at ~10^{-8} Torr to room temperature after which the Ni substrate was removed from the vacuum system and placed into a dry, organic free vial. Under these experimental conditions the ice sublimes 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 analyze the suite of organic compounds present both before and after hydrolysis.

The residual organic materials were analyzed by Gas Chromatography-Mass Spectrometry (GC-MS) and High-Precision Liquid Chromatography (HPLC). In GC-MS the identity of molecules were ascertained from both their retention time and mass fragmentation patterns after derivatization with a silylating agent.[25] GC-MS was used both before and after hydrolysis of the samples in 6 M HCl. After hydrolysis samples were analyzed by HPLC, and the identity of molecules bearing an amino group were determined by retention time and co-injection of authentic standards. The HPLC derivatization and separation procedure[26] we employed makes fluorescent diastereomers of chiral amines which allows one to distinguish between the D- and L- (mirror image) forms of chiral amino acids.

The organic molecules N-formyl glycine, cycloserine (4-amino-3-isoxazolidinone), and glycerol were detected *before hydrolysis,* and glycine, alanine, serine, glycerol, ethanolamine, and glyceric acid were observed by GC-MS *after hydrolysis.* After standard (6 M HCl) hydrolysis, glycine, alanine, serine, and ethanolamine were detected by HPLC (Fig. 1). The yield of glycine,

relative to carbon for the experiment depicted in the figure, was 0.47%. After a mild acid hydrolysis (0.06M HCl) glycine and serine were measured at levels 3-4 times lower then for the standard hydrolysis procedure. Even pure H_2O was adequate to release glycine, albeit at an order of magnitude less than the standard hydrolysis. In all cases where the chiral amino acids serine and alanine were detected, they were determined to be racemic, within the integration error (100.1±1.6% racemic). Since living systems employ L amino acids almost exclusively, the finding that our experimentally derived chiral amino acids were racemic provides a powerful demonstration that they were not contaminants. This is confirmed by controls showing that the amino acids are 1000 times less abundant than in normal experiments if the UV photolysis was omitted (Fig. 1, Trace B).

We recently reported rates of decomposition of amino acids on exposure to UV under interstellar conditions,[27] allowing us to put constraints on the lifetime of amino acids in the interstellar medium (ISM) both in the gas-phase and in ices thin enough to be penetrated by UV light. While those previous results placed limits on amino acids surviving in an ice mantle thin enough to be penetrated by UV radiation, they are not inconsistent 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 majority of amino acids probably do not form until the warm-up when these chemical species react with one another. We detect N-formyl glycine and cycloserine before hydrolysis, but observe that the abundances of these molecules diminish with hydrolysis, and there is a concomitant appearance of glycine and serine. This, and the absence of strong amino acid peaks in the no UV control (Fig. 1 trace B), strongly suggest that at least part of the racemic amino acids we observe by HPLC after hydrolysis had formed as individual molecular species i.e., are not degradation product of macromolecules, such as HCN polymer.[28] Since our ices contain HCN, NH_3, and $H_2C=O$ (formaldehyde, from the photolysis of CH_3OH), it is possible that the formation mechanism is related to the Strecker synthesis (the reaction of these three molecules in liquid water to make glycine).[11] However, we have not yet detected the corresponding nitrile intermediates, and radical

recombination mechanisms have been proposed.[15] These two different kinds of mechanisms would lead to very different predictions as to which atoms in the products derived from which starting materials. If the amino acids formed by a kind of solid state UV-mediated Strecker reaction then the acid (-CO_2H) carbon comes from HCN, while CH_3OH, by way of $H_2C=O$, is the source of the methylene (-CH_2-) in glycine. In a radical recombination mechanism the reverse would probably be true. In future experiments we will attempt to ascertain mechanistic information by measuring how products vary in structure and abundance depending on the composition and concentrations of reactants, temperature, and radiation wavelength, and dose. Similarities between UV photolysis and proton irradiation experiments on identical mixtures,[29] and ion irradiations of various ice mixtures, suggest that ion irradiation of HCN containing ices give comparable results.[30] Thus, whatever the mechanism, cosmic rays should also contribute to amino acids in extraterrestrial ices. If this is true then cosmic rays might be especially significant in thicker ices that UV does not penetrate, such as in the outer solar system.

Given that the water on Murchison was probably deuterium depleted,[12] forming deuterium enriched amino acids by parent body aqueous chemistry poses a conundrum.[13] However, low temperature interstellar ices are believed to be deuterium enriched,[10] so deuterium enrichment of meteoritic amino acids would be a logical consequence of photochemistry of already enriched low temperature ices. Isotopic labeling experiments to explore this and other questions are ongoing in our laboratory.

Recent detections of slightly L-biased mixtures of amino acids in meteorites[31] and astronomical observations of circularly polarized radiation (CPR) in OMC-1[32] have renewed interest in the hypothesis CPR may have led to small enantiomeric excesses through chirally selective photochemistry.[33] However, only relatively inefficient mechanisms based on selective destruction of a racemic starting population have been demonstrated in the laboratory.[34] The result reported in this paper, of an apparently free racemic chiral molecule from an interstellar ice photolysis, now makes it possible to test whether circularly polarized radiation could yield non-

racemic mixtures of amino acids by selective *formation* under astrophysically relevant conditions. We are currently exploring this avenue of research.

