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Correspondence and requests for materials should be addressed to A.C. (armando.cordova@miun.se; acordova@organ.su.se)

Abiotic synthesis of amino acids and self-crystallization under prebiotic conditions

Liying Jiang[2], Pawel Dziedzic[1], Zdenek Spacil[2,3], Gui-Ling Zhao[1], Lennart Nilsson[4], Leopold L. Ilag[2] & Armando Córdova[1,5]

[1]Department of Organic Chemistry, Stockholm University, SE-106 91 Stockholm, Sweden, [2]Department of Analytical Chemistry, Stockholm University, SE-106 91 Stockholm, Sweden, [3]Department of Chemistry, University of Washington, Seattle, WA 98195-1700, USA, [4]Karolinska Institutet, SE-171 77 Stockholm, Sweden, [5]Department of Natural Science, Engineering and Mathematics, Mid Sweden University, SE-851 70 Sundsvall, Sweden.

Building on previous research on the origin and homochirality of life, this study focuses on analyses profiling important building blocks of life: the natural amino acids. The spark discharge variation of the iconic Miller experiment was performed with a reducing gas mixture of ammonia, methane, water and hydrogen. Amino acid analysis using liquid chromatography coupled with tandem mass spectrometry after pre-column derivatizaiton revealed the generation of several amino acids including those essential for life. Re-crystallization of the synthetic products and enantiomeric ratio analysis were subsequently performed. Results from liquid chromatography coupled with either fluorescent detector or tandem mass spectrometry after pre-column derivatization with chiral reagent revealed spontaneous and effective asymmetric resolution of serine and alanine. This work describes a useful analytical platform for investigation of hypotheses regarding the origin and homochirality of amino acids under prebiotic conditions. The formation of numerous amino acids in the electric discharge experiment and the occurrence of high enantiomeric ratios of amino acids in re-crystallization experiment give valuable implications for future studies in unraveling fundamental questions regarding origins and evolution of life.

Amino acids are the fundamental building blocks of proteins, which are the main catalysts that support life. How amino acids were produced under early prebiotic conditions is an essential question to address in order to reveal the possible origin of life. There are numerous investigations about the origin of amino acids in the early earth. For instance, over 80 natural and non-natural amino acids have been detected in the carbonaceous chondrites (meteorites), which implies that amino acids in the terrestrial biosphere could originate from elsewhere in the solar system[1]. Moreover, eight proteinogenic amino acids were abiotically synthesized under hydrothermal conditions, which supports the hypothesis that amino acids first appeared in submarine hydrothermal systems[2]. In the early 50's, Miller showed that amino acids could be synthesized by the action of electric discharges on a reducing atmosphere of methane, ammonia, water and hydrogen thought to represent the atmosphere of the early earth[3]. Later, they demonstrated up to ten natural amino acids and nine non-natural amino acids/amines could be synthesized in an electric discharge experiment[4]. Those and many other variations of electric discharge experiment clearly showed the production of amino acids from simple chemical reactions, which placed the origin of life question within the realm of organic chemistry[4–7]. Notably, in all of those investigations the amino acids detected were formed as racemates (50 : 50 ratio between the L and D forms within the precision of the measurements). However, in studies of meteorites non-natural amino acids with an enantiomeric excess have been reported[8,9]. The fact that all organisms on Earth manifest single handedness of their chiral amino acids, begs the question regarding the origin of this homochirality, which is another key question regarding the origin of life.

To be able to investigate both questions, the origin and homochirality of amino acids in the early earth, reliable and high throughput analytical techniques are required. For chiral separation of amino acids, an approach using either derivatization with chiral reagents, such as o-phthaldialdehyde/*N*-acetyl-L-cysteine (OPA-NAC)[10] and (+)-l-(9-fluorenyl) ethyl chloroformate (FLEC)[11], or employing a chiral column such as Chirasil-Val GC column[5] is needed. For amino acid analysis, paper electrophoresis[3,12] or gas chromatography (GC)/high performance liquid chromatography with UV or fluorescence detection (HPLC-UV/FD)[2,13] were usually used in the early days.

Those techniques are not sufficient for identification of amino acids due to low resolution and sole reliance on the chromatographic retention time. Later when mass spectrometry (MS) techniques became more popular analytical tools, GC-MS or LC-MS methods emerged within the field of amino acids analysis. The identification becomes more confident because of the use of two dimensions i.e. both retention times from chromatography and molecular masses from mass spectrometry. A good example is the study where a meteorite was analyzed for amino acids using LC-time of flight (TOF) MS[1]. The advantage of using TOF MS is to obtain high mass accuracy for reliable identification. But the use of OPA-NAC reagent which is known to form unstable derivatives for quantification in this study is a disadvantage. Among the derivatization reagents for amino acid analysis, the reagent 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate (AQC) showed several attractive features, such as simplicity, speed, stability, self-hydrolysis of excess reagent and versatile derivatization of all primary and secondary amino acids/amines. Consequently, it became the method-of- choice for amino acid analysis in this study. For comparison of different derivatization reagents, the structures of alanine derivatives using OPA-NAC and FLEC for enantiomeric analysis and AQC for amino acid analysis were elucidated in Fig. 1 (a, b and c respectively). Furthermore, tandem mass spectrometry (MS/MS) was also chosen to be used for improvement of identification confidence and detection limit.

