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Enantiomeric separation of complex organic molecules produced from irradiation of interstellar/circumstellar ice analogs

M. Nuevo [a,*], U.J. Meierhenrich [b,c], L. d'Hendecourt [a], G.M. Muñoz Caro [a], E. Dartois [a], D. Deboffle [a], W.H.-P. Thiemann [b], J.-H. Bredehöft [b], L. Nahon [d,e,1]

[a] *Institut d'Astrophysique Spatiale, Astrochimie Experimentale, bât. 121, Université Paris-Sud, F-91405 Orsay Cedex, France*
[b] *Universität Bremen, Physikalische Chemie NW2, FB02, Leobener Str., D-28359 Bremen, Germany*
[c] *Laboratoire A.S.I. et Laboratoire de Chimie Bioorganique, UMR 6001 CNRS, Université de Nice – Sophia Antipolis, Faculté des Sciences, Parc Valrose, F-06108 Nice Cedex 2, France*
[d] *Laboratoire pour l'Utilisation du Rayonnement Electromagnétique (LURE), bât. 209D, Université Paris-Sud, F-91405 Orsay Cedex, France*
[e] *CEA/DRECAM/SPAM and LFP/CNRS URA 2453, bât. 522, CE de Saclay, F-91191 Gif sur Yvette Cedex, France*

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Abstract

Irradiation of interstellar/circumstellar ice analogs by ultraviolet (UV) light followed by warm up in the laboratory leads to the formation of complex organic molecules, stable at room temperature. Hydrolysis of the room temperature residue releases amino acids, the building blocks of proteins. These amino acids exist in two different forms (L and D), but proteins encountered in living beings consist exclusively of L enantiomers. The origin of this property, called *homochirality*, is still unknown. Amino acids can be detected and quantified by chemical techniques such as chiral gas chromatography coupled with mass spectrometry (GC-MS). Enantiomers of chiral organics are also known to interact selectively with circularly polarized light (CPL), leading to a selective production or destruction of the final compounds. This paper describes how we settled an experiment where amino acids are formed by irradiation of interstellar/circumstellar ice analogs with ultraviolet (UV) CPL, produced by a synchrotron radiation beamline, which allowed us to quantify the effect of such polarized light on the production of amino acids. These results can be compared to the enantiomeric excesses measured in primitive meteorites such as Murchison.

Keywords: Astrochemistry; ISM; Circularly polarized light; Synchrotron radiation; Chiral molecules; Chromatography

1. Introduction

1.1. Astrophysical context

About 125 molecules have up to now been detected in the interstellar and circumstellar media (ISM and CSM, respectively), both in the gas and solid phases, the first detection of water ice by Gillett and Forrest dating back to 1973. Towards the lines of sight of astrophysical objects such as dark and dense molecular clouds and dust discs around protostars, observations showed the presence of interstellar solid ices, so-called "dirty ices", on the surface of cold silicate or carbonaceous dust grains. In particular, the recent infrared (IR) spectrometers onboard satellites (ISO, Spitzer) make a harvest of ice spectroscopic signatures. These dense environments display a large variety and complexity in their chemical composition. The most abundant component of these ices is H_2O, followed by CO, CO_2, CH_4, CH_3OH (see

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[*] Corresponding author. Present address: Graduate Institute of Astronomy, National Central University, No. 300, Jhongda Rd, Jhong-Li City, Taoyuan County 32049, Taiwan. Tel.: +886 03 422 7151 # 65970.
E-mail address: mnuevo@astro.ncu.edu.tw (M. Nuevo).

[1] Present address: SOLEIL, L'Orme des Merisiers, St Aubin, BP48, 91192 Gif sur Yvette Cedex, France.

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e.g. Gibb et al., 2004). NH_3 is also present, but with relative abundances of a few percent compared to water ice, lower than the first predictions (Dartois and d'Hendecourt, 2001; Dartois et al., 2002; Taban et al., 2003).

To complete and interpret these observations, the experimental approach is essential to understand the physico-chemical and thermal evolution of interstellar ices. The MICMOC[2] experiment aims at studying the evolution of such ice mixtures in the laboratory. The chemical composition of interstellar ices can be characterized and quantified by comparison between laboratory IR spectra and ISO spectroscopic data (e.g. d'Hendecourt et al., 1996; Gibb et al., 2004; van Dishoeck, 2004; Dartois, 2005).

