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Possibility of Production of Amino Acids by Impact Reaction Using a Light-Gas Gun as a Simulation of Asteroid Impacts

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Abstract In order to investigate impact production of carbonaceous products by asteroids on Titan and other satellites and planets, simulation experiments were carried out using a 2-stage light gas gun. A small polycarbonate or metal bullet with about 6.5 km/s was injected into a pressurized target chamber filled with 1 atm of nitrogen gas, to collide with a ice + iron target or an iron target or a ice + hexane + iron target. After the impact, black soot including fine particles was deposited on the chamber wall. The soot was carefully collected and analyzed by High Performance Liquid Chromatography (HPLC), Fourier Transform Infrared Spectroscopy (FT-IR), and Laser Desorption Time-of-Flight Mass Spectrometry (LD-ToF-MS). As a result of the HPLC analysis, about 0.04–8 pmol of glycine, and a lesser amount of alanine were found in the samples when the ice + hexane + iron target was used. In case of the ice + iron target and the iron target, less amino acids were produced. The identification of the amino acids was also supported by FTIR and LD-ToF-MS analysis.

Keywords Titan · Impact reaction · Amino acid · HPLC · FTIR · LD-ToF-MS

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Introduction

A large amount of organic compounds are abiologically produced in space. This is confirmed by the detection of organic compounds from interstellar molecule clouds (Irvine and Knacke 1989). This is also confirmed by the identification of organic compounds in carbonaceous meteorites such as the Murchison meteorite (Kvenvolden et al. 1970; Cronin et al. 1985; Cronin and Chang 1993). It is considered that organic compounds have been produced by impact reactions in space. Organic compounds produced by impact reactions can be stored in the surfaces and subsurfaces of planets and scattered into space. Recently, it was suggested that organic compounds were produced on early Earth by simulation experiments of oceanic impacts (Furukawa et al. 2009). Because the amino acids were detected from the organic matter produced, a new generation process for material related to the origin of life was suggested. To investigate the origin of life on Earth, various models of the primitive Earth are proposed, and simulation experiments for chemical evolution based on these models were performed (Miller 1953; Schlesinger and Miller 1983; McKay and Borucki 1997). However, it is difficult to know what actually happened in real planetary environments. Titan, the largest moon of Saturn, has attracted much attention in studies of chemical evolution. Titan is about 5150 km in diameter. The mean density, surface pressure and temperature of Titan are 1.88 g/cc, 147 kPa and 90 K, respectively. An attractive feature of Titan is its dense reducing atmosphere, which consists of mainly N_2 and CH_4 (Atreya et al. 2009). On the other hand, it is considered that atmosphere of the primitive Earth contained N_2 and CO_2 as primary components, with CH_4 and CO as accessory components (Kasting 1990). Titan is thus similar to the primitive Earth in that N_2 and CH_4 are contained in the atmosphere. So, amino acids might be produced on Titan's surface by the impacts of meteorites and asteroids just as on the primitive Earth.

The surface of Titan has been revealed by the Cassini space probe sent by NASA/ESA (Beghin et al. 2009). From the observational results obtained by Voyager 1, the existence of a variety of organic compounds-ethane (C_2H_4), acetylene (C_2H_2), propane (C_3H_8), hydrogen cyanide (HCN), cyanogen (C_2N_2), cyanoacetylene (CHCCN) and so on-have been found in the Titan's atmosphere (Hanel et al. 1981; Kunde et al. 1981). Furthermore, from the observational results obtained by the Cassini, it was found that there are lakes of liquid methane/ethane in Titan. Many hydrocarbons and molecular carbon clusters produced in the upper atmosphere by UV reactions fall into these lakes, and likely accumulate there. In addition, it is considered that carbon clusters, hydrocarbon molecules, a part of amino acids produced by high temperature reactions after asteroid impacts to Titan's surface could be also accumulate in methane /ethane lakes.