In this investigation, we performed several electric discharge experiments with different time spans (three and seven days). The resulting solutions were taken for profiling analysis by laser desorption ionization (LDI)-TOF MS and amino acid analysis by either HPLC- or ultra-high performance liquid chromatography (UHPLC)-MS/MS after AQC derivatization. Crystallization was also carried out using the synthesized organic mixture solution obtained from the electrical discharge experiments. The enantiomeric ratios of the amino acids of the selected crystals were then analyzed by HPLC-FD or HPLC-MS/MS after the derivatization with OPA-NAC. The aims of this study are: 1) to profile the natural amino acids that can be abiotically synthesized in electric discharge experiment, thus implying the possible origin of these important building blocks of life on Earth; 2) to examine the possibility of spontaneous asymmetric resolution of amino acids obtained from electric discharge experiment through self-crystallization, thus implying the occurrence of homochirality under prebiotic condition; and 3) to develop a versatile analytical platform that can be a useful tool to investigate the questions surrounding the origin and homochirality of amino acids in the early earth.

Results

We performed our experiments in a "Miller apparatus" using spark discharge and a gas mixture of ammonia, methane, water and hydrogen continuously for three or seven days to get sample A and B respectively. Each sample contained two phases: one is the "prebiotic" mixture in the bottom flask; another one is the electrical discharge mixture in the top flask as shown in Fig. S1. In all cases, numerous organic molecules were assembled as determined by LDI-TOF MS (Fig. 2). Both samples were subjected to amino acid analysis by LC-MS/MS after derivatization with AQC reagent. In sample A, a total of 15 natural amino acids and two non-natural amino acids were identified by UHPLC-MS/MS (Fig. 3). In sample B, a total of 17 natural amino acids and seven non-natural amino acids/amines were identified by HPLC-MS/MS (Table S1). Among them 16, natural and two non-natural amino acids are chiral and their relative abundances are shown in Fig. 4. These results revealed that numerous amino acids could be abiotically synthesized under early earth conditions.

Inspired by Kojo's study in which high enantiomeric ratios can be spontaneously generated for 12 racemic amino acids when an excess of racemic asparagine is coexisting[14], we carried out similar re-crystallization experiments. Firstly, seven racemic amino acids with similar ratio as in sample B were re-crystallized in the presence of an excess of racemic asparagine. The enantiomeric excess (D-enantiomer) of the amino acids in these crystals were determined to be 41 ± 3% for aspartic acid, 36 ± 4% for serine, 30 ± 3% for alanine and 42 ± 1% for asparagine (n = 3) based on HPLC-FD analysis (Fig. S3 and 4). Next, re-crystallization was also carried out for the "prebiotic" mixture of sample B. Simply mimicking likely local environments of the early earth, slow evaporation of water from the organic molecule mixture led to the appearance of crystalline material from these two mixture solutions. HPLC-MS/MS analysis after derivatization with chiral reagent OPA-NAC revealed an enantiomeric ratio (er) L to D of 89 : 11 and 58 : 42 for serine and alanine respectively in crystals from "prebiotic" mixtures (Fig. 5b), while it was 49 : 51 and 51 : 49 for serine and alanine respectively in "prebiotic" mixtures before re-crystallization (Fig. 5c). We also performed multiple crystallizations of "prebiotic" mixture A and analyzed the er of alanine (Table 1). We found that a significant er could be obtained (L:D up to 88 : 12) in favor of the L-enantiomer. It is noteworthy that we discovered that the spontaneous asymmetric resolution could give significant er in favor of the opposite enantiomer (Table 1). Thus, a significant excess of the D-enantiomer of alanine was observed for the frozen and stored mother solutions from the "prebiotic" and electrical discharge mixture of sample A (L:D up to 33 : 67), respectively (Table 1). In fact, freezing of abiotic amino acid mixtures could have likely occurred both on earth and extraterrestrial environments[7]. The *vide supra* results indicate that spontaneous asymmetric resolution of amino acids is possible after they were synthesized under Earth's primitive conditions.

Discussion

Encouraged by Miller's famous experiment where he demonstrated that amino acids can be synthesized from simple chemicals; as well as fast development of analytical techniques, especially by employing mass spectrometry for amino acid analysis, we performed a similar Miller experiment and an exhaustive amino acids analysis of the synthesized compounds in the mixture. An overview of the plethora of synthesized organic compounds was revealed by LDI-TOF MS analysis with a mass to charge (*m/z*) window of 50–1000 Da (Fig. 2). In fact, the subsequent amino acid analysis by LC-MS/MS detected all non-sulphur containing natural amino acids except glutamic acid and several non-natural amino acids/amines. The finding is highly valuable compared to previous publications[4–7] in terms of the species of natural amino acids that were synthesized and detected in this study. The reliable analytical method with low limit of detection developed in this study played an important role.

