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Communication

A Prebiotic Ribosylation of Pyrimidine Nucleobases Promoted by Metal Cations and Clay Minerals

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Abstract: We report a prebiotically relevant solution to the *N*1-ribosylation of pyrimidine nucleobases, a well-known challenge in the RNA World hypothesis. It is found that the presence of metal cations and clay mineral enables the previously unachievable direct ribosylation of uracil, providing by far the highest yield. Spectroscopy and chromatography analyses confirmed the formation of ribosylated uracil. The method can also be extended to the ribosylation of 2-pyrimidinone. These findings are also compatible with the metal-doped-clay model developed by our lab for the unified route of the selection of ribose and subsequent syntheses of nucleotide and RNA.

Keywords: Origins of Life; RNA World; Uracil; Ribosylation; Metal Cation; Clay Mineral

1. Introduction

The RNA World is one of the leading hypotheses for the origins of life.[1] Among all hypothetical synthetic steps, the formation of glycosidic bond between ribose and nucleobase is considered the most problematic link, known as the "nucleosidation problem" (Figure 1).[2,3,4] The weak nucleophilicity on *N*9 of purine or *N*1 of pyrimidine is the major obstacle for the direct ribosylation of nucleobase. Various conditions have been attempted since the pioneering work by Orgel in the 1970s.[5,6] Compared with *N*9 of purine, the nucleophilicity of *N*1 of pyrimidine is even weaker. The direct ribosylation of uracil has been a key stumbling block for decades.[7] There have been very few precedents on the formation of uridine or its isomers via direct ribosylation of uracil. Among the few published examples, Hud was able to theoretically demonstrate that divalent metal ions such as Mg^{2+} might be promotive to the ribosylation of uracil. Cations are believed to lower the activation energy by holding the reactants in close proximity.[8] However, no detectable uridine or its isomers has been reported experimentally. More recently, Zare reported the ribosylation of uracil facilitated by aqueous microdroplets.[9,10] Although no further evidence was given to support the formation of uracil-1-*β*-D-ribofuranoside, the reported high-resolution mass spectroscopy (HRMS) signal indicated that the ribosylation of uracil had taken place, no matter which isomer(s) were formed. The generality of the corresponding geochemical conditions of this model on the primordial Earth remains debatable. To circumvent the low reactivity of pyrimidine, a stepwise strategy was developed by Orgel.[11] Sutherland developed a stepwise route to access pyrimidine nucleotides.[12] Carell reported a multistep synthesis based on the condensation between ribose and *N*-isoxazolyl-urea to form pyrimidine nucleotides.[13] Saladino and Di Mauro reported the detection of the ribosylated uracil catalyzed by meteorite, but without indicating the yield.[14] Moreover, the prebiotic availability of the required proton irradiation is questionable. Nevertheless, the direct nucleosidation between ribose and nucleobase is still attractive due to its simplicity, and a prebiotically general condition is yet to be developed.

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Recently, our lab has demonstrated that metal-doped-clay (MDC) is able to highly selectively adsorb and stabilize ribose from the complex Formose mixture, as well as to promote downstream syntheses towards RNA such as the nucleosidation and phosphorylation.[15] We have attempted the ribosylation of formamidopyrimidine (FaPy) and melamine in the presence of MDC, with both of the nucleobases being non-canonical. It was shown that MDC-retained ribose is highly reactive for these reactions. These results encouraged us to explore if MDC can catalyze or facilitate transformations that are previously unachievable. The ribosylation of canonical nucleobase under similar conditions have not been explored. In particular, it would be interested to see whether MDC has a facilitating effect on the nucleosidation of previously challenging substrates. Herein we present the direct ribosylation of pyrimidine nucleobases facilitated by the use of metal salts and clay minerals.

3. Results

Clay minerals are important catalyst and platform for synthesizing RNA oligomers.[16] Cation-exchanged clays have been reported to catalyse organic transformations such as the formation of acetal.[17] The acetal formation is a condensation between aldehyde and alcohol. Therefore, it is reasonable to assume that MDC is able to catalyze the nucleosidation which is also a condensation between an aldehyde (sugar) and a nucleophile (pyrimidine). Here, we discovered that uracil and ribose could couple to form a set of desirable adducts in the presence of metal salts and kaolinite (Figure 1).

