BioSystems 162 (2017) 53–58

Contents lists available at ScienceDirect

BioSystems

journal homepage: www.elsevier.com/locate/biosystems

A step into the RNA world: Conditional analysis of hydrogel formation of adenosine 5′-monophosphate induced by cyanuric acid

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ARTICLE INFO

Article history:
Received 9 August 2017
Received in revised form 1 September 2017
Accepted 2 September 2017
Available online 5 September 2017

Keywords:
Hydrogel formation
Adenine
Cyanuric acid
π-Stacking
Hydrogen bond
RNA world

ABSTRACT

Nucleotide polymerization occurs by the nucleophilic attack of 3′-oxygen of the 3′-terminal nucleotide on the α-phosphorus of the incoming nucleotide 5′-triphosphate. The π-stacking of mononucleotides is an important factor for prebiotic RNA polymerization in terms of attaining the proximity of two reacting moieties. Adenosine and adenosine 5′-monophosphate (AMP) are known to form hydrogel in the presence of cyanuric acid at neutral pH. However, we observed that other canonical ribonucleotides did not gel under the same condition. The π-stacking-induced hydrogel formation of AMP was destroyed at pH 2.0, suggesting that the protonation of N at position 1 of adenine abolished hydrogen bonding with the NH of cyanuric acid and resulted in the deformation of the hexad of adenine and cyanuric acid. A liquid-like gel was formed in the case of adenosine with cyanuric acid and boric acid, whereas AMP caused the formation of a solid gel, implying that the negative charge inherent to AMP prevented the formation of esters of boric acid with the cis-diols of ribose. Cyanuric acid-driven oligomerizations of AMP might have been the first crucial event in the foundation of the RNA world.

1. Introduction

The central dogma of biology has indicated that the genetic information transmits in the order of DNA → RNA → protein (Crick, 1958). The so-called “chicken or the egg” problem in the evolution of molecular biology appears solvable by the “RNA world” hypothesis (Gilbert, 1986), wherein the roles of information infrastructure and catalysis are postulated to have been performed by RNA alone. The discovery of ribozymes (Kruger et al., 1982; Guerrier-Takada et al., 1983) spurred the foundation of the concept and suggested that the homochiral RNA world could have evolved into the homochiral ribonucleoprotein world (Tamura and Schimmel, 2004; Tamura, 2008).

RNA is composed of nucleotide units. Sutherland and his coworkers showed that activated pyrimidine ribonucleotides can be formed from prebiotic molecules–cyanamide, cyanoacetylene, glycolaldehyde, glyceraldehyde, and inorganic phosphate (Powner et al., 2009). Although the mechanism of synthesis of purine ribonucleotides still needs to be elucidated, the fact that adenine is synthesized from hydrogen cyanide under possible primitive conditions on Earth (Oró, 1961) suggests the plausibility of a prebiotic method of mononucleotide formation (Sutherland, 2016). Given such a possibility, the main problem underlying the foundation of the RNA world would be the polymerization of mononucleotides. Non-enzymatic template-directed oligonucleotide ligation between 3′-OH and 5′-triphosphates has been reported, but the yield is very low (Rohatgi et al., 1996). Ribozymes catalyzing RNA ligation (Ekland et al., 1995; Robertson and Ellington, 1999; Rogers and Joyce, 2001; Kurihara et al., 2014) or polymerization (Johnston et al., 2001; Zaher and Unrau, 2007; Wochner et al., 2011; Attwater et al., 2013) have also been reported, but all of them comprise more than 50 nucleotides.

Oligomerization of mononucleotides under various conditions (with/without template, clay, and metal ions) has been studied (Orgel, 2004). In this nucleophilic reaction, the π-stacking of nucleobases is an important factor in terms of attaining close proximity of the two reacting moieties, 3′-oxygen of the 3′-terminal nucleotide (nucleophile) and α-phosphorus of the incoming nucleoside 5′-triphosphate (Fig. 1). Hud and coworkers reported that the mixing of an artificial nucleoside, 5-β-ribofuranosyl-2,4,6-triaminopyrimidine (TARC), with cyanuric acid in a sodium phosphate and boric acid buffer resulted in the formation of a

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http://dx.doi.org/10.1016/j.biosystems.2017.09.004
0303-2647/

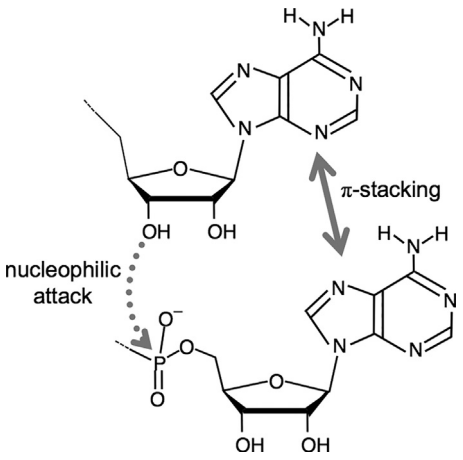

Fig. 1. Schematic presentation of the polymerization of nucleotides. The π-stacking of nucleobases is an important factor in terms of attaining close proximity of two reacting moieties, 3′-oxygen of the 3′-terminal nucleotide (nucleophile) and α-phosphorus of the incoming nucleoside 5′-triphosphate.

hydrogel due to π-stacking interactions (Chen et al., 2014). Moreover, they observed the hydrogel formation of diaminopurine, adenosine and AMP in the presence of cyanuric acid (Li et al., 2016). Cyanuric acid is a plausible ancestral nucleobase known to be prebiotically synthesized by a chemical reaction between ammonia or ammonium ions and carbon dioxide, and it is also found in meteoritic samples (Jeilani et al., 2014).