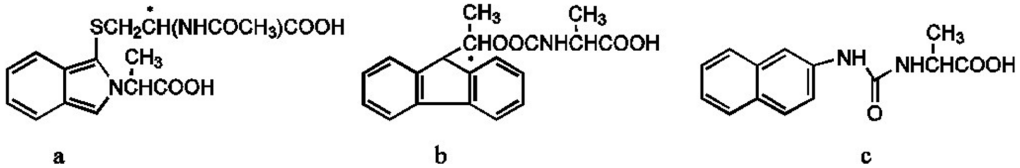

Figure 1 | The structures of alanine derivatives using different reagents: (a) OPA-NAC; (b) FLEC and (c) AQC. * The chiral centre of the reagents. which is used for enantiomeric separation.

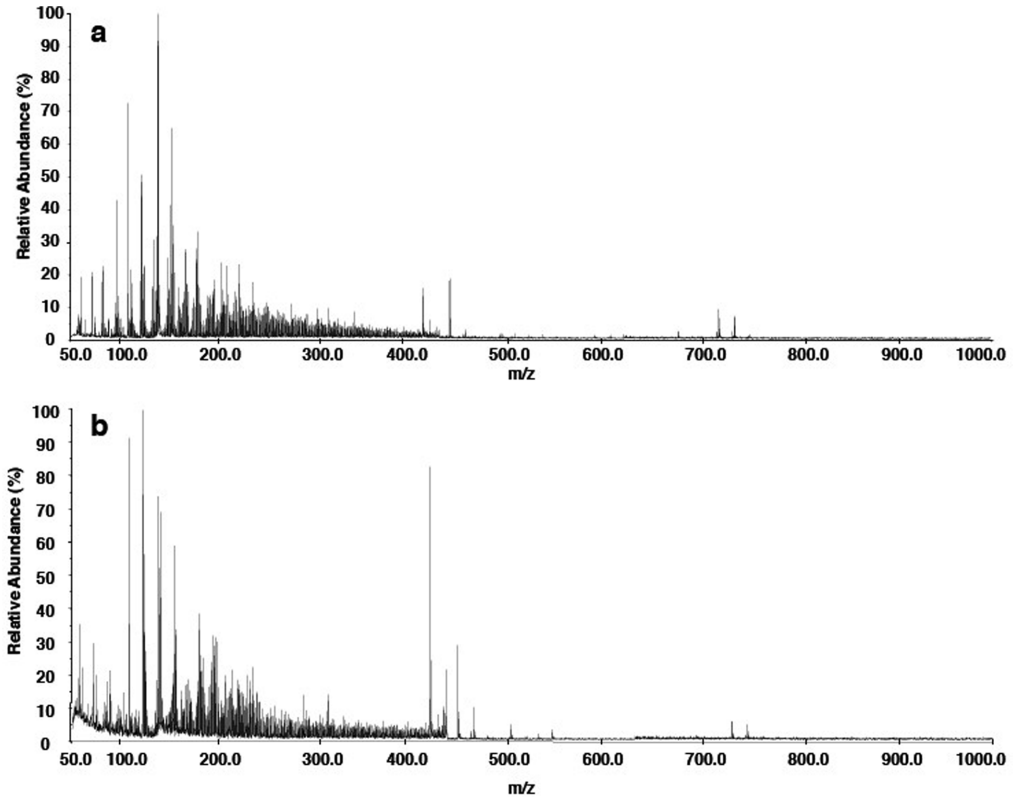

Figure 2 | LDI-TOF spectra of (a) the collected condensed "prebiotic" mixture (b) the collected condensed electrical discharge mixture of the sample B.

Sample A was analyzed by a UHPLC-MS/MS system and pre-column derivatization with AQC tag. There are a number of good features with this method when considering the method reliability and detection limit, namely: 1) Femtomole level detection limit and stable derivatives for one week at room temperature with AQC reagent[15]; 2) Full baseline separation for almost all amino acids when employing the UHPLC C18 column with small particle size (1.7 μm) for high chromatographic efficiency and thus resolution (Fig. S2); 3) Good ionization efficiency of amino acid precursor ions when using soft ionization technique electrospray ionization (ESI) and thus low detection limit compared to the electron ionization (EI) technique used in GC method; 4) Highly reliable identification results generated from MS/MS approach using selected reaction monitoring (SRM) compared to all MS approaches (Table S2) and 5) High ion