1.2. Amino acids

Irradiation of different mixtures of interstellar ice analogs (H_2O, CO, CO_2, CH_4, CH_3OH, NH_3) in the laboratory with UV light at low temperatures is known to lead to the production of complex compounds. Among them, we can find for instance the solid state species NH_4^+, OCN^- and H_2CO, presumably identified in grain mantles towards protostars (Schutte et al., 1996; Schutte and Khanna, 2003). Note that H_2CO is also a well known and abundant gas phase molecule (Loinard et al., 2002; Aikawa et al., 2003). Then, more complex molecules which are stable at room temperature are formed. These molecules appear as a refractory organic residue that can be analyzed by chemical techniques such as gas chromatography coupled with mass spectrometry (GC-MS). Such residues display a great variety of compounds (Meierhenrich et al., 2005a), among them amino acids (Muñoz Caro et al., 2002; Bernstein et al., 2002). Some of these amino acids (20) are the building blocks of proteins in all living beings on Earth, and are known to play a role in prebiotic chemistry.

Most of amino acids (proteinaceous and non-proteinaceous) are chiral, i.e. they exist in two forms, L and D enantiomers, the one being the mirror image of the other. To build proteins, life only uses the L enantiomers. Most of the biochemical reactions display such enantiomeric selectivity, or *homochirality*, and the origin of this particular property remains to be found. In carbonaceous chondrites such as the Murchison meteorite, most of the identified amino acids were found to be non-racemic, i.e. they display an L enantiomeric excess (e.e.). However, we do not know the origin of this excess. Biological terrestrial contamination seems to be excluded since a large majority of the identified amino acids are not proteinaceous, and isotopical analyses on ^{13}C and ^{15}N are in agreement with an extraterrestrial origin for these molecules (Engel and Macko, 1997).

Chiral molecules such as amino acids are known to interact differently with both polarizations of a circularly polarized light (CPL), and enantioselective photodestruction with polarized light has been already observed on leucine in aqueous solutions by Flores et al. (1977) and Bonner and Bean (2000), and recently on solid films of leucine by Meierhenrich et al. (2005b). The extension of our experiment, Chiral MICMOC, was settled in order to perform similar irradiation experiments using UV CPL as a light source. This light was provided by the SU5 VUV beamline at LURE[3] in Orsay, France. The main goal of this experiment was to study the possible formation of amino acids displaying an enantiomeric excess as a result of irradiation by UV CPL, in order to check if CPL is a possible source for homochirality in the ISM, and/or in the primitive Solar nebula (Nuevo et al., submitted). Several reviews presenting sources known or assumed to provide such polarized radiations and their polarization rates in astrophysical environments exist (see e.g. Bailey, 2001; Jorissen and Cerf, 2002).

2. Experiments

In a cryostat cooled by liquid nitrogen to a temperature of about 80 K and evacuated by a turbo-molecular pump to a pressure of about 5×10^{-7} mbar, a gaseous mixture is deposited onto an IR-transparent MgF_2 window. Under those conditions of temperature and pressure, the gases condense onto the window and form a thin ice layer. The gas mixtures are chosen to be related to the composition of the observed interstellar ices, i.e. they contain H_2O, CO_2, CH_3OH and NH_3 in different relative proportions, but as a check some others do not contain water ice. Simultaneously to deposition, the accreting ice layer is UV irradiated.

The deposition and evolution with time of the ices are controlled by Fourier transform IR spectroscopy (Vector 22 spectrometer from Bruker). The UV light sources we used are either a H_2 discharge UV lamp (Opthos) for IAS (Institut d'Astrophysique Spatiale) laboratory irradiations, providing mainly 160- and 121.6-nm (Lyman-α) photons with a $\sim 10^{14}$ photons s^{-1} flux at the sample location (see Weber and Greenberg, 1985 for a measurement of this flux), or the SU5 VUV beamline set at a wavelength of 167 nm (7.45 eV). The SU5 beam, emitted by a variable polarization undulator, was circularly polarized with polarization rates of 91% and 96% for right CPL (RCPL) and left CPL (LCPL), respectively,

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[2] Matière Interstellaire et Cométaire, Molécules Organiques Complexes, i.e. Interstellar and Cometary Matter, Complex Organic Molecules.