In the previous simulation experiment concerning impact reactions on Titan's surface, production of many carbon clusters were confirmed (Mieno and Hasegawa 2008), and the possibility of production of amino acids and nitride polymers was expected. Recently, a few amino acids were detected by GCMS analysis of aerosols produced by a plasma electric discharge (Poch et al. 2012). Additionally, the aerosol analysis with pyrolytic equipment on Cassini's Huygens lander and proton beam irradiation experiments in the laboratory suggest amino acid precursors might be formed near Titan's surface (Israel et al. 2005; Taniuchi et al. 2013). However, amino acid production in experiments simulating asteroid impacts has not yet been reported. Therefore, we carried out laboratory simulation experiment of asteroid impacts using a 2-stage light-gas-gun, and tried to find amino acids and nitride polymers from carbon soot produced by impact reactions under a nitrogen-rich atmosphere.

Methods

The Impact Experiment

The experiment was carried out using a 2-stage light-gas-gun at ISAS/JAXA. This gas gun can accelerate a polycarbonate bullet 7.1 mm in diameter (or a stainless steel bullet 3.2 mm in diameter) to about 6.5 km/s under a vacuum of 0.1 Pa, and the bullet collides with an ice + iron target (an iron target or an ice + hexane + iron target) in a pressurized chamber. A schematic of the pressurized chamber is shown in Fig. 1a. At the end of the large target chamber of the gas gun (Fig. 1b), the pressurized impact chamber was set, which is 255 mm in diameter and 250 mm long, and made of stainless steel. To collect the soot sample produced, the inside walls of the chamber were covered with clean aluminum sheets. The pressurized chamber was at first evacuated by a rotary pump, and then 1 atm of nitrogen gas was introduced. A bullet penetrates the aperture of the chamber, 65 mm in diameter covered with a 0.1 mm thick aluminum film, and hits the iron target 76 mm in diameter and 25 mm thick. The target can be cooled down to about −100 °C by thermal conduction of a copper rod, which is cooled by liquid nitrogen. On the iron target, thin ice/water (water + hexane) layer about 2 mm thick can be set by covering with a thin aluminum-sheet. After the impact, the soot was carefully collected using propanol and a brush.

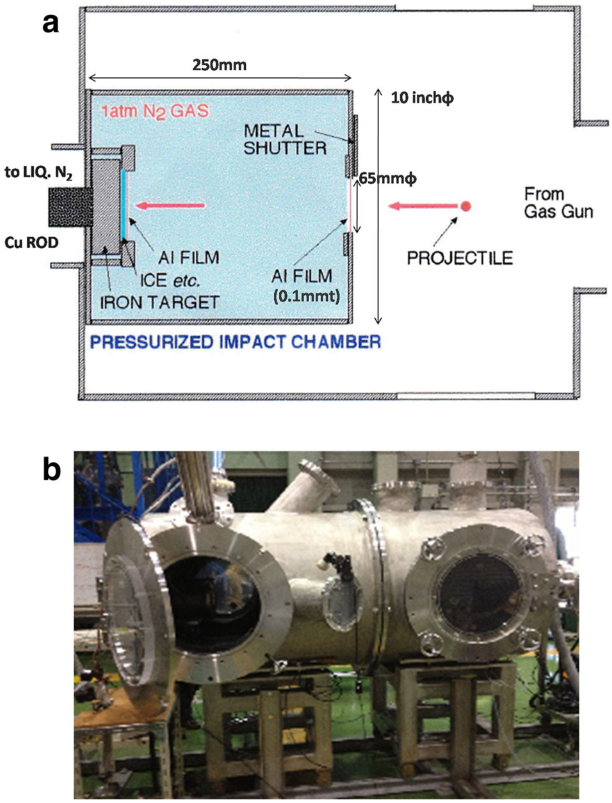

Fig. 1 **a** Schematic of the pressurized chamber. **b** Photograph of the target chamber. The projectile is injected from right side

High Performance Liquid Chromatography (HPLC)