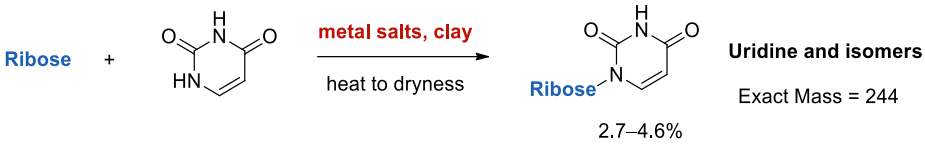

Figure 1. Direct ribosylation of uracil enabled by metal salts and kaolinite

Due to the low reactivity of uracil, stoichiometric amount of ribose was used instead of the one adsorbed on the metal-doped-clay. Muiltiple metal chlorides ($CuCl_2$, $FeCl_2{\cdot}4H_2O$, $MgCl_2$ and $CaCl_2{\cdot}2H_2O$) and clay mineral (kaolinite) were added together with the reactants (D-ribose and uracil). The pH of the mixture was adjusted to 5, and the mixture was heated to evaporate water on a shaking drybath to simulate the hot-dry conditions on the primordial Earth.[18] The dried sample was added water and analyzed by LCMS equiped with a reverse phase HPLC column. It was observed that a set of early eluted peaks (1.7, 2.0 and 2.3 min) were strongly associated with the expected masses of 245 ($[M+H]^+$), 267 ($[M+Na]^+$) and 243 ($[M–H]^-$). To confirm the identity of these signals, a co-injection with authentic β-uridine sample was carried out. There were a group of neighboring peaks between 12 min to 16 min, which should be non-product-related impurities since they did not have all three characteristic masses (245(+), 267(+), 243(–)). The intensity of the impurity peaks remained unchanged; thus, they can be used as the internal standards. It was found that in the LCMS analysis of co-injected sample, besides the signal of β-uridine itself, the TIC signal strengths (scanned for 245.1(+)) at 1.7, 2.0 and 2.3 min also increased sharply (Figure 2, red line in the upper right graph). These observations indicated that the peaks at 1.7, 2.0 and 2.3 min were altered forms of β-uridine (or its isomers). The most probable case was that the adducts tend to coordinate to metal cations, which caused a polarity increase and thus an early elution on the reversed phase HPLC. To further elucidate the effectiveness of the use of metal ion and clay, a control experiment in the absence of metal salts and kaolinite was performed, in which the characteristic MS signals and the corresponding LC peaks were not detected (Figure 3).

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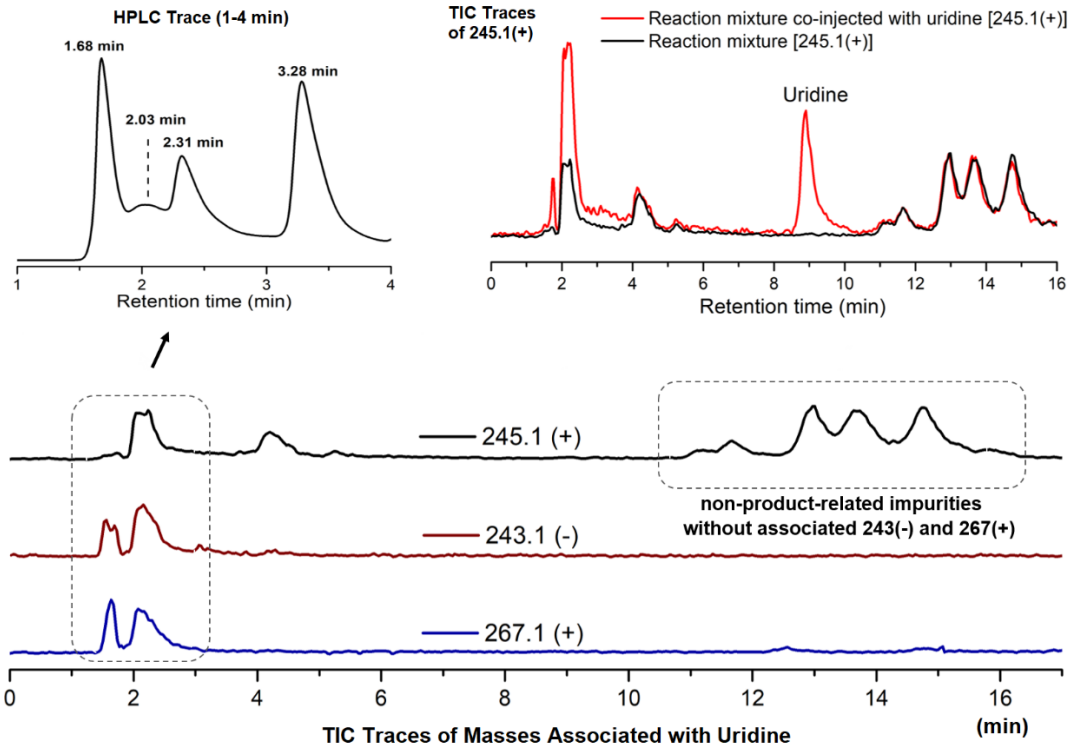