[3] Laboratoire pour l'Utilisation du Rayonnement Electromagnétique.

in the 7–8 eV range (Nahon and Alcaraz, 2004), and provided an integrated photon flux of $\sim 10^{14}$ photons s^{-1} in a 10% bandwidth.

For the LURE experiments, the system used at IAS and described above was transferred at LURE and aligned with the SU5 beamline. The irradiations were performed under the same conditions and ice mixtures prepared in the same way as for the IAS experiments. The ice mixtures were irradiated with RCPL and LCPL, in two different experiments, for irradiation exposures of about 2 days for each polarization.

After deposition/irradiation, the samples are slowly warmed up to room temperature in order to be analysed ex situ by chemical techniques such as chiral GC-MS, able to separate enantiomers of chiral molecules, following the protocol described in details in Abe et al., 1996. The residues are extracted with water and hydrolyzed with 6 M HCl at 110 °C for 24 h in order to set the amino acids contained in the residues free. Then the extracts are derivatized to make the compounds become volatile. The samples are finally injected into the column, where molecular species elute with characteristic retention times. The column we used was a 25 m chiral stationary Chirasil-L-Val phase column from Varian-Chrompack. An Agilent 6890N GC system was applied in a splitless mode. The temperature program ramp was 10 °C min^{-1} starting at 50 °C. At 90 °C the ramp was decreased to 2 °C min^{-1} until a temperature of 110 °C was reached. Then, the ramp continued with 10 °C min^{-1} until 180 °C, which was held constant for 39 min. The chromatogram was obtained in the total ion current (TIC) mode of the Agilent 5973N mass selective detector. Once molecules elute from the column, they pass through a mass spectrometer which gives the mass distribution of the fragments arriving on the detector. The retention times and mass fragments are then compared to standard samples in order to clearly identify the molecules present in the samples. To exclude contamination, experiments using ^{13}C-labeled starting ice mixtures were also performed. Blank samples, in which MgF_2 windows were irradiated without any gas deposition, led to a flat spectrum, demonstrating that possible contamination from the system is negligible.

3. Results

3.1. Production of amino acids with unpolarized UV irradiation

Several residues, produced after irradiation of different ice mixtures by the UV lamp (unpolarized light), have been analyzed by GC-MS. Those analyses show the presence of a great variety of amino acids, and confirm previous results reported by Muñoz Caro et al., 2002 and Bernstein et al., 2002. Among the detected compounds, we find proteinaceous amino acids such as glycine (Gly), alanine (Ala) and valine (Val), but also non-proteinaceous amino acids such as sarcosine (Sar), 2,3-diaminopropanoic acid (DAP) and isovaline (Iva). All these amino acids were also found in carbonaceous chondrites such as Murchison (e.g. Cronin and Pizzarello, 1997; Engel and Macko, 1997; Pizzarello et al., 2003; Meierhenrich et al., 2004) and Murray (Cronin and Pizzarello, 1999).

