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Non-enzymatic oligomerization of 3', 5' cyclic ribonucleotides

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Abstract. The very first origin of RNA is fraught with uncertainty. RNA world might have been preceded by an "unknown-polymer" world, or it might have started its polymerization destiny from highly activated precursors as phosphoramidated nucleotides. It might even have taken origin outside this planet. Each one of these scenarios has its appeals and drawbacks. Alternatively, RNA might have originated by autopolymerization of simple prebiotically plausible compounds, i.e., 3',5' cyclic nucleotides. In this work, we review our observations about spontaneous polymerization of 3', 5' cyclic ribonucleotides and in particular of 3',5' cyclic cGMP. The reaction requires neither template, nor enzymatic activities, is thermodynamically favored, and selectively yields 3',5'-bonded oligoribonucleotides containing as many as 25 nucleotides.

Key words. RNA world – RNA – prebiotic chemistry–origin of life

1. Introduction

To fully understand the processes occurring in present-day living cells, we need to consider how they arose in evolution. The origin-of-life quest has long been split into several attitudes exemplified by the aphorisms "genetics-first" or "metabolism-first". Opposition between these two approaches seems be solved by a unitary and simple chemical frame involving the one-carbon molecule formamide (HCONH2) (Saladino et al. 2012). The steps leading from any putative simple-molecule precursor to an extant-type nucleic informational polymer are: 1) condensation into nucleic bases; 2) formation of nucleosides thereof; 3) phosphorylation of nucleosides; 4) chain-wise linear polymerization; and 5) survival of the formed polymer for a period long enough to allow replication. Formamide plays a positive role in most of these steps, both as a building block and, in defined instances, as a catalytic cofactor. The step 4) entails non-enzymatic autocatalytic self-replication systems based on template-directed synthesis of oligonucleotides as pioneered by Weimann and coauthors (Weimann et al. 1968). These studies established the principle. However, since the formation of a phosphodiester bond is thermodynamically uphill, protein-free template-directed syntheses of phosphodiester- linked oligonucleotides required the use of chemically activated nucleotides. Having observed the abiotic simple formation of cyclic nucleotides from nucleosides (Costanzo et al. 2011), we reported non-enzymatic polymerization from $3',5'$ cyclic cGMP (hereafter abbreviated as cGMP) and $3',5'$ cAMP. In the case of cGMP, polymers rapidly formed at moderate

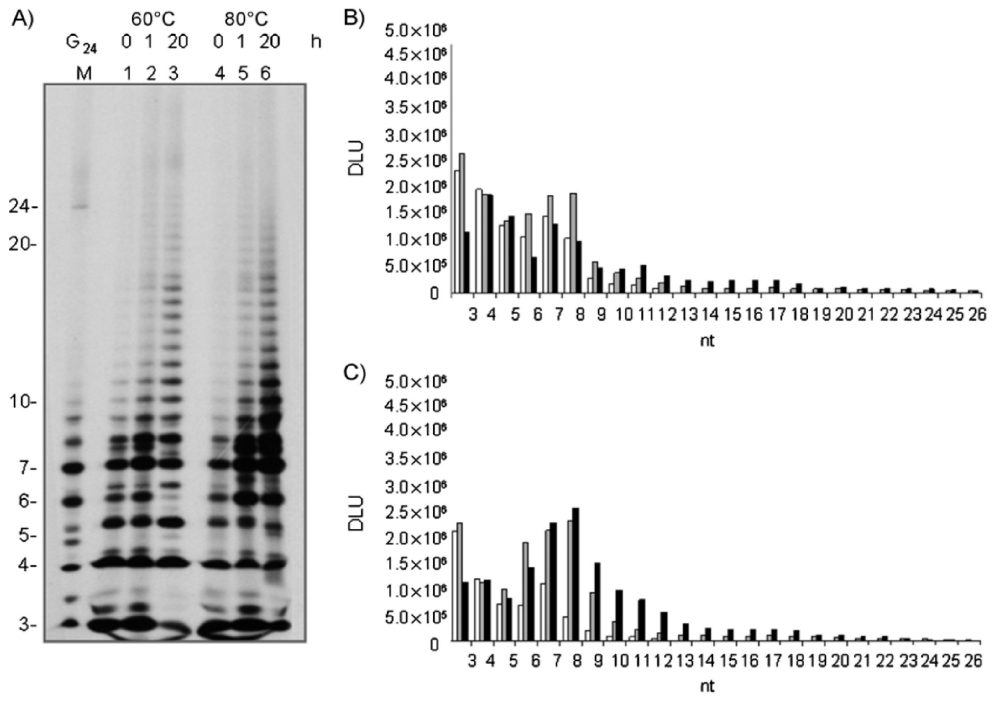

Fig. 1. Polymerization of cGMP in water as a function of temperature and time. Numbering refers to the length in nucleotides (Reproduced from: Costanzo et al. 2012).

temperature up to a length of 25 units, while for 3′, 5′ cAMP only oligomers up to 6 units were detected which polimerize with a slower kinetics. Non-enzymatic oligomerization of ribonucleotides fit well into the RNA world hyphotesis (Gilbert 1986), based on the general idea that, in the development of life on the Earth, evolution based on RNA replication preceded the appearance of DNA and protein syntheses since RNA can both store genetic information and catalyze chemical reactions.