Figure 2. HPLC trace and TIC analysis of the characteristic MS of the ribosylation products

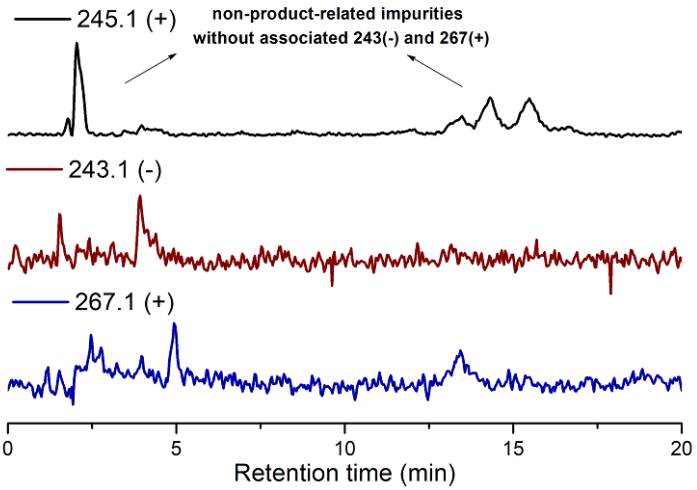

Figure 3. TIC results of the ribosylation of uracil without metal salts and kaolinite

Although the adducts cannot be unambiguously determined to be β-uridine itself, these results at least suggested that the product have the same glycosidic linkage as *β*-uridine but in isomeric forms (*α*-pU, *β*-pU, *α*-fU, and *β*-fU itself). Whether the *β*-furanoside was formed predominantly was not the primary focus of this study. Instead, we would emphasis on the formation of the desired glycosidic linkage (N1), and leave the question open on whether other isomers are convertible to *β*-furanoside under the reaction conditions. The coordination of uridine and isomers to metal ions seemed to be strong, and we were unable to de-combined the product from these cations. However, that total reaction yields could be determined by UV-integral method, no matter the chromophore was bound to a cation or in free form. The maximum overall yield of the ribosylation products was 4.6%, based on the HPLC integration of the peaks at 1.7, 2.0 and 2.3 min. To the best of our knowledge, this is by far the highest-yielding direct ribosylation of uracil, since few precedents reported the yield of this transformation.

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In general, the ribosylation of cytosine gave the 4-ribosylamino adducts as the major product, due to the much greater nucleophilicity of the 4-NH_2 group that readily react with the -CHO group of ribose. Under the currently investigated conditions, we have not yet found an effective way to overcome this problem. We have discovered that cavitation (both acoustic and hydrodynamic) is helpful in forming elevated amount of *N*9 purine nucleoside compared with 6-ribosyamino products,[19] but whether the cavitational condition is compatible with the environment rich in metal salts and clay minerals, remains unclear. Therefore, the ribosylation of cytosine is out of the scope of this study. To expand the scope of this nucleosidation, we explored the reaction with 2-pyrimidinone following the same procedure as the ribosylation of uracil (Figure 4).

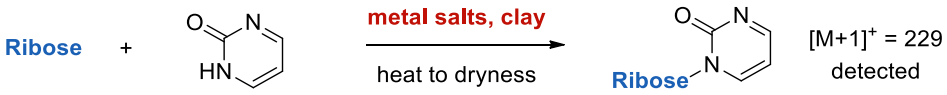

Figure 4. Direct ribosylation of 2-pyrimidinone enabled by metal salts and kaolinite

To do that, the pH of the suspensions of ribose, 2-pyrimidinone, metal salts and kaolinite was adjusted to 5. The mixture was heated at 85 °C heating block for 13 h. The dried mixtures were added water and analyzed by LCMS. The desired MS signal of 229 ($[M+H]^+$) showed up, indicating the formation of ribosylated 2-pyrimidinone. The MS signal of 227 ($[M–H]^-$) was weak, due to the lack of acidic proton in the ribosylation products.[20] The product peaks were again in the solvent front, which implied the possible formation of metal complexes (Figure 5).

