Correction

CHEMISTRY

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High-energy chemistry of formamide: A unified mechanism of nucleobase formation

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The coincidence of the Late Heavy Bombardment (LHB) period and the emergence of terrestrial life about 4 billion years ago suggest that extraterrestrial impacts could contribute to the synthesis of the building blocks of the first life-giving molecules. We simulated the high-energy synthesis of nucleobases from formamide during the impact of an extraterrestrial body. A high-power laser has been used to induce the dielectric breakdown of the plasma produced by the impact. The results demonstrate that the initial dissociation of the formamide molecule could produce a large amount of highly reactive CN and NH radicals, which could further react with formamide to produce adenine, guanine, cytosine, and uracil. Based on GC-MS, high-resolution FTIR spectroscopic results, as well as theoretical calculations, we present a comprehensive mechanistic model, which accounts for all steps taking place in the studied impact chemistry. Our findings thus demonstrate that extraterrestrial impacts, which were one order of magnitude more abundant during the LHB period than before and after, could not only destroy the existing ancient life forms, but could also contribute to the creation of biogenic molecules.

origin of life | asteroid impact | biomolecules | LDB

As the Sun formed from its molecular cloud, it was accompanied by a disk of material that consisted of gas and small dust particles. Over several tens of millions of years, these dust particles accumulated and formed the planets we see today. This process occurred in several stages in the terrestrial planet zone, eventually culminating in massive, potentially moon-forming impacts on the proto-Earth (1). Then, following the solidification of the Moon ∼4.5 Ga, the initially heavy impactor flux declined (2) and increased again during the Late Heavy Bombardment (LHB) some ∼4–3.85 Ga (2). Our best models for the origin of the LHB link the LHB to a dynamic instability in the outer solar system (the so-called Nice model) (3, 4), when Jupiter’s orbit changed as a result of close encounters with ice giants and small cometary bodies. These changes resulted in the release of impactors from their previously stable asteroidal and cometary reservoirs. The synthesis of observation and theoretical constraints indicates that the impactor flux on Earth was ∼10 times higher at the LHB than in the period immediately preceding the LHB and that this flux slowly decayed afterward (5–7). At the peak, the LHB most likely involved an impact frequency of 10\textsuperscript{4} tons of material per year (ref. 5 and Fig. L4). The typical impact speeds are estimated to have increased from ∼9 to ∼21 km/s once the LHB began. The ratio of the gravitational cross-sections of Earth and the Moon is found to be ∼17:1. Thus, for every lunar basin, such as Orientale or Imbrium, ∼17 basins should have formed on Earth (8).

Such high impact activity also had extensive implications for the evolution of early Earth (selected milestones in early Earth’s history are shown in Fig. 1B) (9): The atmosphere was partly eroded and transformed (10, 11), and the hydrosphere was enriched by water (12, 13). Crucially, these impact-related processes most likely also contributed to the transformation of biomolecules and their precursors on Earth’s surface, which would have relevant consequences on the origin of life (14, 15).

Although the impact energies were most likely not large enough to produce ocean evaporation or globally sterilizing events (16), they could have served as local energy sources for biomolecule synthesis (17–19). Therefore, the high-impact activity may not have been harmful for the formation of biomolecules and the first living structures. Conversely, it may have been the source of energy required to initiate chemical reactions, such as the synthesis of biomolecules (20).

One of the current landmarks of prebiotic chemistry is the proposal that formamide could be the parent compound of the components of the first informational polymers (21). Saladino et al. extensively studied the formamide-based chemistry that can lead to the synthesis of nucleobases and nucleotides and their metabolic products (22–24). By choosing the appropriate catalyst, purine, adenine, guanine, and cytosine (catalyzed by lime-stone, kaolin, silica, alumina, or zeolite), thymine (irradiated by sunlight and catalyzed by TiO\textsubscript{2}), and hypoxanthine and uracil (in the presence of montmorillonites) were obtained (23–27).

