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THERMAL AND CHEMICAL STABILITY OF THE SOLID STATE RNA SAMPLES: COULD IT SURVIVE AT THE EXTREME CONDITIONS OF THE EARLY EARTH?

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(Received: August, 9, 2020 Accepted: November, 5, 2020)

Abstract
In this work physical and chemical properties of RNA and its sodium salt (Na-RNA) have been studied at extreme conditions similar to those abundant at the prebiotic stage of chemical evolution. Characterization of dry RNA samples has been performed by thermal analysis (TGA and DTA) and NMR relaxation methods. Aqueous solutions of the above samples have been additionally studied using UV-Vis spectroscopy and dynamic light scattering. It has been shown that the mechanism and rate of thermal destruction, as well as the hydration degree of the samples significantly differ for the two forms of RNA studied. A chemical modification of the solid state RNA samples upon storage includes both oxidation of the nitrogenous bases and hydrolysis of phosphodiester bonds leading to the changes in the absorption spectra and different solubility of the RNA and Na-RNA samples.

Keywords: ribonucleic acid, sodium ribonucleinate, themogravimetric analysis, differential thermal analysis, NMR relaxation.

Introduction

The main problem in verification of numerous scenarios of abiogenesis and chemical / prebiotic evolution under the certain astrochemical and geochemical conditions is their consistency with the minimal physicochemical requirements to the conditions of existence (structure preservation and sustainable functioning) for the crucial substances involved in the above scenarios. In other words, at the given natural temperatures and pressures in the phase diagram a possible prebiotic system should have the same phase composition and functionality as its modern biologically active analog or evolutionary "descendant". However, in the high temperature range the system will decompose while in the ultra low temperature range components of the modern cytoplasm crystallize (which is not always reversible [1] and often disrupts their conformation [2,3] and biological activity [4]). In multicomponent microheterogeneous systems like cytoplasm one component may start to decompose before another one, therefore, the loss of functionality of the whole system will be determined not by the entire composition of the system, but by the limiting component, according to the Liebig principle. Thus, validation of the numerous scenarios of abiogenesis and molecular evolution should first of all include a physicochemical analysis [5] using the phase diagram of the corresponding systems.

Transition from abiogenic to prebiological systems is a set of phase transitions [6-13], and the resulting system is a multiphase system that is stable in a biologically relevant range of terrestrial environmental conditions. If the system is not optimal for a given environment, it does not survive due to the physicochemical natural selection [14,15]. One of the main criteria for the successful transition from chemical to biochemical evolution is reproduction or replication of the genetic code carrier [16,17]. Hence, all the scenarios proposed must ensure the safety of the carrier (or carriers [18,19]) at all stages of molecular evolution. If a certain scenario of molecular evolution includes an episode of disruption of carriers of genetic information, further development of life based on this substance is impossible. Hence, it is necessary to analyze thermal stability of nucleic acids under all temperature conditions in which DNA / RNA could appear during molecular evolution [20,21].

To date, there are several hypotheses linking the development of the ability to replicate, as well as the accumulation of mutations in DNA / RNA, with the cyclic temperature changes in the medium, which could lead to thermal cycling of the code carriers by analogy with the known polymerase chain reaction (PCR) protocols [22-24]. Beyond the temperature range suitable for PCR, this principle is not applicable, since thermodynamics of irreversible processes leads to the fact that the system, brought too far from the equilibrium, not only fails to produce its own copies, but also can not more exist in its initial state, going into another phase field [25-27].

Nowadays there are modern techniques combining thermal cycling of nucleic acids in PCR and their thermal analysis [28], but they operate within a narrow temperature range in which the state of DNA and RNA is well established. Meanwhile, in many scenarios of the origin of life temperature significantly exceeds the PCR threshold less than 100ºC. For example, the so-called "Hot Earth" hypotheses consider temperatures much more than 100ºC, in a deep-water hydrothermal

origin of life concept (which also implies high pressure and supercritical fluids [29]) the temperature reaches 150–200ºC, in volcanic systems the temperature is above 400–800ºC [30-32], and finally in panspermia speculations the rock passing through the dense layers of the atmosphere can be exposed to the temperature difference of $n*10^3$ ºC and heating of the meteorite / micrometeorite surface can exceed $n*10^3$ ºC [33-35]. Therefore, an extended thermal analysis of RNA from 25 to 800ºC is required. Since in real conditions the nucleic acid sample heating is always accompanied by the loss of mass, it is possible to apply thermogravimetry. In order to simulate an anoxigenic atmosphere of the early Earth [36], the experiments can be carried out in nitrogen atmosphere.