In order to detect production of amino acids, the produced soot, which was deposited on the inner wall of the pressurized chamber, was analyzed (Fig. 2). A part of the soot was refluxed in pure water (Wako, Ultrapure water) for 8 h at 100 °C. The water was then filtered using a 0.2 μm filter to remove the impurity, and condensed. The condensed sample was reacted with dabsyl chloride to make dabsyl-amino acids (Chang et al. 1981). The reaction mechanism is shown in Fig. 3. A standard amino acid solution including 17 amino acids and a blank were also reacted in the same way. The prepared samples were analyzed by a HPLC with a UV/VIS detector (Jasco Gulliver System, wave length of 465 nm). 200 μl of the dabsylized sample was injected into the HPLC analyzer. The experiment condition such as eluent and gradient condition was quoted from LC application data from JASCO Corporation (Sato et al. 2010).

Fourier Transform Infrared Spectroscopy (FTIR)

To measure the molecular structure, a Shimadzu 8700 Fourier Transform Infrared spectrometer was used. First, a drop of sample was put on a CaF_2 plate (20 mm in diameter, 1 mm thick) by using a pipette, and dried. And then, the sample and the background were analyzed by FTIR. The range of the wave number is 1000–4000 cm^{-1}. Each spectrum was acquired for 100 scans and averaged.

Laser Desorption Time-of-Flight Mass Spectrometry (LD-ToF-MS)

To measure the mass spectra of the produced soot, a Bruker AutoFLEX LD-ToF-MS was used. ToF-MS analyses were performed in the reflector mode and 50 shots are averaged. The matrix, trans-2-[3-(4-t-butyl-phenyl)-2-methyl-2-propenylidene]malononitrile (DCTB), was dissolved in methanol. Samples were dissolved in methanol on an evaporating dish and mixed with DCTB matrix. Samples were deposited onto a target plate, and then air dried.

Results

Representative HPLC chromatograms are shown in Fig. 4. These chromatograms are compared with those of standard amino acid solution including 17 amino acids and blank. Peaks suggesting the presence of glycine and alanine were detected in the samples when the ice + hexane + iron target and ice + iron target were used. Peaks suggestive of serine and leucine were occasionally detected in the ice + hexane + iron target and the ice + iron target, respectively. Table 1 shows the results of HPLC analysis. It is estimated that 0.04–8 pmol of glycine and 0.07–3.3 pmol of alanine were included in the injected solution in case of the ice +

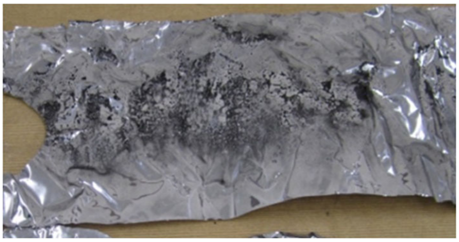

Fig. 2 Black soot deposited on an aluminum sheet set on the inner wall of the pressurized chamber

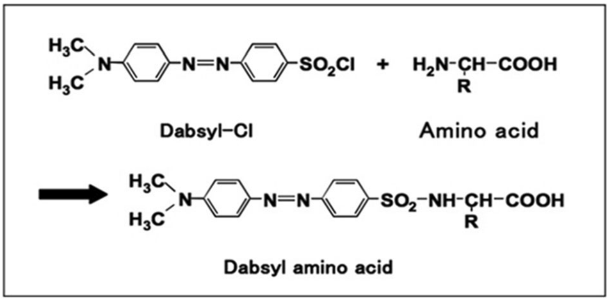

Fig. 3 Schematic of the dabsyl reaction. (quoted from Sato et al. 2010)

hexane + iron target. In samples of the ice + iron target, 0.02–3.2 pmol of glycine and 0.07–2.1 pmol of alanine were included in the injected one. On the other hand, in case of the iron target, amino acids were slightly detected. In order to investigate how much amino acids are included in carbon soot produced by the impact experiment, we performed HPLC analysis in more detail. First, the aluminum sheet on which carbon soot are deposited was cut to a small

