Abiotic synthesis of high-molecular-weight organics from an inorganic gas mixture of carbon monoxide, ammonia, and water by 3 MeV proton irradiation

Yoshinori Takano[a)]
Institute for Marine Resources and Environment, National Institute for Advanced Industrial Science and Technology (AIST), AIST Central 7, 1-1-1 Higashi, Tsukuba, Ibaraki 305-8567, Japan

Akihiro Ohashi, Takeo Kaneko, and Kensei Kobayashi
Department of Chemistry and Biotechnology, Yokohama National University, Hodogaya-ku, Yokohama 240-8501, Japan

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The abiotic formation of high-molecular-weight organics from an inorganic gas mixture of carbon monoxide, ammonia, and water as a result of 3 MeV proton irradiation from a Van de Graaff accelerator was experimentally verified. The inorganic gas mixture was simulated for representative of interstellar medium. The irradiation products included amino acid (AA) precursors, and the molecular weight distribution ranged from several hundred to a maximum of 3000 Da. Both proteinous and nonproteinous AAs were detected after acid hydrolysis. Thus, the primary irradiation products were not free AA analogs, but were AA precursors having high molecular weight. The present results have significant implications regarding the extraterrestrial origins of AA precursors, such as meteoritic organic compounds and the organic composition of interstellar dust particles. [DOI: 10.1063/1.1646757]

The generation of life would have required the fundamental building blocks of bio-organic compounds and is believed to have occurred via a process generally described as ''chemical evolution.'' [1] Organic compounds are thought to have been formed and transformed in interstellar dust particles (ISDs) as they traveled in molecular and diffuse clouds, after which they were preserved in comets in the proto-solar system.[2] It therefore seems that the first steps of abiotic organic compound formation occur in molecular clouds of ISDs. The likely carbon sources for abiotic formation of organics in the ISD environment are carbon monoxide, formaldehyde, and methanol, while the major nitrogen source is ammonia.[3,4] Nitrogen (N_2) may be present in the ISD environment, but it cannot be detected spectrometrically.[5] These interstellar media are constantly irradiated with cosmic and UV rays from neighboring stars.

Recent experiments using UV irradiation of mixtures containing methanol,[6,7] as well as quantitative discussion,[8] have yielded significant results in the field of interstellar organics. However, little is known about the primary irradiation products. Kobayashi *et al.*[9] suggested that the primary products from proton irradiation of the primitive earth atmosphere (carbon monoxide, nitrogen, and water) could be only amino acid (AA) precursors (molecules that provide AAs after hydrolysis) not the free AAs themselves. Miyakawa *et al.*[10] developed the magneto-plasma dynamic arc-jet to synthesize AAs from an amorphous substance composed of carbon, nitrogen, and oxygen. It has been confirmed that AA precursors[8,9,11] and nucleic acid precursors[12,13] were formed by irradiation of primitive earth atmosphere components, although the matrix composition of the irradiation products has not been elucidated. Here, we report the abiotic formation of high-molecular-weight (HMW) organics from an inorganic gas mixture of carbon monoxide, ammonia, and water after high-energy proton irradiation derived from a Van de Graaff accelerator. The present study investigated the primary irradiation products of interstellar dust organics and elucidated their morphological aspects.

A schematic view of the apparatus used for the high-energy proton-irradiation experiment is shown in Fig. 1. Irradiation conditions resembled those found in interstellar dust-clouds, and proton irradiation simulated the main component of cosmic rays. A Pyrex™ glass tube was filled with the following inorganic gas components to simulate the interstellar gas mixture: 350 Torr of carbon monoxide and 350 Torr of ammonia over liquid water, which provided 20 Torr of water vapor at room temperature. Gas mixtures were irradiated with 3 MeV protons generated by a Van de Graaff accelerator at the Tokyo Institute of Technology. Total energy delivered to the gas mixture was 4000 J, as given by the product of the number of particles delivered and ionization