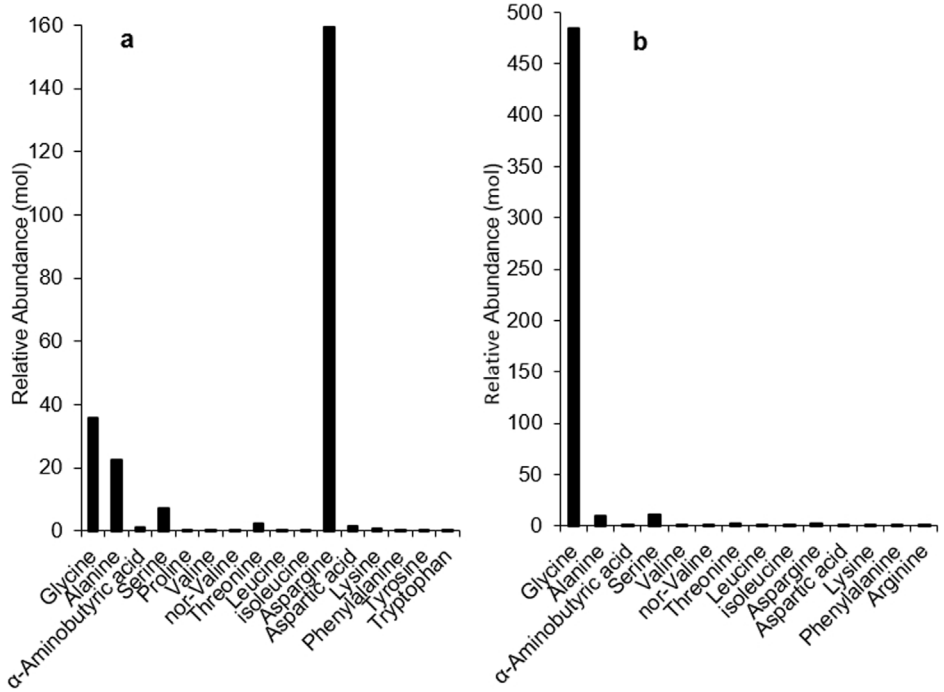

Figure 3 | Relative abundance (in mol) of amino acids in the collected condensed solution of the sample A: (a) "prebiotic" mixture; (b) electrical discharge mixture.

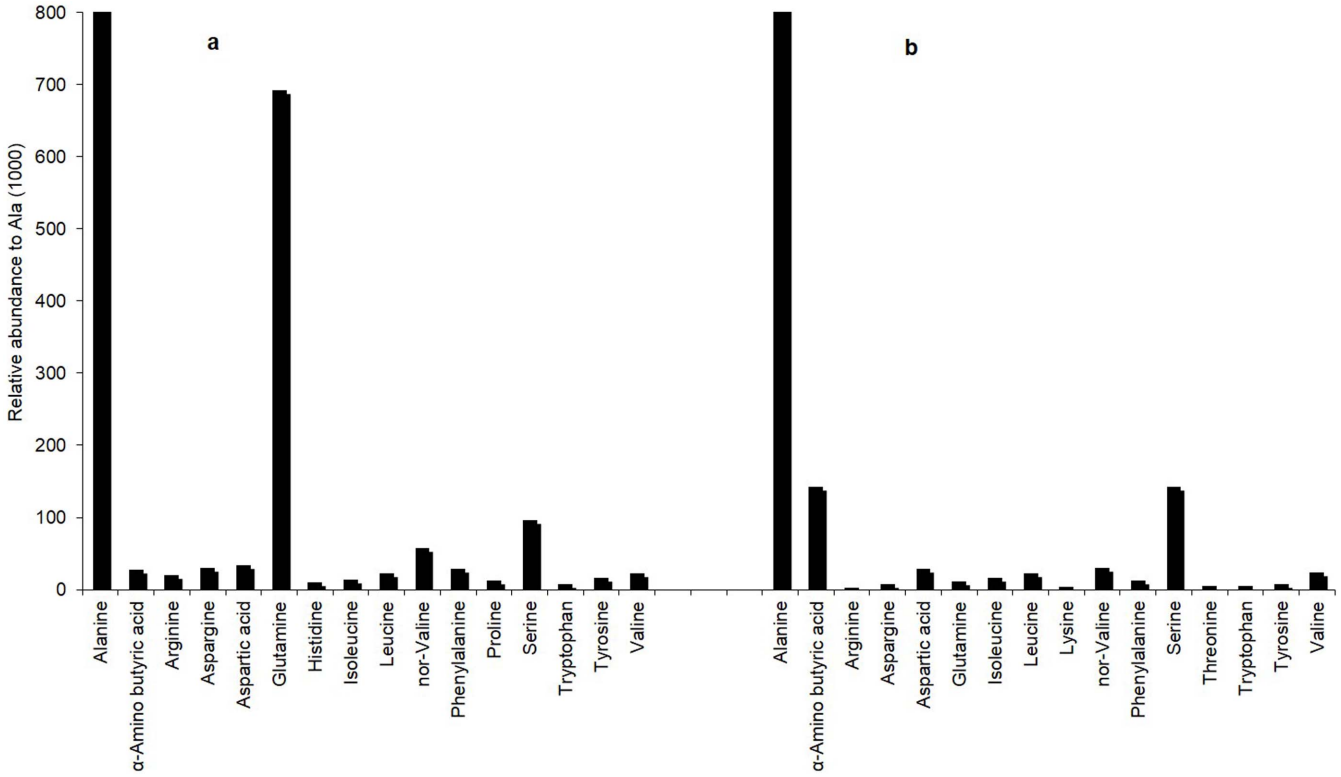

Figure 4 | Relative abundance of amino acids with respect to alanine, which has a value of 1×10^3, of the collected condensed a) "prebiotic" mixture; b) electrical discharge mixture of the sample B.

