Complex structures synthesized in shock processing of nucleobases – implications to the origins of life

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Abstract

Nucleobases are nitrogenous bases composed of monomers that are a major constituent of RNA and DNA, which are an essential part of any cellular life on the Earth. The search for nucleobases in the interstellar medium remains a major challenge, however, the recent detection of nucleobases in meteorite samples and laboratory synthesis in simulated analogue experiments have confirmed their abiotic origin and a possible route for their delivery to the Earth. Nevertheless, cellular life is based on the interacting network of complex structures, and there is substantial lack of information on the possible routes by which such ordered structures may be formed in the prebiotic environment. In the current study, we present the evidence for the synthesis of complex structures due to shock processing of nucleobases. The nucleobases were subjected to the reflected shock temperature of 3500–7000 K (estimated) and pressure of about 15–34 bar for over ~2 ms timescale. Under such extreme thermodynamic conditions, the nucleobases sample experiences superheating and subsequent cooling. Electron microscopic studies of shock processed residue show that nucleobases result in spontaneous formation of complex structures when subjected to extreme conditions of shock. These results suggest that impact shock processes might have contributed to the self-assembly of biologically relevant structures and the origin of life.

Introduction

One of the most challenging puzzles in the search for the origin of life is to understand how cellular life emerged from complex molecular structures that include proteins, nucleic acids and lipids. Nucleobases, the one ring (pyrimidines) or two rings (purines), are the key structural units of biological nucleic acids (DNA and RNA) that store genetic information, the formation of which is considered as a necessary component in the prebiotic chemistry leading to the origin of life. A wide variety of nucleobases have been detected in meteorite samples, which confirms their extra-terrestrial origin (Martins et al., 2008; Callahan et al., 2011; Burton et al., 2012; Martins, 2018). Ribose and related sugars have also recently been identified in primitive meteorites (Furukawa et al., 2019). Several experiments have been performed to investigate pathways for an extra-terrestrial synthesis of these molecules. As a result of these investigations, it is found that complex organic molecules of biological interest such as nucleobases can be synthesized in residues obtained from ultraviolet (UV) irradiated molecular ices containing simple molecules such as NH₃, H₂O, CH₃OH, etc. (Nuevo et al., 2009, 2012; Materese et al., 2010), UV irradiation of formamide solution (Menor-Salván & Marín-Yaseli, 2011; Burton et al., 2011; Burton et al., 2017; Oba et al., 2019). Sugars and deoxyxugar derivatives are also reported in residues of UV irradiated icy mixture of H₂O and CH₃OH and confirming their presence in astrophysical environments and carbonaceous meteorites (Nuevo et al., 2018). These results suggest the possible origin of these biomolecules in interstellar space where ice-covered dust grains are illuminated by UV irradiation. Other mechanisms have been proposed that discuss the plausible prebiotic origin of nucleobases in Earth’s primitive environment. These include synthesis of adenine from ammonium cyanide (Oró, 1960) and hydrogen cyanide (Oró & Kimball, 1961), UV irradiation of formamide solution (Saladino et al., 2006; Barks et al., 2010), UV irradiation of acetylene in a water/ice solution (Menor-Salván & Marín-Yaseli, 2013), and spark discharge of urea in water/ice solution (Menor-Salván et al., 2009). Nonetheless, extra-terrestrial impact by comets, meteorites, asteroids, and interplanetary dust particles have been considered as the primary source of organic materials required for the emergence of life on the early Earth (Chyba et al., 1990; Chyba & Sagan, 1992). A wide variety of organic compounds such as carboxylic acids, amino acids, hydroxyl acids, amines, etc., have been detected in various meteorite samples, indicating the essential role of impact processes in the origin of life (Pizzarello & Shock, 2010, 2017). Impact events are ubiquitous.