2. Sustainability and chaos in the abiotic polymerization of 3',5' cyclic guanosine monophosphate

In the last years, we reported that 3',5' cyclic nucleotides oligomerize and that especially the free acid form of cGMP, due to its unique stacking properties, could play a dominant role in the abiotic generation of the first oligonucleotide sequences (Costanzo et al. 2009). We have described the cGMP oligomerization in water, in formamide, in dimethylformamide and in the dry state (the more efficient in yields). The reaction requires neither template, nor enzymatic activities, is thermodynamically favored, and selectively yields 3',5'-bonded oligoribonucleotides containing as many as 25 nucleotides. The reaction products were analyzed by denaturing PAGE, MALDI ToF MS, ^{31}P NMR analysis and specific RNases assays of the polymerized materials. Polymerization in water was performed by incubating the cGMP solution at the concentration (tipically 3mM), temperature (60-80 °C) and time span (1-20 hrs) appropriate. Fig. 1A shows the polymerization products of 3′, 5′-cGMP dissolved in Tris·HCl (pH 8.4) for 0 (handling time < 5 s), 1 and 20 h at 60 °C (lanes 1–3) and for the

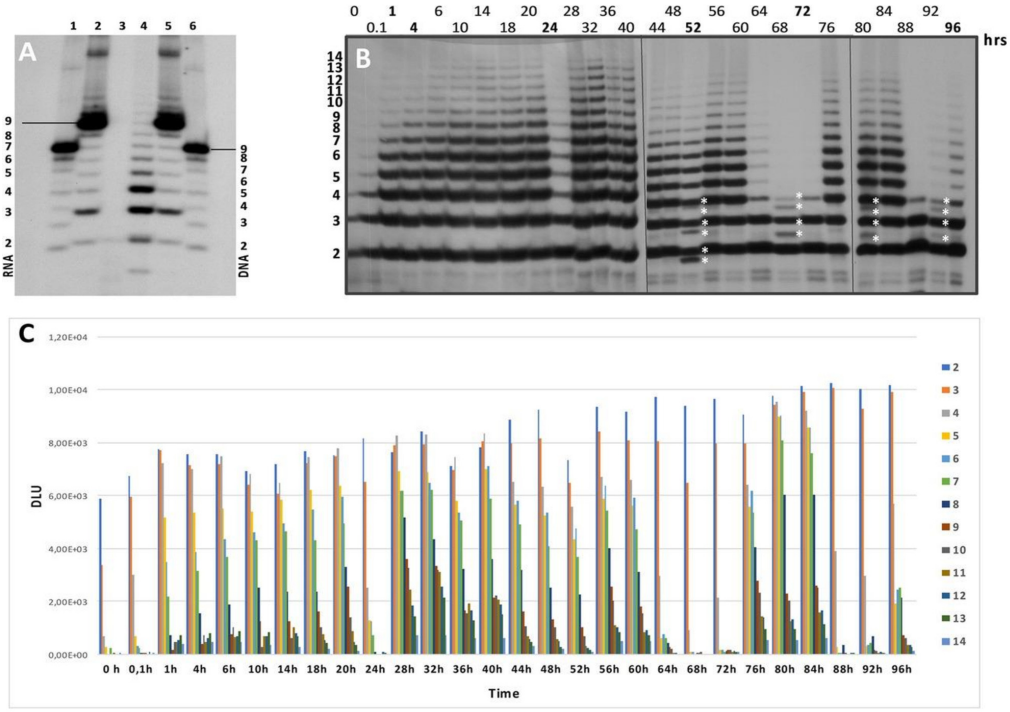

Fig. 2. Polymerization of cGMP in dry state (Reproduced from: Costanzo et al. 2020).

same times at 80 °C (lanes 4–6). The quantitative evaluation of each oligomer is shown in panels B and C (Costanzo et al. 2012).

More recently, cGMP polymerization was revisited in dry form as well as in aqueous solution. In addition, as a novelty, we have followed the reaction on samples which were prepared by subsequent wetting of the dry material. Fig. 2 shows the PAGE analysis of the oligomers formed as a function of time. In this set of experiments the reaction was followed for up to 96 hours.

Fig. 1, Panel A shows the set of markers used to define the length of the oligomerized material. Panel B shows the time dependence of the oligomerization reaction. The analysis was routinely performed by gel electrophoresis and autoradiography, showing that cGMP oligomerizes to a length of at least 14 monomer units. Panels C and D show the length distribution of the oligomers produced as a function of time, and their average length, respectively.The sequential ensemble of the reactions produces oligomers of varying length and quantity. When repeating the whole series of experiments, these alternations persist; nevertheless variations are observed in the onset and in the duration of the oligomerization periods. This trend was constantly observed in at least 10 separate series of experiments. Noteworthy, the concentration of dimers and trimers remains quantitatively constant throughout. The *bona fide* conclusion is that for each experiment the profiles are not exactly repeatable as a precise function of time and as quantity of polymer obtained, and for the time of onset of the degradation processes. However, the alternating character is constantly observed. This hints to the fact that the system is intrinsically complex and that one or more of its parameters are close to being stochastic.

3. Conclusions

From the analysis of the conditions leading to the oligomerization of cGMP on longer time scales we have learnt that the reaction exhibits a qualitatively constant overall behavior leading to oligonucleotide sequences at maximum of about 25 monomer units. Nevertheless, it has disclosed a new feature of the reaction that might be strongly associated with its erratic repeatability. We have found that in terms of the amount and distribution of the oligomers formed on time scales of several days the reaction exhibits a wave-like behavior, which cannot be explained just by considering a kinetics based on the counteracting effects of oligomerization and degradation reactions. In addition, we observe a variation pertaining to the time of the onset of the oligomerization and degradation cycles. Further, we have found that at least one drying step is needed for the sustainable production of oligomers, indicating that phase-separation processes (most likely aggregation) might fundamentally affect the outcome of the reactions.

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