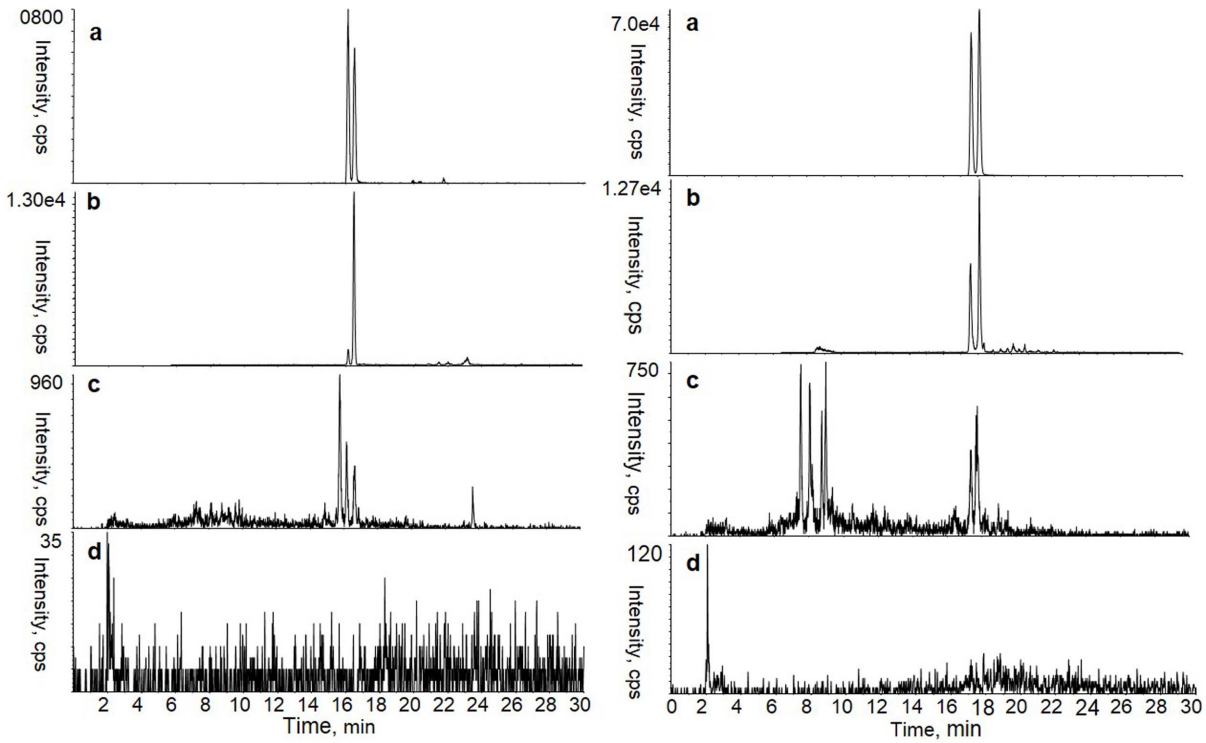

Figure 5 | *(Left lane)* LC-MS/MS chromatograms of D/L-Ser: (a) racemic standard D-Ser ($t_{R,}$ = 16.2 min) and L-Ser (t_R = 16.7 min). (b) D/L-Ser in crystals derived from the "prebiotic" mixture of the sample B. (c) D/L-Ser in the "prebiotic" mixture of the sample B. (d) the blank. *(Right lane)* LC-MS/MS chromatograms of D/L-Ala: (a) racemic standard D-Ala (t_R = 17.4 min) and L-Ala (t_R = 17.8 min). (b) D/L-Ala in crystals derived from the "prebiotic" mixture of the sample B. (c) D/L-Ala in the "prebiotic" mixture of the sample B. (d) the blank.

Table 1 | The crystalization of samples A or B and enantiomeric ratio of alanine

		Prebiotic Mixture Alanine (L:D)[a]	Elect. Discharge Bulb Alanine (L:D)[a]
Crystallization 1 of A	Mother liquid[b]	51 : 49	49 : 51
	Mother liquid[c]	45 : 54	36 : 64
	Crystals[d]	51 : 49	48 : 52
	Supernatant	59 : 41	54 : 46
Crystallization 2 of A	Mother liquid[c]	49 : 51	33 : 67
	Crystals[d]	88 : 12	54 : 46
	Supernatant	55 : 45	36 : 64
Crystallization 3 of A	Mother liquid[c]	49 : 51	NA
	Crystals[c]	55 : 45	NA
	Supernatant	59 : 41	NA
Crystallization of B	Mother liquid[b]	49 : 51	-
	Crystals[d]	58 : 42	-
	Supernatant	NA	-

[a]See experimental section for details.
[b]Fresh mother liquid.
[c]The er of the mother liquid, which was frozen and stored for several months prior to recrystallization experiments.
[d]Er of selected crystals after recrystallization.

transmission efficiency and thus low limit of detection by using four separated segments for 18 different transitions (Table S2). Shortly, this powerful method gives us very confident results for amino acid analysis. For versatile applications in the laboratory without a UHPLC system, we also developed a HPLC-FD-MS/MS method with AQC derivatization for amino acid analysis applied for sample B (see details in Method section). In both samples, the relative abundance of amino acids was estimated by using comparison of chromatographic peak area of each amino acid in the samples with the one in an amino acids standard mixture solution.