in the Solar System, with various impact craters on planetary bodies being a constant reminder of the role of impact events in Solar System formation and evolution. Impact-induced shock may also serve as an essential source for large-scale molecular synthesis due to various chemical pathways of reaction available in the extreme thermodynamic conditions provided by impact events (McKay & Borucki, 1997; Blank et al., 2001; Goldman & Tamblyn, 2013). Synthesis of various amino acids and nucleobases from simple molecules containing nitrogen, oxygen, carbon and hydrogen in simulated impact shock conditions has already been discussed (Bar-Nun et al., 1970; Martins et al., 2013; Furukawa et al., 2015; Rios, 2015). Experiments have also shown the synthesis of nucleobases from formamide as a result of the plasma formed by high-energy impact events (Ferus et al., 2014, 2015, 2019). Ferus et al. (2017) have also demonstrated the formation of nucleobases as driven by impact plasma and electric discharge in a Miller Urey reducing atmosphere containing simple gases. Apart from amino acids and nucleobases, sugars are also synthesized in impact-driven processes (Civiš et al., 2016). So, the routes by which prebiotic availability of nucleobase, either due to endogenous production or as a result of exogenous delivery, have been confirmed. The next key step in this process requires that these molecules had to be brought in closer proximity to facilitate the emergence of nucleosides, nucleotides and their oligomers (Menor-Salván, 2018). Given the possibility of synthesis of all the nucleobases together in impact shock condition (Ferus et al., 2014; Ferus et al., 2015) or synthesis in interstellar ice analogous (Oba et al., 2019) and their detection in meteorites samples (Callahan et al., 2011), and with evidence of intense impact events on Earth during the period of late heavy bombardment (Koeberl et al., 2000), these organics must have undergone multiple impact events leading to some destructive and some constructive consequences. So, it is necessary to further understand the subsequent fate of these nucleobases in extreme conditions of impact to understand the pathways to the origin of life. While amino acids are known to survive in such extreme condition and also resulted in the formation of peptides as a result of impact-driven shock processes (Blank et al., 2001; Otake et al., 2011; Sugahara & Mimura, 2014, 2015), the fate of nucleobases in similar impact conditions remains largely unexplored. In a previous investigation, we have shown synthesis of a variety of complex macroscale structures as a result of shock processing of various amino acids (Singh et al., 2020). Here we report an experimental investigation of impact-induced shock processing of nucleobases utilizing a shock tube to simulate the high-temperature heating effect, due to impact-induced shock. The high temperature (thousands of kelvin) and pressure (tens of bar) that are created in these shock tube experiments certainly do not exactly mimic the actual pressure–temperature values that are produced in natural impact events, where the pressure values can go as high as tens of thousands of bar but these tools offer a similar thermodynamic condition that occurs in post-impact shock condition when decomposition occurs and pressure value falls to standard planetary conditions. Laboratory simulations of such extreme conditions are always challenging. Various experimental techniques such as high-intensity lasers are employed to simulate such events (Gerasimov et al., 1998). Previous experiments have discussed the high-temperature heating effect of impact-induced shock experiments using high-intensity lasers to explore prebiotic chemistry at high temperatures (Ferus et al., 2014, 2019) or to explore rock-melts during meteorite impact (Ebert et al., 2017). Shock tube offers similar characteristics and could be a useful tool to simulate such extreme conditions of impact. Previously, Bar-Nun et al. (1970) had utilized the shock tube setup in their experimental simulation of an impact-induced shock to produce amino acids. In the present investigation, nucleobases were subjected to a strong shock with a reflected shock temperature of 3500–7000 K (estimated) and shock Mach number range 4–6, maintained over a 2 ms time scale and results in the formation of complex structures.