The vast majority of works on thermal analysis of nucleic acids deals with DNA as the main carrier of genetic information in modern biological systems [37-39] which possesses higher thermal and chemical stability (compared to RNA) as a result of natural selection during chemical evolution. However, the widely recognized concept of the RNA-world postulates the primacy of RNA as the earliest carrier of the genetic code [40,41]. In this regard, in order to verify the concepts of abiogenesis suggesting the leading role of RNA at the prebiotic stage of chemical evolution, it is necessary to address physical and chemical properties of RNA in extreme conditions, in particular, at dehydrated state, upon storage and under high temperatures abundant for the geochemical conditions of the emergence and development of life on early Earth.

Experimental

Crystalline powders of yeast ribonucleic acid (RNA, sample **9**) and its sodium salt (Na-RNA, samples **1-8**) were purchased from Reachem (Moscow, Russia) and used as received. Both reagents were of chemical grade and contained low-molecular fractions of the yeast RNA extracts. Solid state RNA and Na-RNA samples have been studied by TGA and DTA using a standard test method ASTME 1131-08 for compositional analysis by thermogravimetry. Characteristics of thermal destruction of materials (10–20 mg) were studied using an automated modular thermal analysis system "Du Pont 9900". Software allowed to control the operation of thermoanalytical modules, to monitor the measurement process in real time, to register the sample mass change (TGA) in the form of data files and to process the results obtained. Processing and analysis of the thermoanalytical curves obtained was carried out using «File Modification V 1.0» and Universal Analysis 2000 software. Thermal balances TGA-951 were calibrated using reference substances recommended by the International Committee for Thermal Analysis (ICTA) and a gold reference (50 ± 0.005 mg). Thermoanalytical measurements were carried out in an inert (nitrogen) atmosphere with the heating rate of 20°C / min. The following characteristics were calculated from the thermoanalytical curves (TG and DTG): temperature ranges of destruction, mass loss within the temperature ranges (%), DTG maximum temperature (ºC), mass loss rate (% / min), chark oxidation rate (% / min).

Molecular dynamics of the most relevant samples **3**, **5**, **8** and **9** was studied by proton magnetic relaxation using a Minispec PC-120 NMR relaxometer (Bruker, Germany) with an operating frequency of 20 MHz. RNA and Na-RNA powders were placed into a specific NMR tube with a diameter of 5 mm and hermetically sealed. All the measurements were carried out at 25 ± 0.5ºC. To estimate the spin-spin relaxation time T_2 and the fraction of protons with different mobility we used the techniques for studying the free induction decay (FID) of the signal after a 90°-pulse and the pulse sequence CPMG [42,43]. Typically, FID is used to evaluate proton mobility in condensed systems, including solid-state polymers, with the relaxation times up to microseconds and the decay time of the free induction signal about 100 μs. When the signal decay is longer than 100 μs, distortions are introduced into the measurements due to the inhomogeneity of the magnetic field. CPMG method removes the influence of magnetic field inhomogeneity, but the measurements can be started only from the time of about 70 μs, i.e. the characteristics of the mobile proton fraction are measured, while the study of the rapidly decaying part of the curve is difficult. In this work, the FID signal has been studied from 10 μs up to 100 μs with the sampling frequency of 1 MHz (90°-pulse duration was 2.7 μs, dead time - 7 μs, the number of repetitions - 100 and the delay between the subsequent pulses was 2 s). T_1 time for all the samples was less than 0.3 s. The time range of the measured curve in CPMG method was 1.5–2 ms. The time between 90°- and 180°-pulses τ = 20 μs, the number of repetitions was also 100 and the time between two pulse sequences was 2 s. Magnetization decay curves were calculated using Origin 6.1 software.