The amino acid analysis determined that asparagine had the highest abundance in the "prebiotic" mixture of the sample A. This result led our thoughts to Kojo's study[14] where it was shown that an excess of asparagine can initiate spontaneous generation of asymmetry in a mixture of racemic amino acids. Thus, we wanted to examine whether this also could be the case for the synthesized organic molecule solutions under possible primitive Earth conditions. The initial re-crystallization experiment of a mixture of eight racemic amino acids with similar relative abundance ratio as in the sample B led to spontaneous generation of asymmetry. We next performed a prebiotic crystallization experiment directly on the samples B and A. It is noteworthy that spontaneous generation of asymmetry of alanine and serine in samples A and B was accomplished. Thus, an excess of asparagine is not necessary to induce spontaneous kinetic resolution. To the best of our knowledge, spontaneous resolution of a racemic amino acid mixture derived under prebiotic conditions has never been observed before.

In conclusion, amino acid analysis using modern analytical techniques revealed that 17 essential amino acids were formed in organic mixtures generated by the electrical discharge Miller experiment. Recrystallization of this synthetic amino acid mixture revealed that spontaneous and effective asymmetric resolution could be accomplished. Thus, complex organic mixtures containing a plethora of amino acids generated under prebiotic conditions can undergo spontaneous generation of asymmetry. Since amino acids can be synthesized in space by the reactions presented herein, the generation of asymmetry under the described conditions should also be possible on other planets inside or outside our solar system. In this context, Mars may have had a possible environment for amino acid synthesis since methane was recently detected on this planet[16].

Methods

Chemicals and reagents. Chemicals and solvents were either purchased *puriss p. A.* from commercial suppliers or purified by standards techniques. Water was purified by Milli-Q water purification system (Millipore, Bedford, MA, USA) and had a resistance >18 $M\Omega.cm^{-1}$. Solvents acetonitrile and methanol were HPLC grade obtained from Sigma-Aldrich. Formic acid (≥98%) was from Scharlau Chemie (Barcelona, Spain).

Electrical discharge experiment (see Fig. S1). The reactions were run on a glass apparatus specially made according to the descriptions of Miller[3]. The bottom flask (250 mL) was filled with Milli-Q purified water (125 mL) and heated to 80°C. Next, the system was evacuated under reduced pressure followed by addition of CH_4, NH_3, and H_2 in three separate balloons (150 mL), respectively. We then began the electrical discharge employing two tungsten electrodes continuously for three or seven days in the top flask (250 mL). The CH_4 and NH_3 were refilled each morning and night in two separate balloons (100 mL) after the system had been evacuated. The gas mixture reacted and the water vapor was condensed and the mixture was collected in the bottom of the apparatus. The color of the mixture turned dark brown during the progress of the reaction. After three or seven days, this mixture and the material in the top flask were collected and directly analyzed by LDI-TOF MS and LC-MS/MS.

Re-crystallization of eight racemic amino acids. Eight racemic L,D-amino acids: D,L-α-aminobutyric acid (84.1 mg), D,L-alanine (100.7 mg), D,L-serine (65.2 mg), D,L-valine (9.7 mg), D,L-asparagine (200.2 mg), D,L-tryptophan (4.3 mg), D,L-phenylalanine (18.6 mg) and D,L-aspartic acid (23.7 mg) were dissolved in 2.5 mL Milli-Q water. The re-crystallization was carried on at room temperature for one week. The subsequent crystals were dissolved in Milli-Q water before enantiomeric analysis by HPLC-FD.

AQC derivatization. The standard/sample was derivatized with AQC (Waters AccQ·Tag kit, Waters, Milford, MA, USA). Typically, 20 μL of standard solution was buffered with 0.5 M borate (80 μL) and derivatized with AQC reagent solution (20 μL). The sample was pre-concentrated before the derivatization step, i.e. the sample solvent was evaporated under nitrogen gas, then the dried sample was re-dissolved in borate (80 μL) before 20 μL of AQC reagent was added for derivatization reaction. Finally the sample was shortly centrifuged and supernatant was taken for injection into LC-MS/MS system.

OPA-NAC derivatization. The standard/sample was derivatized with OPA-NAC prepared according to previous report[10]. Typically, 5 μL of standard/sample was derivatized by mixing with 50 μL of OPA-NAC and the reaction was stopped after 10 min by adding 145 μL of 50 mM sodium acetate. The sample was pre-concentrated before derivatization step, i.e. the sample solvent was evaporated under nitrogen gas. Then the dried sample was re-dissolved in borate and derivatized as above mentioned.