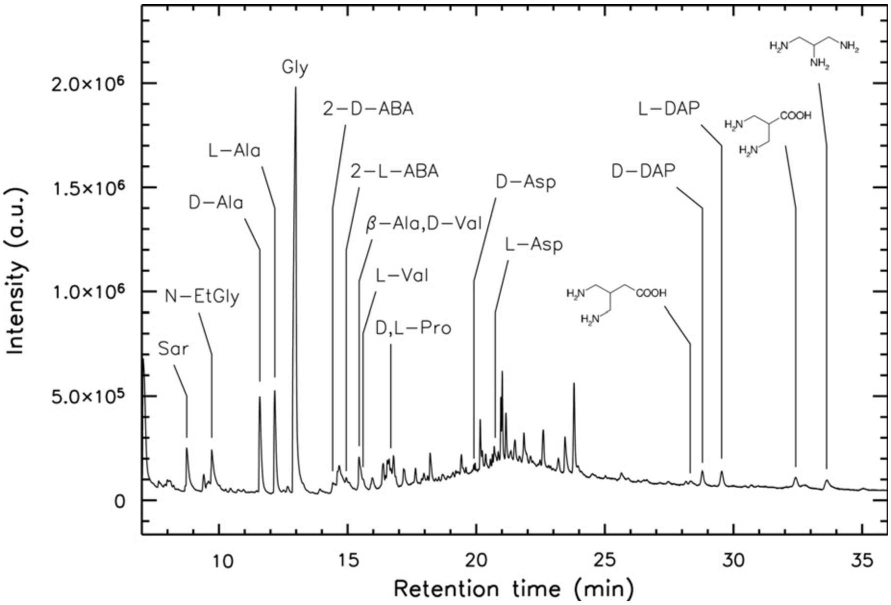

Fig. 1. Total-ion current (TIC) chromatogram obtained after the analysis of a residue produced from the irradiation of a $CH_3OH:NH_3 = 1:1$ starting ice mixture with the UV lamp (unpolarized light). A large variety of amino acids was detected, and for most of the chiral compounds, their enantiomers were clearly separated. The detected amino acids are listed in Table 1.

Table 1
List of all amino acids shown in the TIC chromatogram of Fig. 1 with their associated mass fragments and retention times R_t

Amino acid	MS-fragmentation (amu)	R_t (min)
Sarcosine (Sar)	88, **116**, 144, 189	8.75
N-Ethylglycine (N-EtGly)	58, **130**	9.72
D-Alanine (D-Ala)	88, 102, **116**, 144	11.59
L-Alanine (L-Ala)	88, 102, **116**, 144	12.17
Glycine (Gly)	**102**, 130, 175	12.98
D-2-Aminobutyric acid (D-2-Aba)	102, **130**	14.42
L-2-Aminobutyric acid (L-2-Aba)	102, **130**	14.95
β-Alanine (β-Ala)	98, 102, **115**, 116	15.45
D-Valine (D-Val)	102, 116, **144**	15.45
L-Valine (L-Val)	102, 116, **144**	15.60
D,L-Proline (D,L-Pro)	103, 131, **142**	16.68
D-Isovaline (D-Iva)	116, **144**	16.87
L-Isovaline (L-Iva)	116, **144**	17.18
D-Aspartic acid (D-Asp)	**188**	19.91
L-Aspartic acid (L-Asp)	**188**	20.74
4,4′-Diaminoisopentanoic acid	**116**, 133, 142	28.32
D-2,3-Diaminopropanoic acid (D-DAP)	102, 129, 157, **175**	28.79
L-2,3-Diaminopropanoic acid (L-DAP)	102, 129, 157, **175**	29.54
3,3′-Diaminoisobutyric acid (DAIB)	102, 115, 128, **142**, 188	32.42
1,2,3-Triaminopropane[a]	**102**, 115, 130	33.62

[a] Not an amino acid.

The total-ion current (TIC) chromatogram and a list of all detected amino acids, along with their associated retention times R_t, for a residue produced from irradiation of a $CH_3OH:NH_3 = 1:1$ starting gas mixture are given in Fig. 1 and Table 1, respectively. The identifications of the amino acids were made by comparison with standard samples and data available in the literature (e.g., Huang et al., 1993; Muñoz Caro et al., 2002). Irradiations of ice mixtures with/without water ice both lead to the production of amino acids.

The relative abundances of these amino acids are different according to the starting gas mixture used for the irradiation, but in all cases the most abundant amino acids are the simplest ones, namely Gly and Ala, and the abundance of the compounds generally decreases with molecular complexity. This is also an indirect indication that the processes are only photolytic and not due to biological contamination during manipulation of the samples. The chromatogram of Fig. 1 also shows enantiomeric separations for some chiral proteinaceous amino acids such as alanine, valine or aspartic acid (Asp), as well as for some chiral non-proteinaceous amino acids such as isovaline (Iva) and DAP, which are detected with abundances close to some proteinaceous amino acids. Every chiral compound, displaying two peaks in the chromatogram, was found to be racemic. Glycine, sarcosine or *N*-ethylglycine, which are not chiral, only display one peak.