Particle size distribution in RNA (sample **9**) and Na-RNA (sample **3**) aqueous solutions (C = 10 mg/ml) was estimated using dynamic light scattering method on Zetasizer ZS (Malvern Instruments, USA) instrument. Excitation and emission spectra of the above solutions were recorded using luminescence spectrometer Perkin Elmer LS-50 (Perkin Elmer, USA) at room temperature in 10 mm quartz cells within the wavelength range of 250–800 nm with a resolution of 0.5 nm. Data processing was performed using Origin 6.1 software.

Results

The data obtained reveals a similar thermal decomposition pathway for all the Na-RNA samples (**1-8**) and a significantly different behavior for pure RNA sample (**9**) (Fig. 1*a*). Below 200ºC the yield of volatiles is 15–20 mass % with the mass loss rate of 3.6–4.6 % per minute. Between 200 and 500ºC thermal destruction of the samples occurs with the mass loss of 40–50% and the mass loss rate of 6.5-9% per minute with the temperature maximum of 295-300ºC. Above 500ºC a very low rate of pyrolysis (1.2–0.2% / min) is observed. For pure RNA sample (**9**) in the temperature range below 500ºC there are three distinct stages of thermal destruction with the maxima at 90ºC, 225ºC and 300ºC, and the corresponding thermal decomposition rates of 1.2, 2.8 and 4.5% per minute. Above 500ºC there is also a very low pyrolysis rate of 1.2–0.3% per minute. After changing the inert N_2 atmosphere to air, the carbonized fraction which differs in different samples undergoes oxidation with different rates (Fig. 1*b*). According to DTG curves, a different nature of the carbonized sample oxidation is observed at 800ºC.

The difference in thermal decomposition observed between RNA and Na-RNA samples in a solid state has been proved by NMR relaxation data. The measured FID signal can be described by three components: the first fast-decaying signal from the protons in the "solid" dry phase (CH, CH_2, NH and several OH groups of the crystal and amorphous parts of the sample) with the corresponding spin-spin relaxation time T_{21}, and two slower decaying components (they can include water protons and protons of the RNA structure in OH, NH groups exchanging with it) with the corresponding spin-spin relaxation times T_{22}, T_{23}. The results obtained using FID methods are summarized in table 1.

From the data obtained it can be concluded that sample **9** demonstrates relaxation close to a solid state with the significantly limited proton mobility, while the other samples **3, 5** and **8** contain two types of protons, including low-mobile ones (predominant in sample **3**) and more mobile ones, predominant in samples **5** and **8**, which can be attributed to the higher water content in the latter samples, resulting in plasticizing effect of water on biopolymers (Fig. 2), which is consistent with the above discussed TGA data.

Additional experiments with the aqueous solutions of the stored RNA and Na-RNA samples revealed a significant difference in their solubility with the Na-RNA samples **1-8** readily dissolving into transparent molecular solutions with pH 6.5 and RNA sample **9** forming a turbid colloidal suspension with pH