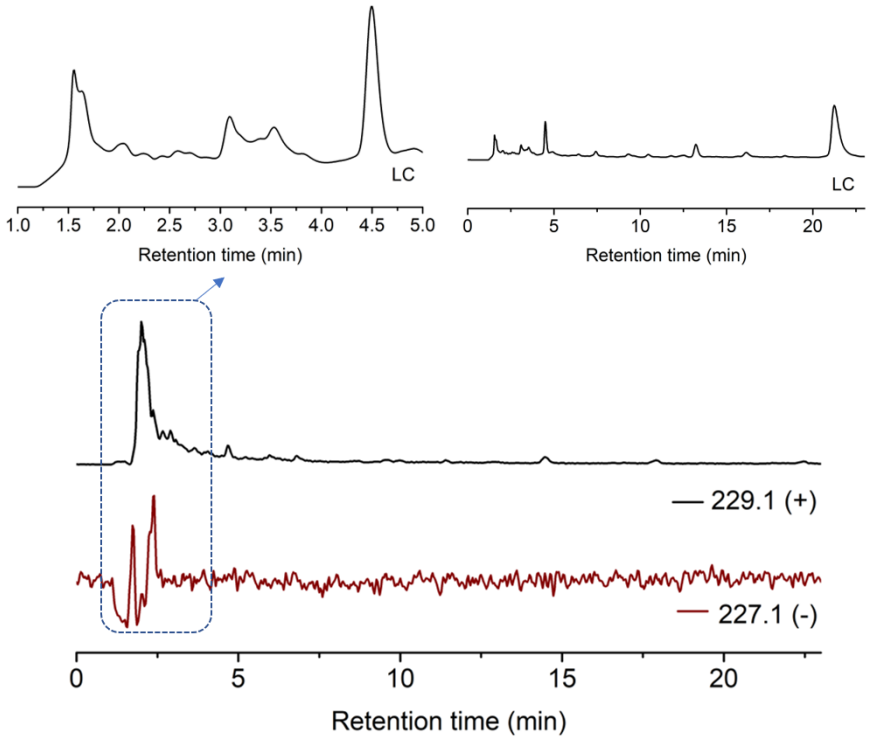

Figure 5. TIC results of the ribosylation of 2-pyrimidinone without metal salts and kaolinite

3. Material and Method

3.1. Chemicals

D-Ribose (CAS 50-69-1) was purchased from TCI (>98%). Cupric chloride (CAS 7447-39-4) was purchased from Shanghai Xinbao (99%). Iron(II) chloride (CAS 13478-10-9) was purchased from Acros (99%). Anhydrous magnesium chloride (CAS 7786-26-2) was pur-

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chased from Adamas-beta (99%). Calcium chloride dehydrate (CAS 10035-04-8) was purchased from General-Reagent (AR, 99–103%). Uridine (CAS 58-96-8) was purchased from Adamas-beta (99%). Uracil (CAS 66-22-8) was purchased from Shanghai Yuanye Bio-Technology Co., Ltd (98%). 2-Pyrimidinone (CAS 557-01-7) was purchased from Bidepharma Co., Ltd (97%). Sodium hydroxide (CAS 1310-73-2) was purchased from Genaral reagent (⩾ 96%). Methanol (CAS 67-56-1) was purchased from Sigma-Aldrich (HPLC grade, >99.9%). Formic acid (CAS 64-18-6) was purchased from Adamas-beta (99%).

3.2. Instrument

Shaking drybath (model# SD1-100) was manufactured by Titan Technology Co., Ltd. Magnetic stirrer (RCT basic) and heating block were manufactured by IKA®. LC-MS analyses were performed on a SHIMADZU LCMS-2020, with SHIMADZU LC-20AD pump, SPD-20A UV detector, SIL-20AC auto-sampler, and electrospray ionization (ESI) MS detection mode with SHARPSIL-U C18 column (S-5 μm, 100 Å, 4.6 mm I.D. × 150 mm), at a detection wavelength of 254 nm and a flow rate of 1.0 mL/min, with water (pH = 3, adjusted with formic acid, as phase A) and methanol (as phase B) as the eluents. The standard injection volume was 30 μL. The gradient conditions were: 0→3 min, 100% A; 3→300 min, 100% A to 100% B. The elution was stopped at 23 min. Reaction yields were calculated from the integration area of the isomer divided by the total integration area of the starting material and products.

3.3. Procedure for the direct ribosylation of uracil enabled by metal salts and kaolinite:

Uracil (22.2 mg, 0.20 mmol), D-ribose (450 mg, 3.0 mmol) and multiple metal salts ($CuCl_2$ (6.72 mg, 0.05 mmol), $FeCl_2{\cdot}4H_2O$ (9.94 mg, 0.05 mmol), $MgCl_2$ (4.76 mg, 0.05 mmol) and $CaCl_2{\cdot}2H_2O$ (7.35 mg, 0.05 mmol)) were added in H_2O (4 mL, [uracil] = 0.05 M, [D-ribose] = 0.75 M, Σ[multi-metal] = 0.05 M). The solution (1.5 mL) was transferred to a sample vial (2.0 mL) containing kaolinite (120 mg). The pH of the mixture was adjusted to 5 with NaOH. The vial was placed on a shaking drybath at 73 °C for 14 h. The dried sample was washed with water (1.5 mL) and filtered. The filtrate was analysed by LC-MS and TIC. The elution method was: 0 → 3 min, 100% buffer A (water/HCOOH buffer, pH = 3), 3 → 300 min, 100% buffer A to 100% phase B (methanol). The overall yield of the ribosylation products was 2.7–4.6%.