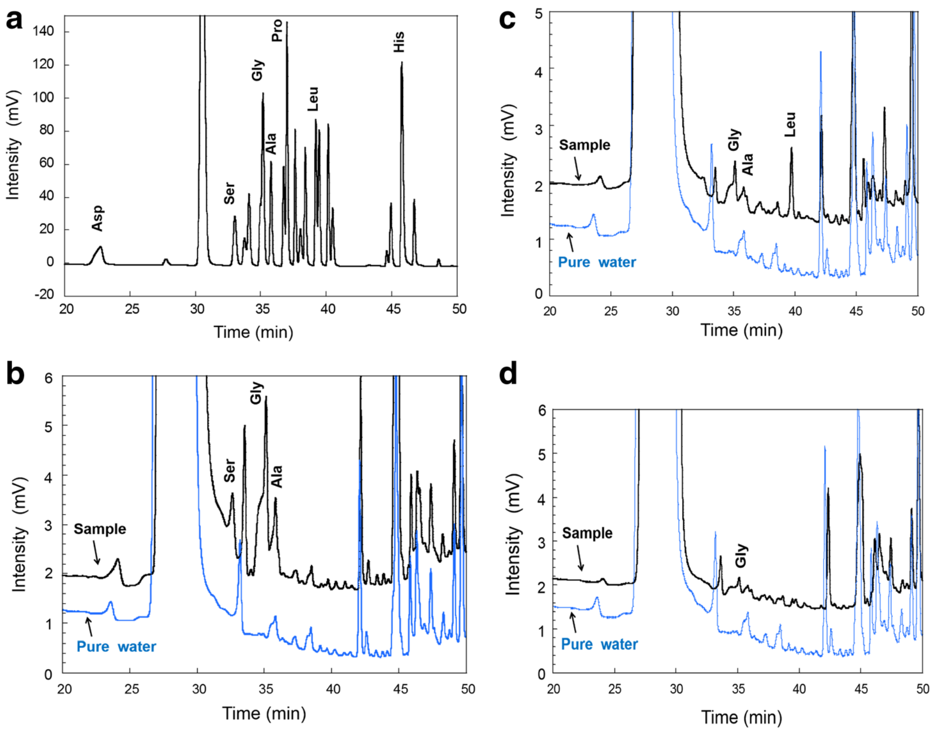

Fig. 4 The representative HPLC chromatograms. **a** Standard amino acid solution. **b** The sample when the ice + hexane + iron target was used. Peaks suggesting serine (Ser), glycine (Gly), alanine (Ala) were detected. **c** The sample when the ice + iron target was used. Peaks suggesting glycine (Gly), alanine (Ala), leucine (Leu) were detected. **d** The sample when the iron target was used. A small peak of glycine(Gly) was detected. In (**b**)~(**d**), *black* chromatogram corresponds to the sample. And *blue* one is to the pure water as blank. The vertical axis indicates the detected intensity of sample

size and its mass measured. Next, all carbon soot was carefully collected from this small aluminum sheet. After collecting, mass of aluminum sheet was measured. From the difference of two values of mass, the mass of the collected soot was calculated. These samples were prepared according to the procedure mentioned above and analyzed by HPLC. Table 2 represents the results of quantitative analysis. It is estimated that approximately 200–400 pmol/mg of glycine and alanine were included in the carbon soot deposited on the aluminum sheet, when the ice + hexane + iron target was used. In case of the ice + iron target, production of glycine and alanine was estimated to be 161 pmol/mg and 131 pmol/mg, respectively. Here, "pmol/mg" represents quantity of the amino acid per unit mass of soot.

Mass spectra obtained from the LD-ToF-MS are shown in Fig. 5. These spectra are compared with those from the matrix standard. In case of the ice + hexane + iron target and the ice + iron target, there were peaks suggesting glycine (M=75.05) and alanine (M=89.09). However, these peaks were not clearly detected in the iron target. In the sample of ice + hexane + iron target and the ice + iron target, there are other clear peaks around m/z=98, 112, 120, 133.