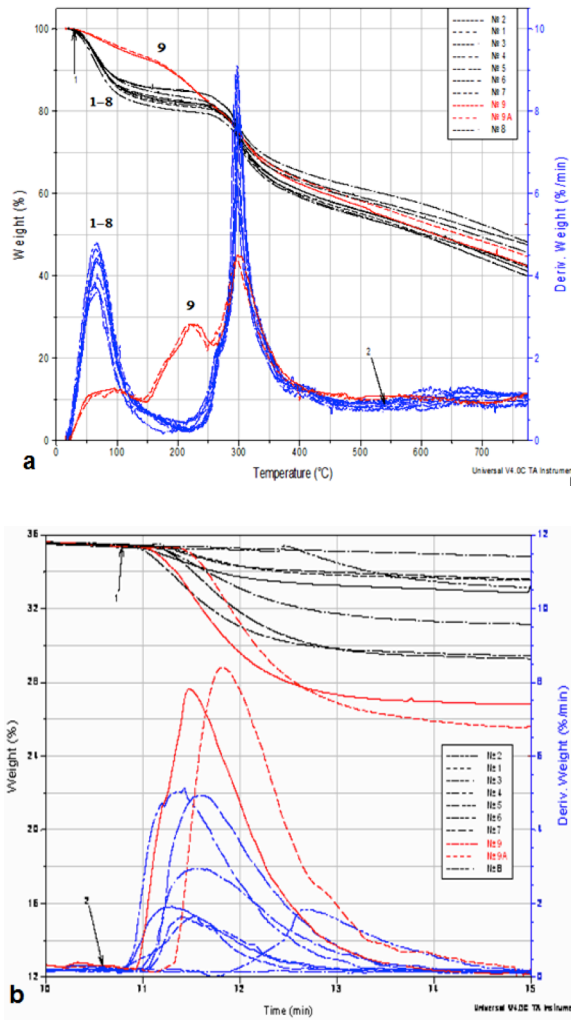

Figure 1. ***a*** - TG (1) and DTG (2) curves of the initial RNA and Na-RNA samples; ***b*** - TG (1) and DTG (2) curves of carbonized sample oxidation at 800°C.

Table 1. Fast and slow FID components and their percentage ratio in RNA samples.

Sample number	T_{21}, μs	%	T_{22}, μs	%	T_{23}, μs	%
3	10	90	22	6	350	4
5	10.7	75	60.2	8	430	17
8	10.2	74	23	14	350	12
9	11	100				

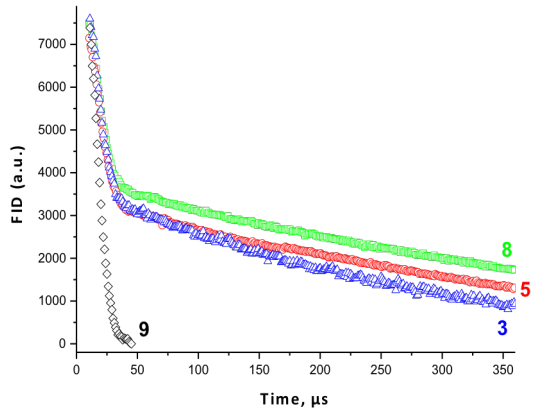

Figure 2. Free induction decay signal, sampled with an acquisition time 1 μs.

3.5 at the same concentration of 10 mg/ml. According to DLS measurements, this suspension demonstrates a wide particle size distribution with the average hydrodynamic diameter of the molecular aggregates being about 200 nm, while the mean particle size in a less polydisperse molecular solution of sample **3** is about 2 nm (Fig. 3). This difference can be attributed to the partial hydrolysis of RNA with the subsequent acidification of the solution preventing the polymer from dissociation. Upon addition of NaOH, neutralization results in ionization of the phosphate groups leading to the increased solubility of the polyanion and the transition from a globular to a linear macromolecule conformation.

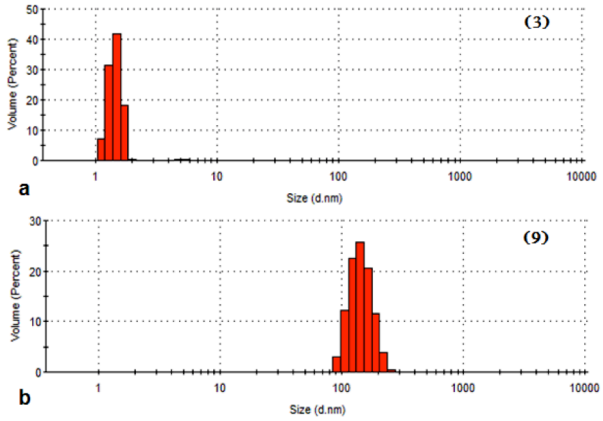

Figure 3. Particle size distribution for Na-RNA (a) and RNA (b) solutions.

For all the samples studied regardless of the chemical form of RNA pronounced spectral changes were also observed upon storage. In addition to the characteristic UV absorption maxima at 230, 260 and 280 nm typical for RNA molecules, a clear bathochromically shifted absorption band was observed at 335 nm with the corresponding fluorescence emission band at 415 nm. Such new bands can be attributed to the oxidation products of the nitrogenous bases, produced upon the RNA sample storage at ambient conditions in oxygen-containing atmosphere without any specific protection. This process can be facilitated by the sample exposure to the UV light, high temperature, or treatment by the reactive oxygen species [44,45]. The primary product of RNA oxidation, according to the literature available, is 8-hydroxyguanosine [46,47]. Further chemical transformations can lead to the formation of a complex mixture of purine derivatives [48]. It is also noteworthy that single-stranded RNA without hydrogen bonds protecting its nitrogenous bases is known to be more susceptible to oxidative damage compared to the double-stranded DNA [49,50]. Oxidative damage of the RNA structure results in the alteration of the normal base pairing, and hence, prevents RNA molecules from correct operation during transcription and translation processes [51].