Our recent studies reported the formation of the canonical nucleobases, as well as purine and glycine, during the dielectric breakdown induced by the high-power laser Asterix in the presence of catalytic materials (meteorites, TiO\textsubscript{2}, clay) (17, 18). Formamide-based synthesis in the high-density energy event (impact plasma) can solve the long-standing enigma of the simultaneous formation of all four nucleobases. The main objective of the present study is to demonstrate a unified mechanism of the formation of the nucleobases through the reaction of

\textbf{Significance}

This paper addresses one of the central problems of the origin of life research, i.e., the scenario suggesting extraterrestrial impact as the source of biogenic molecules. Likewise, the results might be relevant in the search of biogenic molecules in the universe. The work is therefore highly actual and interdisciplinary. It could be interesting for a very broad readership, from physical and organic chemists to synthetic biologists and specialists in astrobiology.

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formamide and its dissociation products in a high-energy impact event relevant to LHB.

The plasma formed by the impact of an extraterrestrial body was simulated using the high-power chemical iodine Asterix Laser System (PALS). During the dielectric breakdown in gas (laser-induced dielectric breakdown or LIDB) generated by a laser pulse of energy of 150 J (time interval of ~350 ps, wavelength of 1.315 μm, and output density of 10^14 to 10^16 W/cm²), all expected outcomes for a high-energy density event occurred: shock rises in temperature to 4,500 K (28), the formation of a shock wave, and the generation of secondary hard radiation (UV-VUV, XUV, and X-ray). LIDB generated by the PALS laser is shown in Fig. 2A, and the laser hall is shown in Fig. 2B.

The dielectric of gaseous nitrogen represents an atmosphere in which hot, dense plasma is formed, and the liquid sample represents a lagoon containing formamide (29). The unstable radicals produced in the formamide dissociation have been identified and quantified using time-resolved discharge emission spectroscopy (30, 31) and the models of plasma chemistry described in our previous work (17). The stable products were analyzed using high-resolution IR spectroscopy. The impact event was simulated using LIDB in formamide liquid. Finally, the canonical nucleobases were detected using GC-MS, and quantum chemical calculations were used to propose a plausible mechanism for their formation (see Materials and Methods for experimental setup and quantum chemical calculations).

**Results and Discussion**

The prebiotic scenario by Saladino et al. proposed that formamide could have been concentrated in lagoons and was exposed to hard UV radiation at elevated temperatures and in presence of various catalysts (32). As the Nice model (3, 4) suggests, during LHB such a lagoon must also have been exposed to plasma during an extraterrestrial body impact event, which could initiate a cascade of chemical reactions, eventually leading to the formation of nucleobases.

To form the nucleobases, formamide is first decomposed into reactive radicals and simple gaseous molecules. Using time-resolved spectroscopy and chemical models of a LIDB plasma, CN• (detected in the electronic states X 3Σ−, A 2Σ+ and NH• (detected in the ground state X 3Σ−) radicals have been identified as the most abundant species in the plasma. The emission spectrum of the plasma together with the absorption spectrum of the products are shown in Fig. 3A. Absorption gas-phase spectra demonstrate the formation of stable molecular products. The absorption bands are characteristic of HCN, CO, NH₃, CO₂, CH₂OH, and N₂O. The individual concentrations of these gaseous dissociation products and the resulting yield of each of the nucleobases are summarized in Table 1.

In the LIDB simulation of the impact plasma (17), the radicals’ total yield reaches 55% CN• and 4% NH•, together with 41% of stable CO. Due to the very rigid structure of CN• (from the strong triple bond) and complicated spectrum, this molecule is able to adopt a series of excited electronic configurations. Additionally, due to very favorable Frank–Condon factors, this species can be also excited to very high vibrational and rotational states, which results in observations of a great number of transitions in the visible, IR, and microwave regions. These properties make this transient species an ideal reactant in the high-energy plasma environment. The CN• radical (33–35) was discovered in interstellar space in envelopes of giant stars (36). In a previous paper (18), we showed that the stepwise addition of CN• radicals to formamide combined with recombination steps with atomic H can give rise to the formation of 2,3-diaminomaleonitrile (DAMN) in a highly exergonic reaction. DAMN is generally considered to be the common precursor of all nucleobases.