Materials and methods

Shock processing of nucleobases

To simulate the high-temperature heating effect of impact-induced shock, we have utilized Material Shock Tube 1 (MST1; Biennier et al., 2017; Singh et al., 2020) facility in the Department of Solid State and Structural Chemistry Unit, Indian Institute of Science, Bangalore, India, and the High-Intensity Shock Tube for Astrochemistry (HISTA; Singh et al., 2020) in the Physical Research Laboratory, Ahmedabad, India. Both the shock tubes are similar in their design, and experiments were carried out and repeated at both the shock tube facilities. A schematic diagram of MST1 is shown in Fig. 1. The shock tube consists of two sections namely, a driver section and a driven section of the length of 2 and 5 m, respectively. The tube has an inner diameter of 80 mm. These two sections are separated by an aluminum diaphragm of about 2 mm thickness. Suitable V-grooves of length 85 mm (as shown in Supplementary Fig. S5) are made perpendicular to each other with a depth of one-third of the thickness of the used diaphragm; this becomes the weakest point to burst the diaphragm instantaneously. The driven section is filled with low-pressure test gas (P1) and the driver section is filled with high pressure (Pd) gas until the diaphragm bursts instantaneously, which produces shock waves in the driven section. For a fixed diaphragm thickness, the shock Mach number increases by decreasing test gas pressure in the driven section. Also, it is possible to increase the Mach number by increasing the thickness of the diaphragm material. Shock strength increases with an increase of the shock Mach number. At the end of the driven section, arrangement is made to place the sample to study the interaction of shock wave with the test sample. The sample is mounted on a horizontal plate between a manually operated ball valve and the reaction chamber. The interaction of the strong shock heated gas with the sample occurs at the end flange of the 300 mm long reaction chamber. The single nucleobases, as well as mixtures of nucleobases mixed in equal weight proportions (as listed in Table 1, procured from Sigma Aldrich with purity >99%), were uniformly distributed over the sample holder parallel to the flow of gas inside the shock tube. Before starting the experiment, the complete shock tube is sterilized with acetone 3–4 times to avoid any impurities from previous experiments. After the sample is loaded, the driven section of the shock tube is purged 2–3 times with ultra-high purity (UHP) argon (99.999%) to remove any residual gas impurity present inside the shock tube then pumped to vacuum down to 3 × 10–4 mbar using a turbomolecular pumping system and then filled with UHP argon up to the desired pressure (pressure values are estimated before shock, to produce a particular temperature) to perform different experiments listed as test gas pressure in Table 1. A rotary pump connected to a gas outlet port of the driver section is used to evacuate up to 0.01 bar. High-pressure helium gas is rapidly filled to the driver section at a very high
mass flow rate until the burst of the aluminium diaphragm. The sudden bursting of the diaphragm results in the formation of a shock wave that travels through the driven section and is reflected from the end flange of the reaction chamber. The sample experiences this reflected shock in the reaction chamber. After shock processing, the high-pressure gas inside the chamber is released slowly and the solid residue left in the reaction chamber was collected in airight vials and stored in a vacuum desiccator until further analysis. Three pressure sensors (PCB Piezotronics 113B22) surface mounted at three different locations, each 0.5 m apart, on the driven section, were used to obtain a pressure signal recorded using a Tektronix digital storage oscilloscope (TDS2014B). The signals recorded from the oscilloscope, showing the signature for both incident and reflected shock are shown in Fig. 2.

The shock Mach number in the driven section is calculated by the following relation:

\[ M_s = \frac{V_s}{a_1}, \quad \text{where} \quad V_s = \frac{\Delta L}{\Delta t} \]  

(1)

where \( \Delta t \) is the time (s) taken by a shock wave to travel across two pressure sensors located at a known distance between two ports, \( \Delta L \) (0.5 m) is used to find out the shock speed \( V_s \), \( a_1 \) (m s\(^{-1}\)) is the speed of sound in test gas and \( M_s \) is the shock Mach number.

The kinetic energy (KE) of the test gas in the driven section travel at high velocity \( V_s \), instantaneously stops at the end flange of the driven section of the shock tube called as stagnation point condition or isotropic condition. The KE of gas molecules is converted to heat energy, so the pressure and temperature shoot up simultaneously at the end flange of the shock tube. The pressure jumps across the primary and reflected shock are recorded by using a PCB-Piezotronics pressure sensor located at end of the driven section of the shock tube as shown in fig. 2. A corresponding pressure jumps \( (P_2/P_1) \), \( (P_3/P_1) \) and temperature jumps \( (T_2/T_1) \) and \( (T_3/T_1) \) for a given shock Mach number are estimated using the following Rankine–Hugoniot (R–H) relations at an isentropic condition (Gaydon & Hurle, 1963):

\[ \frac{P_2}{P_1} = \frac{2 \gamma (M_s)^2 - (\gamma - 1)}{\gamma + 1} \]  