3.4. Control experiment of the ribosylation of uracil without metal salts and kaolinite:

Uracil (8.33 mg, 0.075 mmol) and D-ribose (168.9 mg, 1.125 mmol) were added in H_2O (1.5 mL, [uracil] = 0.05 M, [D-ribose] = 0.75 M). The pH of the mixture was adjusted to 5 with hydrochloric acid. The prepared mixture was reacted on a shaking drybath at 73 °C for 14 h. The dried sample was washed with H_2O (1.5 mL) and filtered. The filtrate was analysed by LC-MS and TIC. The elution method was: 0 → 3 min, 100% buffer A (water/HCOOH buffer, pH = 3), 3 → 300 min, 100% buffer A to 100% phase B (methanol). No ribosylated uracil was detected.

3.5. Procedure for the direct ribosylation of 2-pyrimidinone enabled by metal salts and kaolinite:

2-Pyrimidinone (9.6 mg, 0.10 mmol), D-ribose (225.2 mg, 1.50 mmol), kaolinite (120 mg), $FeCl_2{\cdot}4H_2O$ (5.0 mg, 0.25 mmol), $CuCl_2{\cdot}2H_2O$ (4.3 mg, 0.25 mmol), $MgCl_2$ (2.4 mg, 0.25 mmol) and $CaCl_2{\cdot}H_2O$ (3.7 mg, 0.25 mmol) were added in H_2O (2 mL, [2-pyrimidinone] = 0.05 M, [D-ribose] = 0.75 M, [$FeCl_2{\cdot}4H_2O$] = 0.125 M, [$CuCl_2{\cdot}2H_2O$] = 0.125 M, [$MgCl_2$] = 0.125 M, [$CaCl_2{\cdot}2H_2O$] = 0.125 M). The pH of the solution was adjusted to 5 with aqueous NaOH. The prepared mixture was heated in a 2 mL sample vial in 85 °C bath on heating block (IKA®) for 13 h. Water (1mL) was added to the dried mixture. A small part of reaction mixture (100 μL) was taken and diluted to 1 mL with H_2O for LCMS analysis.

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The standard injection volume was 30 μL. The gradient conditions were: 0→3 min, 100% A; 3→300 min, 100% A to 100% B. The elution was stopped at 20 min.

4. Conclusions

In conclusion, we have demonstrated that the previously unachievable direct ribosylation of uracil can be enabled in the presence of metal salts and clay minerals. This is compatible with the metal-doped-clay model proposed by our lab for the RNA World hypothesis. To the best of our knowledge, this is the first example of the direct coupling of ribose and canonical pyrimidine nucleobase with the correct regioselectivity in one step, under prebiotically general conditions. The mechanism for this promoting effect of metal and clay, the further optimization of reaction yield, and the ribosylation of cytosine are currently under investigation in our lab.

Author Biography

Xiao Wang is an associate professor and principal investigator at Nanjing University, formerly an instructor at Harvard Medical School. Prof. Wang obtained his PhD (2009) from University of Pittsburgh under the guidance of Prof. Dennis Curran on natural product synthesis, and completed his postdoc at MIT with Prof. Steve Buchwald on homogeneous catalysis and flow chemistry. His research at NJU focuses on developing sustainable syntheses of life-related molecules such as peptides and nucleosides (*Nat. Catal.* 2019, *2*, 98), and looking retrospectively at their prebiotic formation and chemical evolution on the primordial Earth (*Cell Rep. Phy. Sci.* 2021, *2*, 100375; *Chem* 2021, DOI: 10.1016/j.chempr.2021.09.002).

Author Contributions: X.W. proposed the research subject. Q.-Q.C and Z.-R.Z. carried out the experiments and the analysis of data. All authors wrote the manuscript. Q.-Q.C and Z.-R.Z. contributed equally to the project.

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Conflicts of Interest: The authors declare no competing interest.

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[20] This is different from the case of uracil, which has an acidic and deprotonatable proton at *N*3, thus the negative MS of the adducts ($[M–H]^-$ = 243.1) was much stronger.