Except cytosine, all canonical nucleobases were detected using a GC-MS derivatization technique in the samples of formamide

**Table 1. Composition of the liquid-phase products formed in the irradiated samples**

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Adenine, %</th>
<th>Guanine, %</th>
<th>Cytosine, %</th>
<th>Uracil, %</th>
<th>Yield, mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCONH₂</td>
<td>61.9</td>
<td>24.0</td>
<td>0.0</td>
<td>14.1</td>
<td>3.71</td>
</tr>
<tr>
<td>HCONH₂ + chondrite</td>
<td>54.5</td>
<td>33.0</td>
<td>0.0</td>
<td>12.5</td>
<td>3.51</td>
</tr>
<tr>
<td>HCONH₂ + clay</td>
<td>79.4</td>
<td>4.4</td>
<td>13.1</td>
<td>3.1</td>
<td>47.07</td>
</tr>
<tr>
<td>DAMN</td>
<td>7.8</td>
<td>4.5</td>
<td>86.0</td>
<td>1.8</td>
<td>24.74</td>
</tr>
</tbody>
</table>
Fig. 3. The main methods used to study the formamide dissociation in a high-density energy event along with examples of the measured spectra are shown in A. The reactive radical products have been detected using emission spectroscopy. Stable molecular gases formed as the dissociation products of formamide, such as HCN, are fingerprinted by gas-phase IR absorption spectroscopy. Nucleobases, formed from formamide in the high-energy impact event, are detected by GC-MS derivatization technique. (B) Mechanistic model for the transformation of formamide in plasma and its afterglow in a high-density energy event. Formamide reacts with transient species formed from its own dissociation, resulting in all of the canonical nucleobases present in contemporary genetic materials. AICN, 4-amino-5-cyanoimidazole.
exposed to LIDB (the mass spectrum of guanine is reported in Fig. 3A). Composition of the products formed upon irradiation of formamide and DAMN using a high-power laser are shown in Tables 1 and 2. We can see that the high-power laser irradiation of neat formamide produces adenine, guanine, and uracil, whereas DAMN suspensions produce all of the canonical bases. When formamide was irradiated in the presence of clay, all four bases were detected. In our opinion, the role of clay is to protect the adsorbed cytosine against deamination to uracil in a further reaction step. To demonstrate this, we irradiated formamide in presence of an olivine chondrite meteorite Northwest Africa (NWA) 6472 (37). Olivin is a silicate mineral with very low or negligible sorption capacity. Indeed, the results of the irradiation with and without chondrite meteorite (Table 1) are roughly similar and clearly show that cytosine is not formed in presence of olivin, whereas clay supports formation of all studied bases.

We suggest the following general model for the high-energy synthesis of nucleobases from formamide (Fig. 3B). The synthesis is initiated by a reaction of formamide with CN$^+$ radical forming several intermediates detected by means of IR spectroscopy. See ref. 18 and Materials and Methods. This part of the reaction pathway leads to DAMN. The photoisomerization of DAMN produces 2,3-diaminofumaronitrile (DAFN), which binds to another CN$^+$ radical to readily cyclize into a trisubstituted pyrimidinyl radical. This moiety serves as the precursor for cytosine and uracil (17). In Fig. 4 we present the computed free-energy profile for the elementary steps of this conversion, which includes a recombination step with an NH$_2$ radical, followed by the elimination of the functionalities at C5 and C6 in hydrogenation steps. Similar to the previously documented steps of this reaction pathway (17, 18), these steps are exergonic and proceed with a relatively low activation energy. The product, 2,4-diaminopyrimidine, deaminates first to form cytosine and then to form uracil. The theoretical description of these deamination steps is extremely complicated because it is highly dependent on the applied reaction conditions (primarily on the catalyst). Therefore, it is beyond the scope of the current study, which is primarily focused on the noncatalyzed synthetic pathways.