A representative FTIR spectrum is shown in Fig. 6. The stretching vibration of CH at 2950 cm^{-1} and the stretching vibration of CN at 1250 cm^{-1} were identified provisionally. The stretching vibration of −C≡N at 2250 cm^{-1} characteristic of nitrile

Table 1 The results of HPLC analysis

Sample	Projectile	Target type	Glycine (pmol)	Alanine (pmol)
130627B	Polycarbonate	Ice + hexane + iron	0.04	N.D.
130628E1	”	”	1.4	1.2
130628E2	”	”	8	3.3
130628E3	”	”	0.64	0.33
130628E4	”	”	0.88	0.33
130813B	”	”	1.24	0.07
130814F	”	”	0.36	0.07
130815I	”	”	1.56	0.5
130627A	Polycarbonate	Ice + iron	2	0.7
130628D	”	”	0.88	0.07
130628G	”	”	0.32	N.D.
130813A1	”	”	0.32	0.07
130813A2	”	”	1.2	2.1
130813A3	”	”	3.2	1.2
130813A4	”	”	0.02	N.D.
130814E	”	”	0.72	0.23
130627C	Polycarbonate	Iron	N.D.	N.D.
130628F	”	”	0.08	0.13
130813C	”	”	N.D.	N.D.
130814D1	”	”	0.14	N.D.
130814D2	”	”	0.14	N.D.
130814G	”	”	N.D.	N.D.
130815J	Stainless steel	Hexane + iron	0.56	0.2
130815K	”	”	0.72	0.4

The amount of glycine and alanine included in 200 μl of inject solution is represented

Table 2 The results of quantitative HPLC analysis

Sample	Projectile	Target type	Glycine (pmol/mg)	Alanine (pmol/mg)
130628E	Polycarbonate	Ice + hexane + iron	244	237
121220D	”	”	429	339
121220C	”	Ice + iron	161	131

The production of glycine and alanine in the collected soot are shown as quantity of them per unit mass of soot

was not observed. Also, NH broad band around 2675–3050 cm^{-1} characteristic of glycine or alanine was not obtained. Because the FTIR analysis in this study was carried out with a drop of sample, there is room to be still improved.

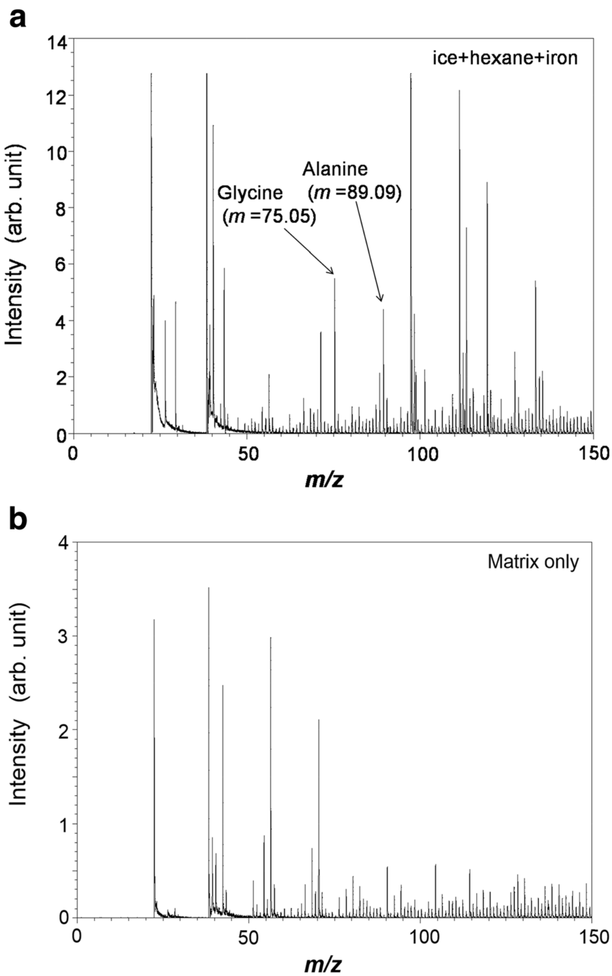

Fig. 5 The representative Laser-Desorption Time-of-Flight mass spectra. **a** The sample when the ice + hexane + iron target was used. **b** Matrix only. Other significant peaks are around *m/z*=98, 112, 120, 133 (positive ion mode, 50 shots are averaged)