(2)

\[ \frac{T_2}{T_1} = \frac{(2 \gamma (M_s)^2 - (\gamma - 1)) - [(\gamma - 1)(M_s)^2 + 2]}{(\gamma + 1)(M_s)^2} \]  

(3)

where \( \gamma = C_p/C_v \) is the specific heat ratio of test gas, \( C_p \) and \( C_v \) are specific heat of the gas at constant pressure and volume, respectively, reflected shock pressure \( (P_3) \) is recorded using a pressure sensor located at the end of the shock tube. The pressure jump \( (P_3/P_1) \) across the reflected shock wave can also be estimated theoretically using the following equation:

\[ \frac{P_3}{P_1} = \frac{2 \gamma (M_s)^2 - (\gamma - 1)}{\gamma + 1} \left[ (3\gamma - 1)(M_s)^2 - 2(\gamma - 1) \right] \]  

(4)

The estimated temperature jump across the reflected shock wave \( (T_3/T_1) \) for the measured value of shock Mach number in the driven section is computed using the following equation:

\[ \frac{T_3}{T_1} = \frac{2(\gamma - 1)(M_s)^2 + (3 - \gamma)\{(3\gamma - 1)(M_s)^2 - 2(\gamma - 1)\}}{(\gamma + 1)(M_s)^2} \]  

(5)

where \( P_1 \) is the initial test gas pressure in the driven section, \( P_2 \) is the primary shock pressure, \( T_1 \) is the ambient temperature (298 K)
for the test gas, $T_2$ is the primary shock temperature, and $T_5$ is the reflected shock temperature.

Experimentally we measured reflected shock pressure ($P_5$) and estimated the reflected shock temperature ($T_5$) under isentropic conditions using equation (5). It is not possible to measure actual shock temperature within the test time of 1–2 ms time scale. Using a platinum resistance thermometer, we can only measure heat transfer rate, but not the actual temperature. Knowing the ambient temperature, it is possible to estimate the reflected shock temperature experienced by the test gas. Reflected shock temperature is a function of $M_s$ and $\gamma$ of the test gas. The powder sample loaded inside the shock tube experiences this stagnation pressure and temperature simultaneously at the end of the shock tube for a short duration. During this process, the sample experiences superheating and cooling at the rate of about $10^5$ K s$^{-1}$ at medium-reflected shock pressure. The presented experiments were performed with reflected shock temperatures ranging from 3740 to 6900 K (estimated) and reflected shock pressure of about 15–34 bar for 1–2 ms timescale. Typical experimental values of Mach number, reflected shock pressure and estimated shock temperature are listed in Table 1.

Sample preparation for scanning electron microscope (SEM) and high-resolution transmission electron microscope (HRTEM) analysis

SEM imaging was performed to investigate the morphology of both unshocked samples and shock processed residue. Carbon tape was pasted on the SEM sample stub, on which the sample was spread and then gold coating was applied on the top of the surface of the sample to make it conductive and to avoid surface charging during SEM analysis. The SEM was conducted utilizing a ZEISS ULTRA 55 at an operating voltage of 5 kV at different magnifications ranging from 30× to 10 000×.

An HRTEM analysis was also performed to investigate the internal structure of shock-processed residual samples at higher magnification. The small quantity of sample was placed in 1.5 ml of acetone solvent and then sonicated for 10–20 min. If the solution looks dark, the sample may agglomerate and is not suitable for obtaining good HRTEM images. Acetone is further added to make the solution more dilute and a micropipette is used to collect the suspended particles from the dilute solution and a drop of this solution is dispersed on a 200 mesh carbon-coated copper grid. The sample is kept in vacuum desiccators before loading into the microscope. HRTEM studies were performed utilizing a Titan Themis from FEI, at an operating voltage of 300 kV and magnification of ~25 000×.

Microscopic techniques such as SEM, HRTEM, etc. provide great insights at micro and nanoscale revealing the basic architecture, morphology, surface properties, microstructures, and interfaces of biopolymers which is an essential tool to determine their ideal application (Venkateshaiah et al., 2020). The remarkable application of the microscopic technique in the interpretation of various self-assembling systems and its application in the origin of life research has also been discussed by Jia & Kuruma (2019).