Here, we not only further studied the formation of hydrogel of adenosine or AMP in the presence of cyanuric acid, but also the hydrogel formation of other canonical ribonucleotides. We discuss the results in terms of prebiotic RNA formation.

2. Methods

2.1. Hydrogel preparation

Sodium phosphate buffer 1 (PB1) (pH 7.0) was prepared by titrating 500 mM disodium phosphate to 500 mM monosodium phosphate until it reached pH 7.0. Sodium phosphate/boric acid solution (PBA) (pH 7.0) was prepared by titrating 500 mM disodium phosphate to 500 mM boric acid until it reached pH 7.0. Sodium phosphate buffer 2 (PB2) (for each pH value) was prepared by titrating 200 mM disodium phosphate to 200 mM monosodium phosphate until it reached each pH value shown in Fig. 4. Sodium phosphate solution (PS) (pH 2.0) was prepared by titrating 200 mM phosphate to 200 mM monosodium phosphate until it reached pH 2.0. The solutions of the mixture of nucleic acids with/without cyanuric acid were prepared in 0.6-mL tubes by dissolving them in the solvents described in the figure legends. The mixed solutions were heated to 95 °C for 5 min and left at room temperature for 5 min, then cooled to 4 °C for more than 30 min. The gel formed in a tube was taken out by turning over the tube at 4 °C.

2.2. Circular dichroism (CD) spectroscopy

The CD spectra of adenosine with/without cyanuric acid in PBA (pH 7.0) and those of AMP with/without cyanuric acid in 5 M NaCl at 4 °C were recorded on a J-805 CD spectrometer (JASCO Corporation, Tokyo, Japan). The cell path length was 0.2 mm. The scanning speed was 50 nm min^{-1}.

3. Results

3.1. Formation of AMP hydrogel in the presence of cyanuric acid

We first focused on AMP, since adenine is easily synthesized from hydrogen cyanide under possible primitive Earth conditions (Oró, 1961). AMP dissolved in PB1 (pH 7.0) in the presence of cyanuric acid formed a solid hydrogel, and retained its shape even after being taken out of the tube (Fig. 2). The decrease in the concentration of both AMP and cyanuric acid caused deformation of the solid gel, and the concentrations of around 30 mM were inferred to be the threshold for gel formation in the experimental conditions (Fig. 2).

3.2. Hydrogel formation in the cases of other canonical nucleotides (UMP, GMP, CMP)

We next focused on the gel formation of other canonical nucleotides (UMP, GMP, CMP) in the presence of cyanuric acid. In contrast to AMP, none of the other canonical nucleotides (UMP, GMP, CMP) gelled under the same experimental conditions in PB1 (pH 7.0) (Fig. 3).

3.3. Effect of pH on the formation of AMP hydrogel in the presence of cyanuric acid

Since 200 mM sodium phosphate functions as a buffer between pH 5.8 and pH 8.0 (Sørensen, 1909), we surveyed the results of AMP gel formation with cyanuric acid in PB2 at pH intervals of 0.2 within this range. As shown in Fig. 4A, solid gels were similarly formed at each pH, and pH had no effect on the formation of AMP gel between pH 5.8 and pH 8.0. However, gel was not formed in PS (pH 2.0), even in the presence of cyanuric acid (Fig. 4B).

3.4. Effect of solvents on the formation of hydrogel of adenosine or AMP in the presence of cyanuric acid

To evaluate the effects of the solution content on the formation of both adenosine and AMP gel, several conditions were set. In the case of adenosine (with cyanuric acid), H_2O, PB2 (pH 7.0), 1 M HEPES-NaOH (pH 7.0), or 200 mM NaCl caused solid gel formation (Fig. 5A). In the case of 5 M NaCl, white aggregates were observed, possibly because of the decrease in the solubility of adenosine and cyanuric acid due to the excess of NaCl. The solutions containing boric acid (PBA) (pH 7.0), or 400 mM boric acid/500 mM NaCl also caused gel formation, but interestingly, the gels were liquid-like and could not retain their shapes when removed from the tube (Fig. 5A).

In the case of AMP (with cyanuric acid), no hydrogel formation was observed in H_2O or 1 M HEPES-NaOH (pH 7.0) (Fig. 5B). In contrast, PB2 (pH 7.0), 200 mM NaCl, 5 M NaCl, or 400 mM/500 mM NaCl caused solid gel formation (Fig. 5B). In the case of 5 M NaCl, white aggregates were also found. Although solid gel formed in PBA (pH 7.0), the gel was much softer than that formed in PB2 (pH 7.0) (Fig. 5B).

3.5. CD spectra of adenosine and AMP in the presence of cyanuric acid

Because the CD spectra of TARC are known to change depending on the presence of cyanuric acid (Chen et al., 2014), we measured the CD spectra of adenosine and AMP in the presence or absence of cyanuric acid. The concentration of adenosine or AMP was 7 mM.

The adenosine solution showed a negative peak at around 260–270 nm, and cyanuric acid solution alone did not show large peaks. However, adenosine with cyanuric acid exhibited a positive peak at around 290 nm (Fig. 6A). On the other hand, AMP solution