Discussion

It was confirmed that amino acids were detected in this experiment. But it is necessary to consider the possibility of contamination from outside. The contamination from a target chamber to a pressurized impact chamber is considered. Although the aperture of the chamber is covered by an aluminum film before firing, it is torn afterwards and may permit the inflow of impurities. In this study, a metal shutter closes the aperture to prevent the inflow of impurities soon after firing. Therefore, it is thought that there is no contamination from the gun region. As another source of contamination, we propose aluminum sheets. The impurities including the amino acid might attach onto it originally. To prevent these impurities, the surface of the aluminum sheet is cleaned with pure alcohol before setting it in the inner wall of the pressurized impact chamber. To confirm the influence of the impurities from the aluminum sheet, we collected the impurities from an aluminum sheet which was not used for the impact experiment, and performed HPLC analysis as mentioned above. Figure 7 shows the result of HPLC. Peaks corresponding to the amino acids were not detected in this chromatogram. Thus, it is considered that the amino acids detected in the analysis in this study are derived from impact reactions.

To prove the synthesis, it is useful to check the D/L ratio of alanine. In case of amino acids produced biologically, an excess of L-enantiomers occurs. In addition, it is necessary to investigate the production of amino acid which are rare on Earth and are often founded in meteorites, for example, α -aminoisobutyric acid or isovaline (Zhao and Bada 1989). These analyses are under preparation.

The production of amino acids was confirmed under the condition of the hexane + iron target/ using a stainless steel bullet. This production suggests that water or hexane is important integrant for production of amino acids.

The production rate of amino acids estimated in this study was compared with that of amino acids found in the Murchison meteorite (Table 3). Contents of glycine and alanine are an order of magnitude larger than those of the Murchison meteorite. In this study, we measured the amino acid in the produced carbonaceous soot. However, as the amino acid in the Murchison meteorite was measured from whole rock, it is difficult to compare both values directly.

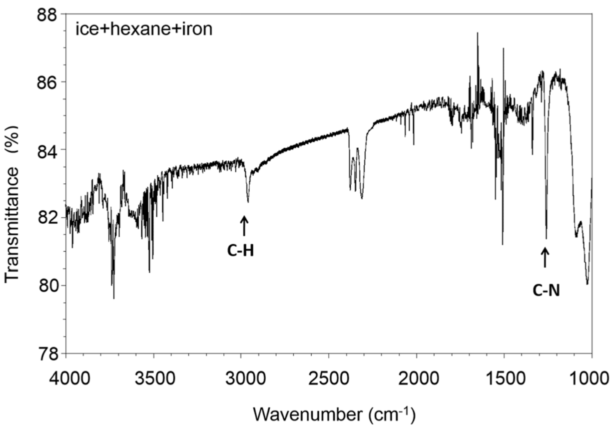

Fig. 6 The representative FTIR spectrum. The spectrum is corrected to remove the influence of carbon dioxide and water vapor in the air. The stretching vibration of CH at 2950 cm^{-1} and the stretching vibration of CN at 1250 cm^{-1} were identified provisionally

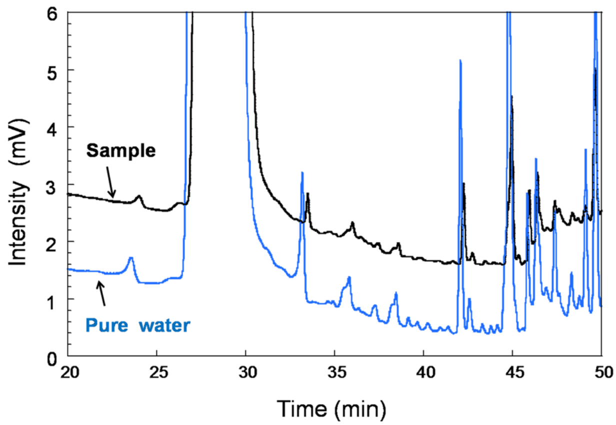

Fig. 7 The chromatogram of sample collected from an aluminum sheet to check the possibility of the contamination. *Black* chromatogram corresponds to the sample. And *blue* one is to the pure water as blank. The *left* vertical axis indicates the detected intensity of sample

