BORATE GUIDED REGIOSELECTIVE PREBIOTIC NUCLEOTIDE SYNTHESIS. Hyo-Joong Kim[1,2] Steven A. Benner[1,2] and Yoshihiro Furukawa[3], [1]Foundation for Applied Molecular Evolution, 13709 Progress Blvd N112, Alachua, FL 32615, USA, [2]Firebird Biomelecular Sciences, 13709 Progress Blvd N134, Alachua, FL 32615, hkim@ffame.org, sbenner@ffame.org [3]Department of Earth Science, Graduate School of Science, Tohoku University, 6-3, Aza-aoba, Aramaki, Aoba-ku, Sendai 980-8578, Japan. furukawa@m.tohoku.ac.jp

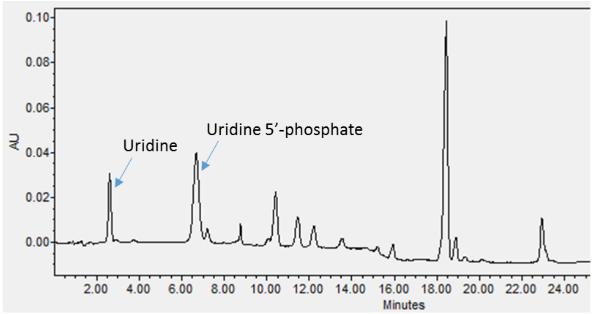
Introduction: RNA world hypothesis offers a coherent model for the origin of life, but requires that RNA play roles in both genetics and catalysis in the first Darwinian systems [1][2]. Further, it requires a mechanism to generate RNA from prebiotic chemical mixtures [3]. Activated nucleoside 5'-phosphates are known to yield oligomeric RNA by non-enzymatic polymerization [4]. However the prebiotic availability of 5'-activated nucleotides is not known. To have streamlined synthesis of RNA from nucleosides, it is important to show that nucleosides can be selectively phosphorylated at 5' position.

About 40 years ago, Oegel and colleagues proposed that urea-inorganic phosphate mixtures might be prebiotic phosphorylating agents [5]. However, this phosphorylation reagent was observed to give many different phosphates from nucleosides, yielding complex mixtures of 5'-, 2'-, and 3'- mono, di, and tri nucleoside phosphates as products. They could improve selectivity for 5'-phosphation, but only at lower reaction temperatures and lower yields [6].

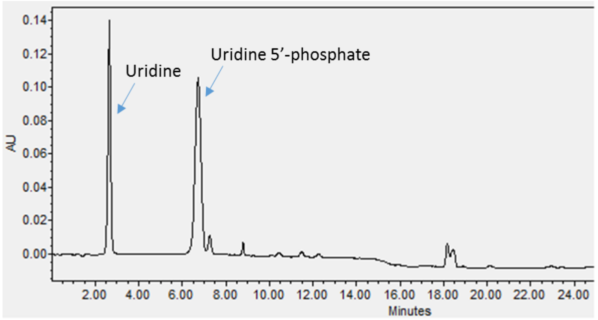
Borate is well known make complexes with 1,2-cis diol [7][8], and this complex was shown to preferentially stabilize ribose [9][10] and guide regioselective phosphorylation of adenosine in formamide [11]. In this presentation, the combination of phosphorylation in urea-phosphate agent and regioselectivity guided by borate ion will be reported.

Results: A mixture (50 μL) of a nucleoside (20 mM), NaH_2PO_4 (20 mM), and urea (200~800 mM) was placed in Eppendorf tube with open lid with or without sodium borate (20 mM). The mixture was heated at 90 °C for 12 hours, resuspended in water and analyzed by rpHPLC. All four ribonucleosides (A, G, C, U) were treated under the same conditions (guanosine was 10 fold diluted because of its low solubility in water). All nucleosides gave similar results. Absent borate, nucleoside 5'-monophosphates were produced together with many other phosphorylation products. However, with 20 mM borate, only two major products were observed: unchanged nucleoside and nucleoside 5'-monophosphate. Moreover, the borate-urea phosphorylation mixtures gave ~50% yield of nucleoside 5'-monophosphate, much higher than selective phosphorylation when urea was replaced by formamide.

This discovery of a high-yield selective phosphorylation of nucleosides with borate and phosphate minerals illustrates how prebiotic chemistry and mineral environments help solve key problems in the RNA first model for life's origins. This requires, of course, semi-arid environments. If not present on early Earth, such environments might have been found on early Mars.



Phosphorylation of uridine without borate



Phosphorylation of uridine with borate

References:

[1] Woese C. (1967) *The evolution of the genetic code. In The Genetic Code, Harper & Row, NY*, 179-195. [2] Crick F. H. C. (1968) *J. Mol. Biol. 38*, 367-379. [3] Orgel L. E. (2004) *Crit. Rev. Biochem. Mol. Biol. 39*, 99-123. [4] Adamala K. and Szostak J. W. (2013) *Science 342*, 1098-1100. [5] Lohrmann R. and Orgel L. E. (1971) *Science 171*, 490-494. [6] Reimann R. and Zubay G. (1999) *Origins Life Evol. Biosphere 29*, 229-247. [7] Scorei R. (2012) *Origins Life Evol Biosphere 42*, 3-17. [8] Prieur B.E. (2001) *First Steps in the Origin of Life in the Universe, edited by J. Chela-Flores, T. Owen and F. Raulins, Kluwere, Netherlands*, 03-106. [9] Ricardo A. et al. (2004) *Science 303*, 196-196. [10] Kim H. et al. (2011) *J. Am. Chem. Soc. 133*, 9457-9468. [11] Furukawa Y. et al. (2015) *Astrobiology 15*, In press.

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