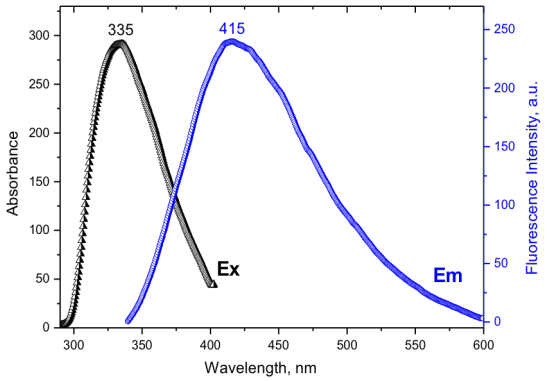

Figure 4. Excitation and emission spectra of Na-RNA solution (sample **3**).

Discussion

Thus, there are at least three types of abiogenetic conditions that impose temperature restrictions on the possibility of RNA participation in prebiological processes. Firstly, it is not possible to consider the “RNA world” operating under hydrothermal conditions at temperatures of about 400°C (within the framework of the so-called “hot scenario”). As it is correctly noted in [52], "one may deny the possibility of the existence of the RNA world under hydrothermal environments on the basis of the qualitative understanding that RNA should normally be weak at high temperatures". The above cited paper shows that approaching a temperature of 200–300ºC leads to the RNA degradation, since the maximum temperature for maintaining its functionality is close to the survival temperatures of hyperthermophilic organisms (100–120ºC).

Secondly, despite the well-known concepts on the possible presence of RNA in meteorites and cosmic dust involved in the panspermia hypothesis (pre-RNA

world [53,54]), it is difficult to believe in the safety of RNA in such objects and the possibility of its safe delivery to the Earth in this way. The inevitable RNA decomposition at temperatures above 300ºC will not allow it to reach the planet surface even in the absence of modern dense layers of the Earth atmosphere. Even less plausible is the hypothesis of the presence of RNA on the surfaces of other astrobiological objects, for example, on Venus, where the surface temperature reaches 280°C.

Variations in the concept of the RNA world localized within the aquatic microreservoirs in volcanic aquifers [55], as well as hypotheses appealing to the effect of lightning in volcanic conditions, also contradict the known data on the temperature in such systems, at which the lifetime of such aquatic microreservoirs could not be sufficient enough for proceeding of molecular / chemical evolution or selection of molecular quasispecies in the RNA world [56-58]. The only exceptions to this rule are marine underwater volcanoes: the extremophile hyperthermophiles found in them can live at temperatures of the order of 100°C [59], which, however, is many times lower than standard volcanic temperatures. It is known that a hot core of the volcanic plume has temperature above 600°C, while magmatic gas - up to 1000°C [60]. Thus, in the "volcanic" versions of the RNA world, thermobarogeochemistry of these systems contradicts the structural chemistry of nucleic acids, which makes impossible molecular / chemical evolution in populations of RNA molecules in volcanic communities.

Conclusions

The performed experiments revealed a significant difference in the thermal decomposition behavior and hydration degree of the solid state samples of ribonucleic acid and its sodium salt, as well as their solubility difference upon storage due to the chemical transformation of the samples. According to the TGA data, the first structural transformation for the RNA samples occurs at 70°C and a complete decomposition is observed at 300°C, which prevents from speculations on the possibility of RNA participation in the prebiotic chemical reactions at higher temperatures.

Acknowledgments

The authors gratefully acknowledge Dr. Bychkova A.V. (N.M. Emanuel Institute of Biochemical Physics RAS) for performance of the DLS measurements and Alexandrov P.L. (Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, RAS) for the technical assistance. The work was carried out in the framework of the State Assignment of Ministry of Science and Higher Education of the Russian Federation (project 0082–2018–0006, registration code AAAA-A18-118020890097-1).

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