LDI-TOF MS analysis. LDI spectra were obtained using Voyager DE-STR time-of-flight mass spectrometer (Applied Biosystems, Framingham, MA, USA) in positive reflector mode equipped with a 2.0 m flight tube (3.0 m in reflector mode) and 3 ns pulsed 337 nm nitrogen laser. Typically, 0.5 μL of 1% trifluoroacetic acid (TFA) in water and 0.5 μL of graphitized carbon black (GCB 4)[17] suspended in water were placed on the sample plate and 1 μL of sample solution was then added. Two-point external calibration using 4-hydroxy benzoic acid (monoisotopic mass at 138.0317 Da) and syringic acid (monoisotopic mass at 199.0528 Da) as calibrants resulted in 80 ppm mass accuracy. The resolving power typically achieved in acquired mass spectra was around 5000 at full width half maximum (FWHM). Voyager Control Panel 5.10 and Data Explorer 4.0.0.0 software were used for gathering and processing raw data.

UHPLC-MS/MS for AQC derivatized amino acid analysis. UHPLC-MS/MS analysis was carried out on an Accela pump and Accela auto-sampler UHPLC system

(Thermo Fisher Scientific, San Jose, USA) coupled with a TSQ Vantage triple quadrupole mass spectrometer (Thermo Fisher Scientific, San Jose, USA). The software package Xcalibur 2.1 was used to analyze acquired data.

HPLC separation was carried out with an ACCQ-TAG™ ULTRA C18 column (100 × 2.1 mm, 1.7 μm particle size, Waters, Ireland) and a binary mobile phase (solvent A: 5% acetonitrile in water with 0.1% formic acid; solvent B: acetonitrile with 0.1% formic acid) delivered at a flow rate of 200 μL/min. The linear gradient elution program was as follows: 0.0 min = 0% B; 10.0 min = 5% B; 25.0 min = 15% B; 40.0 min = 22.5% B; 40.1 min = 80% B; 43.0 min = 80% B; 43.1 min = 0% B and 55.0 min = 0% B. Between two sample injections, one blank operated with a washing program and two blanks operated with the same method for samples were injected to avoid any carry-over on the LC column and stabilize the LC gradient. The washing program was as follows: 0.0 min = 50% B; 20.0 min = 100% B; 20.1 min = 0% B; 30.0 min = 0% B.

AQC derivates of analytes were analyzed in positive ion detection mode using SRM scan type with the ESI technique. The first and third quadrupoles were operated as mass analysers with a resolution at 0.7 FWHM. In order to improve the ion transmission efficiency, SRM scan time in total 40 min was divided into four time segments. Different SRM transitions and scan times in each time segment were list in Table S1. The other MS instrument parameters were optimized as below: spray voltage (4000 V), vaporizer temperature (300°C), capillary temperature (300°C), sheath gas pressure (30 psi), ion sweep gas pressure (0 psi), auxiliary gas pressure (20 psi), S-lens (110), de-clustering voltage (−6 V) and argon collision gas pressure (1.0 mTorr), collision energy (25 V).

HPLC-FD-MS/MS for AQC derivatized amino acid analysis. The system used was API 2000TM LC-MS/MS (Applied Biosystems, Foster City, CA, USA) consisting of a binary pump, auto-sampler, bench-top triple quadrupole mass analyzer with additional fluorescence detector. Analyst 4.2 software was used to explore acquired chromatographic data.

A standard mixture that consists of 18 natural amino acids and nine non-natural amino acids/amines was analyzed using Hypersil GOLD C18 (100 × 2.1 mm, 3 μm particle size) analytical column (Thermo Scientific, San Jose, CA) in gradient elution mode with flow rate at 0.25 mL/min. Eluent A was 0.1% formic acid in water and acetonitrile (95 : 5); eluent B was 0.1% formic acid in acetonitrile, injecting 50 μL of sample. The elution program was applied as follows: 0.0 min = 100% A; 7.0 min = 100% A; 28.0 min = 0% A; 36.0 min = 0% A. Fluorescence detection was carried out with excitation wavelength at 250 nm and emission wavelength at 395 nm. In MS/MS method, AQC singly- or doubly-labelled analytes were chosen as precursor ions and the protonated AQC fragments (m/z = 171.1) were chosen as product ions, as shown in Table S3.

HPLC-FD for OPA-NAC derivatized enantiomeric amino acid analysis. Separation of OPA-NAC derivates was achieved on an Alltima C_{18} (4.6 × 250 mm, 5 μm particle size) column with a mobile phase A (200 mg ammonium acetate with 5% methanol in water, pH 6.8) and a mobile phase B (200 mg ammonium acetate with methanol). The gradient program was as follows: 0.0 min = 0% B; 8.0 min = 15% B; 20 min = 15% B; 45 min = 45% B; 55 min = 65% B; 60 min = 65% B; 60.1 min = 0% B and 75 min = 0% B. The flow rate was 500 μL/min and the injection volume is 5 μL. Fluorescence detection was carried out with excitation wavelength at 360 nm and emission wavelength at 450 nm. Multiple measurements for each enantiomeric ratio determination were performed (technical replicates).