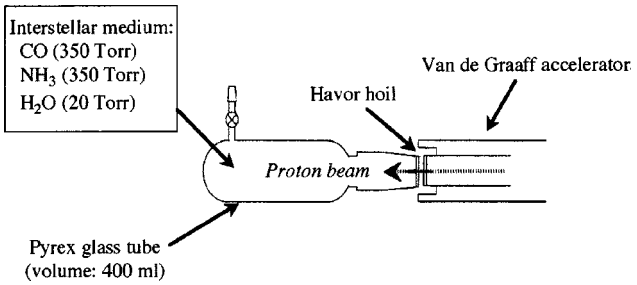

FIG. 1. Schematic view of 3 MeV proton-irradiation apparatus for experimental prebiotic formation of HMW organics, including containing AA precursors.

[a)]Electronic mail: takano.yoshinori@aist.go.jp

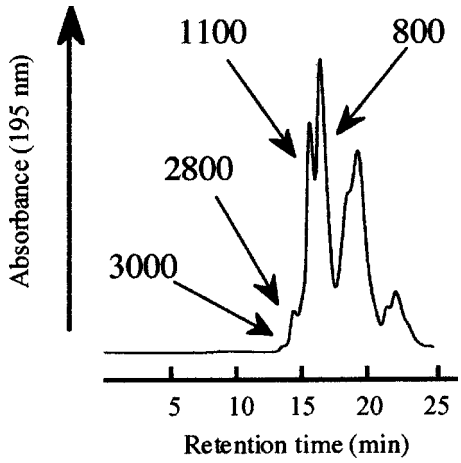

FIG. 2. Gel filtration chromatogram of proton-irradiated sample from the gas mixture of carbon monoxide (350 Torr), ammonia (350 Torr), and water (20 Torr). Values indicate molecular weights of the products.

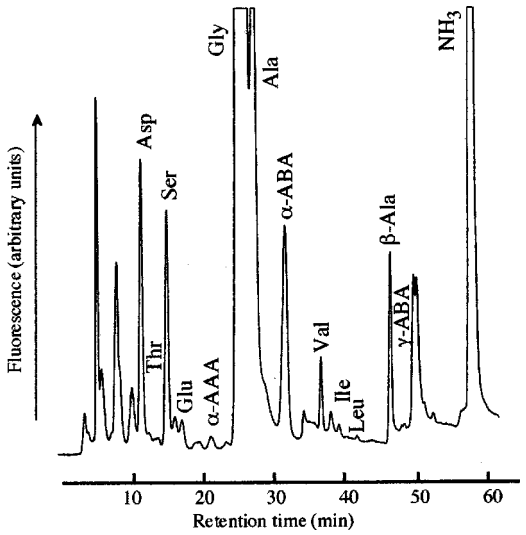

FIG. 3. Typical ion-exchange chromatogram of proton-irradiated sample from inorganic gas mixture of carbon monoxide (350 Torr), ammonia (350 Torr), and water (20 Torr). The fraction was subjected to acid hydrolysis by 6 M HCl for 24 h at 110 °C. Abbreviations. Asp: aspartic acid, Thr: threonine, Ser: serine, Glu: glutamic acid, α-AAA: α-aminoadipic acid, Gly: glycine, Ala: alanine, α-ABA: α-aminobutyric acid, Val: valine, Ile: isoleucine, Leu: leucine, β-Ala: β-alanine, and γ-ABA: γ-aminobutyric acid.

energy loss of a single particle in the gas mixture. Deionized water was further purified with a Millipore Milli-Q LaboSystem™ and a Millipore Simpli Lab-UV (Japan Millipore Ltd., Tokyo, Japan) in order to remove both inorganic ions and organic contaminants. Prior to use, all glassware was heated in a high-temperature oven (Yamato DR-22) at 500 °C in order to eliminate any possible contaminants.