Results and discussion

Single nucleobases and a mixture of nucleobases (as shown in Table 1), were mixed in equal weight ratios and loaded in a shock tube for shock processing. After the shock processing, a blackish residue was left in the reaction chamber which was collected and further subjected to SEM to reveal the intricate details present in the residue. The SEM micrographs revealed that a variety of complex structures appeared after shock processing. No such structures were observed in the unshocked mixture of nucleobases. SEM measurements of the mixture of nucleobases before shock only show some micron-sized particles in the sample, as shown in Supplementary Figs. S1 and S2 of mixtures of adenine, guanine, cytosine and thymine (AGCT) and adenine, guanine, cytosine and uracil (AGCU), respectively. Single nucleobase adenine and cytosine were also shock processed at a temperature of ~4000 K. SEM observations of these shock processed residues did not show any complex structures. Large clumps of size few tens of microns were seen as shown in Supplementary Figs. S3 and S4.

The SEM micrographs of a mixture of AGCT residue, shock processed at an approximate temperature of 3700 K, show the formation of many smooth globule structures with a diameter of 30–50 μm as shown in Fig. 3, as well as the formation of long (~500 μm) periodically twisted filaments, were seen in shock processed samples. Various long folded threads spanning up to a few mm length were also observed, as shown in Fig. 3(d). All these structure formations occur as a result of shock processing of a mixture of nucleobases as the shock heated gas interacts with the sample in the reaction chamber.

The same mixture of nucleobases was further subjected to a higher reflected shock temperature of ~6360 K. Interestingly, a variety of large-scale ordered structures were seen in shock processed residue (Fig. 4), as were long threads of few mm lengths running along with a variety of structures were observed (Fig. 4). The various features observed at higher magnification show that some threads are more twisted and folded.

Further ribose was added to the mixture of AGCT bases, all mixed in equal weight proportion (1:1:1:1:1), and shocked processed at a temperature of ~6900 K. The SEM micrographs of residues also show the formation of long-range (few mm) threads along with large clumped structures. The threads were found to be twisted and folded, as shown in Fig. 5. The structures as such did not show any significant change in morphology compared to the mixture of only AGCT.
Mixtures of four RNA bases (AGCU were also shock processed at a temperature of ∼6320 K. Various thread structures with a size hundreds of microns were observed in SEM observation (Fig. 6). However, the number of threads present in this sample was not as prominent as we observed in the mixture of AGCT also these threads were not so twisted or folded compared to the mixture of AGCT and AGCT with ribose.

Thus a variety of morphological features including long threads and ribbons, twisted and folded appearance, and globule structures were observed in the shock processing of nucleobases. To get more information on the morphological characteristics of these structures, we subjected the shock processed residue to HRTEM. The HRTEM micrographs of the AGCT mixture showed the presence of fine twisted and folded features at a nanometre scale, as shown in Fig. 7(a) and (b). The AGCU mixtures also showed few sheets with uniform and twisted appearance at a nanometre scale (Fig. 7(c) and (d)). These observations indicate that the property of self-organization seems to be not profound in AGCU bases. Although a possible explanation for these results requires a detailed investigation, a possible reason could be the presence of thymine instead of uracil providing more stability due to the presence of the methyl group in thymine. The self-organization property of the mixture of AGCT bases also favours the early role of DNA and its components in the prebiotic evolution of life, confirming the previously suggested theories on the origin of life that require a heterogeneous genetic system made up of both RNA and DNA (Bhowmik & Krishnamurthy, 2019; Kim et al., 2020; Xu et al., 2020).

The shock-processed residues were further characterized by Infrared (IR) spectroscopy. The IR spectroscopy of the shock-processed residue of AGCT mixture at a temperature of ∼3700 K reveals the formation of globule features, twisted filaments and long folded threads.
processed residue and unshocked sample was performed for single nucleobases as well as the mixture of nucleobases over a wavenumber range of 4000–500 cm\(^{-1}\), and are shown in the Supplementary Figs. S6–S11. Various characteristics peaks corresponding to vibrational modes of nucleobases can be seen in both the shocked and the unshocked samples. This indicates that nucleobases remain intact against the impact of the strong shock.