This demonstration of amino acid formation from UV photolysis of simple molecules under astrophysical conditions suggests that outer Solar System or interstellar ice photochemistry could have contributed to the amino acids and other compounds (i.e., glycerol) observed in carbon-rich meteorites. The formation of these molecules by ice photchemistry would potentially provide a more parsimonious explanation for the deuterium enrichment and enantiomeric excesses of meteoritic amino acids than parent body aqueous chemistry. Furthermore, this work implies widespread formation of amino acids in the dense interstellar clouds from which planetary systems form, thus these molecules should have be present in other solar systems.

References and Notes

1. J. M. Greenberg, in *Comets*, ed. L. L. Wilkening, (University of Arizona Press, Tucson 1982), pp. 131-163.; S. A., Sandford, *Meteoritics*, **31**, 449 (1996).

2. S. A. Sandford, and L. J. Allamandola, *Astrophys. J.* **417**, 815 (1993).

3. A. C. A. Boogert, *et al.*, *Astron. Astrophys.* **353**, 349 (2000).; E. L. Gibb et al., *Astrophys. J.* **536**, 347 (2000).; L. d'Hendecourt. *et al.*, in *The Universe as Seen by ISO*, ESA SP-427, **Vol II**, 589. (1999).

4. P. A. Gerakines, W. A. Schutte, P. Ehrenfreund, *Astron. Astrophys.* **312**, 289 (1996).; W. A. Schutte, in *Laboratory Astrophysics and Space Research*, eds. P. Ehrenfreund, C. Krafft, H. Kochan, V. Pirronello, (Kluwer, Dortrecht, Netherlands 1999), pp. 69-104.

5. S. C. Tegler et al., *Astrophys. J.* **411**, 260 (1993).

6. J. M. Greenberg, *Astrophys. J. Lett.* **189**, L81 (1974).; C. Sagan, B. N. Khare, *Nature* **277**, 102 (1979).; M. P. Bernstein, S. A., Sandford, L. J. Allamandola, S. Chang, M. A. Scharberg, *Astrophys. J.* **454**, 327 (1995).; J. P. Dworkin, D. W. Deamer, S. A., Sandford, L. J. Allamandola, *Proc. Nat. Acad. Sci.* **98**, 815 (2001).

7. J. Oró, *Nature* **190**, 389 (1961).; V. I. Goldanskii, *Nature* **269**, 583 (1977).; S. Chang, In *Space Missions to Comets.* eds. M. Neugebauer, D. Yeomans, J. C., Brandt, R. W. Hobbs NASA Conf. Publ. 2089 59. (1979).: C. F. Chyba, P. J. Thomas, L. Brookshaw, C. Sagan, *Science* **249**, 366 (1990).; M. P. Bernstein, S. A. Sandford, L. J. Allamandola *Scientific American* **281**, 42 (1999).

8. J. R. Cronin, S. Pizzarello, D. P. Cruikshank, in *Meteorites and the early Solar System.* eds. J. F. Kerrige and M. S. Mathews, 819 (1988).; J. R. Cronin, S. Pizzarello, S. Epstein, R. V. Krishnamurthy, *Geochim. Cosmochim. Acta* **57**, 4745 (1993).: G. W. Cooper, J. R. Cronin, *Geochim Cosmochim Acta* **59**, 1003 (1995).

9. E. Zinner, In *Meteorites and the Early Solar System*, J. F. Kerridege & M. S. Matthews eds. (University of Arizona Press, Tucson 1988), pp. 956-983.; J. R. Cronin, S. Chang, in *The Chemistry of Life's Origins*. J. M. Greenberg *et al.*, eds. (Kluwer, Dortrecht, Netherlands 1993), pp. 209-258.; E. Deloule, F. Robert, *Meteoritics* **29**, 459 (1994).; J. F. Kerridge *in Formation and Evolution of Solids in Space*, eds. J. M. Greenberg and A. Li. (Kluwer, Dortrecht, Netherlands 1999), pp. 447-XX.

10. W. M. Irvine, P. F. Goldsmith, A. Hjalmarson, in *Interstellar Processes*. eds. D. J. Hollenbach, H. A. Thronson, (D. Reidel, Netherlands 1987), pp. 561-609.; T. C. Teixeira, J. P. Devlin, V.Buch, J. P. Emerson, *Astron. Astrophys.* **347**, L19 (1999). S. A., Sandford, M. P. Bernstein, J. P. Dworkin, *Meteoritics and Planetary Science* **36**, 1117 (2001).

11. A. Strecker, *Ann. Chem.*, **75**, 27 (1850).

12. F. Robert, S. Epstein, *Geochim. Cosmochim. Acta* **46**, 81 (1982).; J. R., Kerridge *Orig. Life Evol. Biosphere* **21**, 1707 (1991).