50 μl of the irradiated sample was injected into a gel filtration high-performance liquid chromatography (HPLC) system composed of an HPLC pump (TOSOH DP-8020) and a UV detector (TOSOH UV-8020). The columns used were a TSKgel G2000 SWxL (7.8 mm i.d.$\times$300 mm) for gel filtration, and an Inertsil ODS-3 (4.6 mm i.d.$\times$250 mm) for reversed-phase chromatography. The mobile phase was a mixture of 25 mM acetonitrile (75%) and 0.1% trifluoroacetic acid (25%). Molecular weights were calibrated with polyethylene glycol (PEG) and human serum albumin molecular weight standards.

As shown in Fig. 2, unexpectedly HMW organic compounds were formed from the inorganic gas mixtures: The molecular weight distribution ranged between several hundred and ~3000 Da, and peaks corresponding to 2800, 1100, and 800 Da were estimated. The yellow-colored product was dissolved in water, thus showing the complex organics formed by proton irradiation contain hydrophilic groups, such as -OH, -NH-, and similar bonds. An aliquot of the irradiation products was hydrolyzed with 6 M HCl at 110 °C for 24 h. AAs in the hydrolyzed and unhydrolyzed fractions were then analyzed in an ion-exchange HPLC system using a post-column derivatization with *o*-phthalaldehyde and N-acetyl-L-cystein. The HPLC system used was composed of two HPLC pumps (Shimadzu LC-6A), a cation exchange column (Shimpak ISC-07/S1504, 4 mm i.d.$\times$150 mm), a post-column derivatization system, and a Shimadzu RF-535 fluoromeric detector (excitation wavelength: 355 nm; emission wavelength: 435 nm).[14] Column temperature was maintained at 55 °C. Gradient elution was performed using eluents A (0.07 M sodium citrate perchloric acid, *p*H 3.2, containing 7% ethanol) and B (0.2 M sodium citrate boric acid-NaOH, *p*H 10).

A representative ion-exchange chromatogram of the proton-irradiation products is shown in Fig. 3. Relative concentrations of the hydrolyzed amino acids are shown in Table I. A wide variety of proteinous AAs, such as glycine, alanine, and aspartic acid, as well as nonproteinous AAs, such as β-alanine and α- and γ-aminobutyric acids, were detected in the hydrolyzed fraction. The major components were the C_2, C_3, and C_4 AAs of glycine, alanine, and aspartic acid, respectively. In the unhydrolyzed fraction, only small amounts of glycine were detected. This demonstrates that AA precursors rather than free amino acids were formed from the inorganic gas mixture. In order to quantitatively evaluate the yields of AAs, G-values (number of molecules formed per 100 eV) of glycine after acid hydrolysis were preliminary given as 2.2×10^{-2}.[15] Glycine was the predominant individual AA among the HMW organics. This strongly suggests that extraterrestrial AAs are contained within HMW matrices in cometary or meteoritic complex organics.

TABLE I. Molar ratio of AAs formed by proton irradiation from the interstellar type gas mixtures of carbon monoxide, ammonia, and water. % mole stands for ratio of individual AAs versus total hydrolyzed AAs.

	Amino acid	% mole
Proteinous	Glycine	88.88
	Alanine	4.73
	Serine	1.14
	Aspartic acid	1.13
	Valine	0.15
	Glutamic acid	0.06
	Threonine	0.02
	Isoleucine	tr.
	Leucine	tr.
Nonproteinous	α-Aminobutyric acid	3.16
	β-Alanine	0.60
	α-Aminoadipic acid	0.10
	γ-Aminobutyric acid	0.03
	β-Aminoisobutyric acid	tr.
	Total	100.00

Cometary organics are considered to be a possible source of the terrestrial biosphere.[3,16] Complex organic compounds were discovered in the coma of Comet Halley, but the presence of free AAs was not confirmed.[17] The simplest AA, glycine, has been the target of telescopic observation of interstellar media in efforts to detect extraterrestrial AAs.[18] Because of its key role in the formation of proteins and other biomolecules, this search is worth resuming, despite the lack of success to date. The possible presence of glycine in molecular clouds has been reported,[18] but the signal has not been confirmed.[19] Recently, detection of interstellar glycine was reported by Kuan *et al.*[20] Hence, the discovery of interstellar glycine[20] may strengthen the exogenous study that interstellar molecules could have played a pioneering role in the prebiotic chemistry of the early Earth.