If amino acids are really produced by the impact reactions, we have to consider its production process. In the previous study, HCN was detected in experiment similar to this study (Kurosawa et al. 2013). If HCN is also produced in this study, it is expected that the processes such as amino acid production on early Earth might have occurred. This processes start from H_2O, CH_4, NH_3, and produces aminoacetonitrile as an intermediate. Glycine is then produced via hydrolysis of aminoacetonitrile (Strecker 1850; Shibasaki et al. 2008). Hexane is pyrolized in 600–700 °C and produces hydrogen, methane, ethylene. Therefore, this reaction process might have happened in this study if ammonia was produced. If aminoacetonitrile was produced, it is considered that there is another process which is called polypeptide theory (Akabori 1955). In this process, polyglycine is produced according to the polymerization of aminoacetonitrile and that then various side-chains are introduced into the glycine residues of polyglycine. It is considered that the peak for $m/z=112$

Table 3 Comparison of the amino acids that were detected in this study with the amino acids found in the Murchison meteorite (Engel and Macko 1997)

Amino acid	Murchison meteorite (nmol/g)	This study (nmol/g)		
		Ice + hexane + iron		Ice + iron
		130628E	121220D	121220C
α-Aminoisobutyric acid	20.1	N.D.		N.D.
Isovaline	8	N.D.		N.D.
Glycine	24.5	244	429	161
β-Alanine	12.8	237	339	131
L-Alanine	10.4			
L-Leucine	2.5	N.D.	N.D.	N.D.
Serine	N.D.	N.D.	N.D.	N.D.
Aspartic acid	4.7	N.D.	N.D.	N.D.
Glutamic acid	10.8	N.D.	N.D.	N.D.

and the one for $m/z=133$ obtained from LD-ToF-MS may be acetonitrile dimer and glycylglycine, respectively. In addition, the peak for $m/z=98$ is very likely to be a fragment of alanylalanine. Theoretical analysis is now underway.

Conclusion

To investigate the possibility of production of amino acids by impact reactions on Titan, experiments were carried out using a 2-stage light gas gun. We collected the soot produced after the impact, and analyzed it by HPLC, FT-IR and LD-ToF-MS. HPLC analysis showed peaks corresponding to glycine and alanine in the samples for which the ice + hexane + iron target and ice + iron target were used. In case of the iron target, only a small amount of amino acids were detected. Mass spectra also showed peaks corresponding to glycine ($M=75.05$) and alanine ($M=89.09$). From the FTIR spectrum, the stretching vibration of CH at 2950 cm^{-1} and the stretching vibration of CN at 1250 cm^{-1} were identified provisionally. These results suggest that amino acids could be produced by the impact reaction.

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References

Akabori S (1955) Asymmetric synthesis of amino acids and formation of fore-proteins. Kagaku (Science) 25:54

Atreya SK, Lorenz RD, Waite JH (2009) Volatile origin and cycles: nitrogen and methane. In: Brown RH et al (eds) Titan from Cassini-Huygens, chapter 7 pp 177–199

Beghin C, Canu P, Karkoschka E, Sotin C et al (2009) New insights on Titan's plasma-driven Schumann resonance infrared from Huygens and Cassini Data. Planet Space Sci 57:1872–1888

Chang JY, Knecht R, Braun DG (1981) Amino acid analysis at the picomole level. Biochem J 199:547–555

Cronin JR, Chang S (1993) Organic matter in meteorites: molecular and isotopic analysis of the Murchison meteorite. In: The chemistry of life's origins, pp 209–258

Cronin JR, Pizzarello S, Yuen GU (1985) Amino acids of the Murchison meteorite II. Five carbon acyclic primary β-, γ-, δ-amino alkanoic acids. Geochim Cosmochim Acta 49:2259–2265