These observations provide further evidence that our sample was not contaminated, since chiral amino acids were found to be racemic, and because proteinaceous and non-proteinaceous amino acids were detected with comparable quantities. Finally, to our knowledge, the chromatogram of Fig. 1 shows the first detection of isovaline (Iva) in laboratory simulations of interstellar ice analogs. This amino acid is interesting because it was found to display the highest L enantiomeric excess ever measured in the Murchison meteorite (Pizzarello et al., 2003).

3.2. Irradiation with UV CPL

During the LURE experiments, $H_2O:^{13}CH_3OH:NH_3 = 1:1:1$ mixtures were irradiated with the SU5 beamline, with both polarizations (Nuevo et al., submitted). The so-obtained residues displayed IR spectra and GC-MS chromatograms very similar to those obtained after unpolarized irradiation with the H_2 discharge UV lamp at IAS. They were also extracted with water, hydrolyzed in 6 M HCl at 110 °C and then derivatized before being injected into the chiral chromatography column.

To quantify the effect of CPL on LURE samples, the remaining gas mixtures were then irradiated at IAS with the H_2 discharge UV lamp. Those IAS samples are known to produce amino acids in racemic proportions (Fig. 1), although they display small apparent e.e.s due to the chromatography technique used. Thus, we could compare the measured excesses found for the LURE samples to the apparent ones found for the IAS samples, in order to determine the real contribution of CPL to the e.e.s measured in the residues produced at LURE.

The e.e.s were determined for two chiral amino acids, namely alanine (Ala, proteinaceous) and 2,3-diaminopropanoic acid (DAP, non-proteinaceous). However, the quantities of organic residues produced in such experiments were very low, because of the small quantities of ices which were irradiated and the limited photon dose received by the samples at LURE. It was thus difficult to quantify e.e.s, in particular for DAP, whose peaks had a lower signal to noise ratio since this amino acid is much less abundant than Gly or Ala (Nuevo et al., submitted).

The results seem to show a weak e.e. for these two amino acids, of the order of 1%, probably induced by UV CPL. Moreover, the systematic errors induced by the use of such chemical techniques, and which are not taken into account in most of e.e. determinations, were estimated to be of the order of 0.2% in our experiments (Nuevo et al., submitted). Of course, other sources of error, such as those due to the peak integration method, must be taken into account. Therefore, these results are within the limits of detection of our instruments and methods, and they cannot allow us to know if the process(es) leading to the creation of this enantiomeric excess was (were) a selective photo-destruction or photo-production. However, assuming they are significant, they are in good agreement with the e.e.s measured in meteorites for α-hydrogenated amino acids, in particular for alanine, where an L-excess of 1.2% in Murchison is reported (Cronin and Pizzarello, 1999). Other comparisons of e.e.s between amino acids produced by irradiation of interstellar ice analogs by UV CPL and amino acids found in meteorites should be made to confirm these results, for instance for α-methyl amino acids such as isovaline, reported to display e.e.s up to 15% in Murchison (Pizzarello et al., 2003).

4. Conclusion

Our laboratory experiments clearly show that irradiation of interstellar ice analogs with UV leads to the production of a large variety of amino acids in detectable quantities and confirm the results obtained previously (Muñoz Caro et al., 2002; Bernstein et al., 2002). These compounds can be analyzed and separated by chromatography techniques such as chiral GC-MS. It is thus possible to determine enantiomeric excess for those which are chiral.

The effect of UV circularly polarized light on the formation of non-racemic chiral molecules was estimated after irradiation of similar ice mixtures with the SU5 UV CPL beamline at LURE. The excesses measured for alanine and DAP, the most abundant proteinaceous chiral and non-proteinaceous chiral amino acids found in our residues, respectively, were found to be low, at most 1% (Nuevo et al., submitted). Such experiments should be repeated to improve the measurements of e.e.s in so-formed organic residues.

Until now, complex organics such as amino acids have not been detected in space. Glycine was reported to be present in dense molecular clouds such as Orion and Sgr B2 (Kuan et al., 2003), but its detection is still controversial (Snyder et al., 2005). However, a large variety of amino acids was found in meteorites such as Murchison. Comparison of both distribution and e.e.s between those data and the results obtained for the production of amino acids by irradiation with UV (circularly polarized) should help us to better understand the processes leading to their (enantioselective) formation and their role in prebiotic chemistry.

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