HPLC-MS/MS for OPA-NAC derivatized enantiomeric amino acid analysis. Separation of OPA-NAC derivates was achieved using Sunfire C18 (150 × 2.1 mm, 3.5 μm particle size) analytical column (Waters, Milford, MA, USA) in gradient elution mode with flow rate at 0.20 mL/min, injecting 50 μL. Eluent A was 5 mM ammonium acetate (pH 5.4) in water and methanol (95 : 5); eluent B was 5 mM ammonium acetate in methanol. The elution program was applied as follows: 0.0 min = 100% A; 25.0 min= 0% A; 30.0 min = 0% A. Fluorescence detection was carried out with excitation wavelength at 360 nm and emission wavelength at 450 nm.

1. Glavin, D. P. *et al.* Amino acid analyses of Antarctic CM2 meteorites using liquid chromatography - time of flight-mass spectrometry. *Meteorit. Planet. Sci.* **41**, 889–902 (2006).
2. Hennet, R. J. C., Holm, N. G. & Engel, M. H. Abiotic synthesis of amino acids under hydrothermal conditions and the origin of life: a perpetual phenomenon? *Naturwissenschaften* **79**, 361–365 (1992).
3. Miller, S. L. A production of amino acids under possible primitive earth conditions. *Science* **117**, 528–529 (1953).
4. Ring, D., Wolman, Y., Friedmann, N. & Miller, S. L. Prebiotic synthesis of hydrophobic and protein amino acids. *Proc. Nat. Acad. Sci. U. S.* **69**, 765–768 (1972).
5. Johnson, A. P. *et al.* The Miller volcanic spark discharge experiment. *Science* **322**, 404 (2008).
6. Parker, E. T. *et al.* Primordial synthesis of amines and amino acids in a 1958 Miller H2S-rich spark discharge experiment. *Proc. Nat. Acad. Sci. U. S.* **108**, 5526–5531 (2011).
7. Levy, M., Miller, S. L., Brinton, K. & Bada, J. L. Prebiotic synthesis of adenine and amino acids under Europa-like conditions. *Icarus*, **145**, 609–613 (2000).
8. Cronin, J. R. & Pizzarello, S. Enantiomeric excesses in meteoritic amino acids. *Science (Washington, D. C.)* **275**, 951–955 (1997).
9. Pizzarello, S. *et al.* Large enantiomeric excesses in primitive meteorites and the diverse effects of water in cosmochemical evolution. *Proc. Nat. Acad. Sci. U. S.* **109**, 11949–11954 (2012).
10. Zhao, M. & Bada, J. L. Determination of α-dialkylamino acids and their enantiomers in geological samples by high-performance liquid chromatography after derivatization with a chiral adduct of o-phthaldialdehyde. *J. Chromatogr. A* **690**, 55–63 (1995).
11. Einarsson, S., Josefsson, B., Moeller, P. & Sanchez, D. Separation of amino acid enantiomers and chiral amines using precolumn derivatization with (+)-1-(9-fluorenyl)ethyl chloroformate and reversed-phase liquid chromatography. *Anal. Chem.* **59**, 1191–1195 (1987).
12. Taube, M., Zdrojewski, S. Z., Samochocka, K. & Jezierska, K. Bildung von Aminosäuren und Aminosäurevorstufen aus primitiven Verbindungen bei der Hochtemperatursynthese. *Angewandte Chemie* **79**, 239–239 (1967).
13. Kvenvolden, K. A. *et al.* Evidence for extraterrestrial amino-acids and hydrocarbons in the Murchison meteorite. *Nature (London)* **228**, 923–926 (1970).
14. Kojo, S., Uchino, H., Yoshimura, M. & Tanaka, K. Racemic D L-asparagine causes enantiomeric excess of other coexisting racemic D, L-amino acids during recrystallization: a hypothesis accounting for the origin of L-amino acids in the biosphere. *Chem. Commun. (Cambridge, U. K.)* 2146–2147 (2004).
15. Cohen, S. A. & Michaud, D. P. Synthesis of a fluorescent derivatizing reagent, 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate, and its application for the analysis of hydrolysate amino acids via high-performance liquid chromatography. *Anal. Biochem.* **211**, 279–287 (1993).
16. Mumma, M. J. *et al.* Strong Release of Methane on Mars in Northern Summer 2003. *Science (Washington, DC, U. S.)* **323**, 1041–1045 (2009).
17. Shariatgorji, M., Amini, N., Thorsen, G., Crescenzi, C. & Ilag, L. L. Mu-trap for the SALDI-MS screening of organic compounds prior to LC/MS analysis. *Anal Chem* **80**, 5515–5523 (2008).

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Author contributions

A.C. and L.L.I. designed the project. L.J., P.D., Z.S., G.L.Z. and L.N. performed the experiment. L.J., P.D. and Z.S. analyzed the data. L.J., A.C. and L.L.I. wrote the manuscript.

Additional information

Supplementary Information accompanies this paper at http://www.nature.com/scientificreports

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