Free AAs are unlikely to be abundant in extraterrestrial environments, and taken together with the presence of AA precursors in the hydrolyzed fraction of meteorites,[21,22] the present data are consistent with the notion that HMW organics are formed in ISD clouds. Consequently, HMW extraterrestrial organics containing AA precursors delivered by comets and/or meteorites may have played a pioneering role in the early stages of chemical evolution under primitive Earth conditions.[3,16]

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[1] S. L. Miller, Science **117**, 528 (1953).
[2] J. M. Greenberg and A. Li, Biol. Sci. Space **12**, 96 (1998).
[3] H. Cottin, M. C. Gazeau, and F. Raulin, Planet. Space Sci. **47**, 1141 (1999).
[4] J. M. Greenberg and A. Li, in *Exobiology: Matter, Energy, and Information in the Origin and Evolution of Life in the Universe*, edited by J. Chela-Flores and F. Raulin (Kluwer Academic, Dordrecht, 1998), pp. 275–285.
[5] *Chronological Scientific Tables, 2002*, edited by National Astronomical Observatory of Japan, Tokyo, 2002.
[6] M. P. Bernstein, J. P. Dworkin, S. A. Sandford, G. W. Cooper, and L. J. Allamandora, Nature (London) **416**, 401 (2002).
[7] G. M. Munoz Caro, U. J. Meierhenrich, W. A. Schutte, B. Barbier, A. A. Segavia, H. Rosenbauer, W. H. P. Thiemann, A. Brack, and J. M. Greenberg, Nature (London) **416**, 403 (2002).
[8] Y. Takano, H. Masuda, T. Kaneko, and K. Kobayashi, Chem. Lett. **10**, 986 (2002).
[9] K. Kobayashi, T. Kaneko, T. Saito, and T. Ohima, Origins Life Evol. Biosphere **28**, 155 (1998).
[10] S. Miyakawa, H. Tamura, A. B. Sawaoka, and K. Kobayashi, Appl. Phys. Lett. **72**, 990 (1998).
[11] T. Kasamatsu, T. Kaneko, T. Saito, and K. Kobayashi, Bull. Chem. Soc. Jpn. **70**, 1021 (1997).
[12] K. Kobayashi and T. Tsuji, Chem. Lett. **1977**, 903 (1997).
[13] S. Miyakawa, H. Yamanashi, K. Kobayashi, H. J. Cleaves, and S. L. Miller, Proc. Natl. Acad. Sci. U.S.A. **99**, 14628 (2002).
[14] K. Kobayashi, T. Kaneko, T. Kobayashi, H. Li, M. Tsuchiya, T. Saito, and T. Oshima, Anal. Sci. **7**, 921 (1991).
[15] Y. Takano, K. Ushio, H. Masuda, T. Kaneko, K. Kobayashi, J. Takahashi, and T. Saito, Anal. Sci. **17**, 1635 (2001).
[16] C. F. Chyba and C. Sagan, Nature (London) **355**, 125 (1992).
[17] J. Kissel and F. R. Krueger, Nature (London) **326**, 755 (1987).
[18] L. E. Synder, Origins Life Evol. Biosphere **27**, 115 (1997).
[19] M. Ohishi, Viva Origino **28**, 175 (2000).
[20] Y. Kuan, S. B. Charnley, H. Huang, W. Tseng, and Z. Kisiel, Astrophys. J. **593**, 848 (2003).
[21] J. R. Cronin, S. Pizzarello, and D. P. Cruikshank, *Meteorites and the Early Solar System*, edited by J. F. Kerridge and M. S. Matthews (The University of Arizona Press, Tucson, 1988), pp. 819–857.
[22] A. Shimoyama, C. Ponnamperuma, and K. Yanai, Nature (London) **282**, 394 (1979).