13. E. T. Peltzer, J L. Bada, G. Schlesinger, S. L. Miller, *Adv. Space. Res.* **4**, 69 (1984).; N. R. Lerner, E. Peterson, S. Chang, *Geochim. Cosmochim. Acta* **57**, 4713 (1993).; N. R. Lerner, *Geochim. Cosmochim. Acta,* **59**, 1623 (1995).

14. V. K. Agarwal, et al., *Orig. Life Evol. Biosphere,* **16**, 21 (1985).

15. W. H. Sorrell *Astrophys. J. Lett.* **555** L129 (2001).; D. Woon, E. Bakes, personal communication.

16. J. H. Lacy, H. Faraji, S. A. Sandford, L. J. Allamandola, *Astrophys. J. Lett.* **501**, L105 (1998).

17. J. E. Chiar, et al., *Astrophys. J.* **537**, 749 (2000).

18. M. A. Disanti, M. J. Mumma, T. R. Geballe, J. K. Davies, *Icarus* **116**, 1 (1995).

19. L. J. Allamandola, S. A., Sandford, A. G. G. M. Tielens, T. M. Herbst, *Astrophys. J.* **399**, 134 (1992).; E. Dartois, K. Demyk, L. d'Hendecourt, P. Ehrenfreund, *Astron. Astrophys.* **351**, 1066 (1999).

20. M. D., Hicks, U. Fink, *Icarus* **127**, 307 (1997).; R. Meier *et al. Science* **279**, 1707 (1998).

21. W. M. Irvine, *Nature* **383**, 418 (1996).

22. L. J. Allamandola, S. A., Sandford, G. Valero, *Icarus* **76**, 225 (1988).

23. P. Warneck, *Appl. Opt.* **1**, 721 (1962). This lamp produces $\sim 2 \times 10^{15}$ photons/cm^2-sec, the flux being nearly evenly divided between the Lyman α line and a roughly 20 nm wide molecular transition centered at 160 nm. Under such conditions, the time that elapses between photons arriving in the same molecular neighborhood is ~ 13 orders of magnitude longer than molecular relaxation times, so multi-photon processes are not relevant.

24. S. S. Prasad, S. P. Tarafdar, *Astrophys. J.* **267**, 603 (1983).

25. The Ni substrate was removed from the vacuum system, cut in two, and one half was treated with Bistrimethylsilyltrifluoroacetamide containing 1% trimethyl chlorosilane (from Alltech) and anhydrous pyridine in a ratio of 1:3. This solution was sonicated and stirred for 30 minutes, after which 1 µL was injected into the GC-MS. Such samples were *never* exposed to liquid water at any time either during formation or analysis. The other half of the Ni substrate was shaken with 18 MΩ Millipore water, the foil was removed, and this solution was added to an equal volume of J.T. Baker Ultrex II Ultrapure Reagent 12M HCl, and heated for 16 hours at 100°C in an organic free sealed ampoule. This material is dried in vacuo to remove the acid and volatile acids, then analyzed by GC-MS as above. GC-MS was performed on a Thermoquest-Finnigan GCQ ion trap 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°/minute to 240°C.

26. Most samples for HPLC were hydrolyzed as above, and after drying, 100 µL of 10 mM pH 9.5 sodium borate was added to the tube which, after mixing, is re-dried in vacuo. This helps to remove volatile amines. The sample was then analyzed by the OPA/NAC fluorescent labeling protocol from M. Zhao, J. L Bada, *Journal of Chromatography A.*, **690**, 55 (1995) on a Hewlett Packard 1100 series HPLC with a Supelco Discovery C-18, 5 µm resin, 4.6x250 mm analytical column with a 5 µL sample loop.

27. P. Ehrenfreund, M. P. Bernstein, J. P. Dworkin, S. A. Sandford, L. J. Allamandola, *Astrophys. J. Lett.* **550**, L95 (2001).

28. C. N. Matthews, R. E. Moser, Nature **215**, 1230 (1967).

29. M. H. Moore, R. L. Hudson, P. A. Gerakines, *Spectrochimica Acta A* **57**, 843 (2001).; H. Cottin, C. Szopa, M. H. Moore, *Astrophys. J. Lett.,* in press (2001).

30. K. Kobayashi, et al., *Advances in Space Research* **16**, 21 (1995).; Gerakines personal communication

31. J. R. Cronin, S. Pizzarello, *Science*, **275**, 951 (1997).

32. J. Bailey, et al., *Science*, **281**, 672 (1998).; E. Rubenstein, W. A. Bonner, G. S. Brown, J. Bailey, *Science* **283**, 1415 (1999).

33. E. Rubenstein, W. A. Bonner, H. P. Noyes, G. S. Brown, *Nature* **306**, 118 (1983).; C. Cerf, A. Jorissen, *Space Science Reviews* **92**, 603 (2000).

34. W. A. Bonner, B. D. Bean, *Orig. Life Evol. Biosphere* **30**, 513 (2000).

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