Engel MH, Macko SA (1997) Isotopic evidence for extraterrestrial non-racemic amino acids in the Murchison meteorite. Nature 389:265–268

Furukawa Y, Sekine T, Oba M, Kakegawa T, Nakazawa H (2009) Biomolecule formation by oceanic impacts on early Earth. Nat Geosci 2:62–66

Hanel R, Conrath B, Flasar FM, Kunde V, Maguire W, Pearl J, Pirraglia J, Samuelson R, Herath L, Allison M, Cruikshank D, Gautier D, Gierasch P, Horn L, Koppany R, Ponnamperuma C (1981) Infrared observations of the Saturnian system from Voyager 1. Science 212:192–200

Irvine WM, Knacke RF (1989) The chemistry of interstellar gas and grains. In: Atreya SK, Pollack JB, Matthews MS (eds) Origin and evolution of planetary and satellite atmospheres. University of Arizona Press, Tucson, pp 3–34

Israel G, Szopa C, Raulin F, Cabane M, Niemann HB, Atreya SK, Bauer SJ, Brun JF, Chassefière E, Coll P, Conde E, Coscia D, Hauchecorne A, Millian P, Nguyen MJ, Owen T, Riedler W, Samuelson RE, Siguier JM, Steller M, Sternberg R, Vidal-Madjar C (2005) Complex organic matter in Titan's atmospheric aerosols from in situ pyrolysis and analysis. Nature 438:796–799

Kasting JF (1990) Bolide impacts and the oxidation state of carbon in the Earth's early atmosphere. Orig Life Evol Biosph 20:199–231

Kunde VG, Aikin AC, Hanel RA, Jennings DE, Maguire WC, Samuelson RE (1981) C4H2, HC3N and C2N2 in Titan's atmosphere. Nature 292:686–688

Kurosawa K, Sugita S, Ishibashi K, Hasegawa S, Sekine Y, Ogawa N, Kadono T, Ohno S, Ohkouchi N, Nagaoka Y, Matsui T (2013) Hydrogen cyanide production due to mid-size impacts in a redox-neutral N2-rich atmosphere. Orig Life Evol Biosph 43:221–245

Kvenvolden K, Lawless J, Pering K, Peterson E, Flores J, Ponnamperuma C, Kaplan IR, Moore C (1970) Evidence for extraterrestrial amino acids and hydrocarbon in the Murchison meteorite. Nature 228:923–926

McKay CP, Borucki WJ (1997) Organic synthesis in experiment impact shocks. Science 276:390–392

Mieno T, Hasegawa S (2008) Production of carbon clusters by impact reaction using light-gas gun in experiment modeling asteroid collision. Appl Phys Express 1:067006-1-3

Miller SL (1953) A production of amino acids under possible primitive earth conditions. Science 117:528–529

Poch O, Coll P, Buch A, Ramirez SI, Raulin F (2012) Production yields of organic of astrobiological interest from H_2O-NH_3 hydrolysis of Titan's tholins. Planet Space Sci 61:114–123

Sato Y, Bounoshita M, Iwaya K, Miyaji T, Saito M (2010) High-speed separation of dabsyl-amino acids by ultra high-performance liquid chromatography. Bunseki Kagaku 59:823–828

Schlesinger G, Miller SL (1983) Prebiotic synthesis in atmospheres containing CH_4, CO, and CO_2, I. Amino acids. J Mol Evol 19:376–382

Shibasaki M, Kanai M, Mita T (2008) The catalytic asymmetric reaction. Org React 70:1–119

Strecker A (1850) Ueberdie künstlicheBildung der Milchsäure und einenneuen, demglycocollhomologen. Ann Chem Pharm 75:27–45

Taniuchi T, Takano Y, Kobayashi K (2013) Amino acid precursors from a simulated lower atmosphere of Titan: experiments of cosmic ray energy source with 13C- and 18O-stable isotope probing mass spectrometry. Anal Sci 29:777–785

Zhao M, Bada JL (1989) Extraterrestrial amino acids in Cretaceous/Tertiary boundary sediments at StevnsKlint, Denmark. Nature 339:463–465