The chemistry described in the previous paragraph can also be applied to derive purine bases from DAMN. The difference is that the pathway includes an additional reaction step in which DAFN cyclizes to 4-amino-5-cyanoimidazole. This synthetic pathway may lead either directly to adenine or to 2,6-diaminopurine, the latter being the precursor of guanine, as shown in Fig. 3B. We have not tried to detect this intermediate by GC-MS technique due to its well-known hydrolytic instability, as demonstrated for example by Levy et al. (38).

**Conclusion**

Our results demonstrate that during the era of LHB, nucleobases may have been synthesized in an impact plasma via reactions of the dissociation products of formamide, such as CN$^+$ and NH$_2$ radicals, with the formamide parent molecule itself, without the need of a catalyst. Our proposal thus extends the original idea of Saladino et al. (39), who suggested formamide to be the precursor of nucleobases in a prebiotic environment (in the presence of UV radiation and a wide range of catalysts, or at elevated temperatures).
temperatures). We suggest that during the LHB period, the environment influenced by extremely frequent impact events was potentially favorable for nucleobase synthesis. The first biosignatures of life are dated to roughly coincide with the LHB or near the end of it. In conclusion, all these findings suggest that the emergence of terrestrial life is not the result of an accident but a direct consequence of the conditions on the primordial Earth and its surroundings (40).

Materials and Methods

Stable products formed in the laser-induced dielectric breakdown have been evaporated to the multipass absorption cell and analyzed using Bruker IFS 125 high-resolution Fourier transform spectrometer in the spectral range of 700–4,300 cm⁻¹. Unstable radicals have been detected using time-resolved spectroscopy of glow discharge plasma in the infrared region of 1,800–5,250 cm⁻¹. Finally, stable nucleic bases have been detected using the gas chromatography mass detection technique in samples dried in vacuum and volatilized using derivatization agent. The experimental procedures are explained in more detail below.

Experimental Procedure of Formamide Dissociation Product Analysis.

The samples of liquid formamide were irradiated in a glass vessel with 10 shots. After irradiation, a sample of the latter analysis standards were shock-heated at a temperature of 30 m directly connected to the Bruker spectrometer IFS 125 HR (Bruker). The measurements were performed with a resolution of 0.02 cm⁻¹ in the middle IR range (MIR) of 700 to 4,300 cm⁻¹ using a HgCdTe liquid nitrogen-cooled semiconductor detector. Three hundred scans were accumulated to acquire a reasonable signal-to-noise ratio. For quantification, the following pure gases were used as calibration standards: HCN (Meter Technology; 98 ppm/He), CO (Linde Gas; 5.3), CO₂ (Linde Gas; 5.0), NH₃ (Linde Gas; 3.8), CH₄ (Linde Gas; 5.5), C₂H₆ (Linde Gas; 5.3), and CH₂O (Sigma Aldrich; 99.9% [wt/wt]). HNCO was prepared via the thermal decomposition of cyanic acid in a quartz tube heated to ~600 °C. The HNCO vapors were concentrated in a nitrogen trap after synthesis, purified by vacuum distillation and then expanded as a standard gas into the multipass cell.

To monitor unstable formamide dissociation products, time-resolved emission spectra were measured using a Bruker IFS 120 spectrometer connected to a computer equipped with a field-programmable gate array processor that was programmed to synchronize the data acquisition and a discharge trigger. This method was described in detail in our work dealing with spectroscopy of methane discharge (30). In the current study, the mixture of 0.05 Torr of formamide with 3 Torr of the He carrier gas was led to the discharge cell being continuously pumped with a rotary oil pump. The emission spectra were measured using CaF₂ optics with an InSb detector and germanium filter in the range of 1,800–5,250 cm⁻¹.