While from previous investigations it has been revealed that rich chemistry can occur at such extreme conditions, the formation of such microstructures was never revealed before. At present, the chemical composition of these microstructures is unknown. Given that nucleobases can still remain intact in the shock processed residue as revealed from IR data, these structures could possibly be the outcome of the assembly of nucleobases. Such structures play an essential role in the biological function because of their unique architecture. Self-organization of such complex structures on a macroscopic scale under simulated impact shock conditions is an important step in the prebiotic development of life. The formation of such long-range ordered structures in a prebiotic context has always been a difficult task. Life requires a high degree of structural order as well as complexity (Mayer, 2020). Any system which lacks one or another will lead to a dead-end, for example, asphalt or tar (Benner et al., 2012) which is highly complex, or conversely crystals that are highly ordered (Mayer, 2020). A mechanism for the simultaneous increase in order and complexity will have significance for the

**Fig. 5.** SEM micrographs of a shock processed residue of nucleobases AGCT with ribose at a temperature of \(\sim 6900\) K: (a) long threads feature mm in length, (b) various thread-like structures can be seen at higher magnification and (c & d) magnified image shows more twisted and folded threads and flat ribbons.

**Fig. 6.** SEM micrographs of a shock processed mixture of nucleobases AGCU at a temperature of \(\sim 6320\) K: (a & b) long threads and (c & d) threads at higher magnification.
prebiotic chemistry. The complex organized structures as synthesized by shock processing of biomolecules, thus have an important consequence for the origin of life. Many attempts have been made on the synthesis of such microstructure in primitive Earth environments and justifying their importance for the origin of life studies (Fox, 1973; Folsome, 1976; Valladas-Dubois & Prudhomme, 1983; Simionescu et al., 1985; Yanagawa et al., 1988). The formation of such structures is not only limited to terrestrial environments but seem to be relevant in the interstellar space with discoveries of self-assembled membranous structures observed in carbonaceous meteorites (Deamer & Pashley, 1989) and further confirmation by experimental investigation of similar self-assembled vesicle structures formed as a result of UV photolysis of interstellar ice analogues containing simple molecules (Dworkin et al., 2001). However, our method of shock processing of nucleobases provides a pathway by which these self-assembled organized structures could have appeared on the Earth, without the presence of any additional catalytic activity or atmospheric conditions, only triggered by shock energy provided by impact events.

Many competing models are suggested to explain the origin of life, such as the RNA world, the compartmentalistic approach, the prebiotic metabolism approach and many others. There is still no united model available for the origin of life (Luisi, 2016). However, the universal presence of cellular life on the Earth demands the emergence of cell-like architectures and functionality (Szostak et al., 2001; Mann, 2012). The first step in this process is to search for a system that can spontaneously organize itself into structures that can act as a prototype that mimics various characteristics of minimal cellularity (Mann, 2012; Zhang, 2012). The formation of complex structures as revealed in the present investigation is a possible answer to the first step in the path of prebiotic cellularity. Evidently, many profound challenges remain that need to be answered to search for the origin of life and find its signature elsewhere (Lingam & Loeb, 2021). In future endeavours, we will explore the physical and chemical characteristics of these structures, which will provide significant elucidation of our understanding of the role played by complex molecules and impact events in the origin of life.

Conclusion

The novel shock tube method is used to produce extremely high temperatures (3500–7000 K estimated) shock and pressure of (15–34 bar) for a small duration to interact with the nucleobases. The shock processing of nucleobases has shown a plausible pathway by which nucleobases can spontaneously be self-assembled in complex structures when subjected to impact shock conditions. SEM and HRTEM analysis of shock processed residue provides an insight into the synthesis of a large variety of complex structures, including globules, long helical threads, and ribbons of up to millimetre length scale and twisted patterns at the nanometre scale. The formation of such structures in plausible prebiotic conditions implies their prebiotic significance in the subsequent stage of evolution, these structures may be incorporated with other prebiotic polymers in the right conditions and serve as the basis for the life-like self-assembling system. In future work, we will pursue more experiments to form new complex structures at different shock temperatures and to investigate the new structures by different characterization techniques.
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