In our previous work, we also refer detection of selected intermediates in the initial steps of nucleobase synthesis mechanism involving direct reaction of formamide with CN radicals (18). The NH₂ deformation band and CN stretching mode of 2-amino-2-hydroxy-acetonitrile (AHAN) and 2-amino-2-hydroxy-malononitrile (AHMN) have been discovered in the gas-phase MIR spectra in the 820, 920, and 1,650 cm⁻¹ regions (Fig. 51). The spectra have been recorded in the same manner as the measurement of other species in this work. In this experiment, the formamide precursor was exposed to LiDDB in presence of NiFe meteorite. We assume that this material binds CN species and possibly blocks the subsequent reactions. AHAN together with AHMN are therefore accumulated in the reaction mixture up to the detection level of 100 ppm.

GS-MS Identification.

The sensitive method of gas GC-MS based on one-step derivatization was used for the targeted analysis of nucleobases. All of the measurements were performed using a Focus GC chromatograph (Thermo Fisher Scientific Corp.) in combination with an ion-trap mass spectrometer ITQ 700 (Thermo Scientific). Derivatized samples were separated on GC capillary column CB-20 (Sigma Aldrich/Supelco; 80% [wt/wt] dimethylpolysiloxane and 20% [wt/wt] polydiethylene). Ten microliters of the derivatization agent N-(tert-butyldimethylsilyl)-N-methyl-trifluoroacetamide [Sigma Aldrich; 99% (wt/wt)] MTBSTFA and 30 μL pyridine aprotic solvent [Sigma Aldrich; 99.9% (wt/wt)] were added to 1 mg irradiated sample and dried in a vacuum at ambient temperature. The vial was then heated at 240 °C for 2 h, and 0.1 μL liquid sample was injected into the split injector heated to 280 °C. The measurement was performed using a column temperature ramp of 50 °C to 280 °C and a temperature gradient of 20 °C min⁻¹. The samples were analyzed for nucleobases by comparing their mass spectra and retention times to those of adenosine, cytosine, uracil [Sigma Aldrich; ≥99% (wt/wt)], and guanine standards [Sigma Aldrich; 98% (wt/wt)]. Besides the RNA bases, thymine, purine, glycine, and HCN tetramer have also been identified among the products using the standard National Institute of Standards and Technology library. The mass spectra of the latter analysis standards are shown in Fig. 52. The method of GC-MS nucleobase analysis has been optimized (e.g., mainly the temperature ramp and derivatization process) based on the results of the analytical standards detection (for illustration, see Fig. 53).

Fig. 54 shows GC-MS chromatograms of four samples treated with high-power laser irradiation. The figure includes chromatograms of pure formamide, DAMN, formamide + clay, and formamide + chondrite. It is important to note that because nucleobases were present in the studied samples in relatively low concentration, it was not possible to analyze them from the total ion current chromatograms, thus only the intensity of major masses were used for quantification. The evaluation has been conducted with the characteristic masses. The respective masses are as follows: cytosine, m/z = 282; uracil, m/z = 283; adenine, m/z = 306; and guanine, m/z = 322.

Theoretical Calculations.

Geometries were optimized at the B3LYP/6-311+G(d,p) level of theory with the 6-311+G(d,p) basis set. The geometrical parameters of the studied compounds were calculated at the same level. The molecular structures of the studied compounds were optimized with the Gaussian 09 program (G09; Gaussian Inc.).

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Supporting Information

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Fig. S1. Gas-phase absorption spectrum of formamide sample treated by LIDB in the presence of NiFe meteorite. Absorption bands of AHAN and AHMN intermediates appear in blue.

Fig. S2. The mass spectra (15–500 m/z) of cytosine, uracil, adenine, and guanine standards after derivatization by MTBSTFA.
Fig. S3. GC-MS chromatogram of nucleobases derivatized by MTBSTFA. It is important to note that adenine standard exhibits two characteristic chromatographic peaks observed only in a high-concentration standard solution. For identification of real samples, we only used the adenine peak with the retention time of 6.79 min.
Fig. S4. GC-MS chromatograms obtained after derivatization of samples irradiated by laser: formamide only, formamide + chondrite, formamide + clay, and DAMN. A total ion current chromatogram and also chromatogram of selected ions (cytosine, m/z = 282; uracil, m/z = 283; adenine, m/z = 306; and guanine, m/z = 322) is presented for every sample.