Retraction for *Chemical Communications*:

First prebiotic generation of a ribonucleotide from adenine, D-ribose and trimetaphosphate

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We, the named authors, hereby wholly retract this Chemical Communication.

Signed: Graziano Baccolini, Carla Boga and Gabriele Micheletti, Università di Bologna, Italy, October 2011.

Retraction endorsed by Dr Robert D. Eagling, Managing Editor. Retraction published 10 October, 2011.

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COMMUNICATION

First prebiotic generation of a ribonucleotide from adenine, D-ribose and trimetaphosphate†

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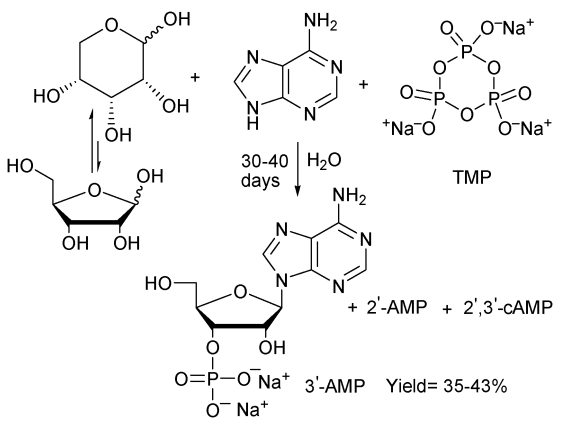
Adenosine monophosphate isomers are obtained by self-assembling of adenine, D-ribose and trimetaphosphate in aqueous solution in good yields. This generation of a ribonucleotide from its three molecular components occurs in a one-pot reaction at room temperature for about 30–40 days and with high chemio-, regio-, and stereo-selectivity. Similar results are obtained with guanine. A mechanism is also proposed.

The idea that RNA might have formed spontaneously at some stage on early Earth has inspired many searches for obtaining some feasible prebiotic syntheses of ribonucleotides, the building blocks of RNA.[1] For many years, efforts to understand the prebiotic synthesis of a ribonucleotide have been based on the assumption that a ribonucleotide could have assembled from three molecular components: a nucleobase, a ribose sugar and a phosphate. But, so far, the main difficulties have been in how to combine these three components.[2,3] In particular, the most frustrating has been the failure to find an efficient procedure[3b,4] to join together the nucleobase and the ribose thus casting doubt on the feasibility of these molecules to spontaneously combine in the primordial Earth. For this reason, the idea that a molecule as complex as RNA could have assembled spontaneously from its components now is viewed with increasing scepticism.[2] Recently, Sutherland *et al.*[5] accepting the impossibility of a spontaneous assembling of the three simple components have explored a totally different approach for pyrimidine ribonucleotide synthesis in which the sugar and the nucleobase emerge from a common precursor after several steps in different reaction conditions. Now, we report that a spontaneous self-assembling of the three components is permitted giving natural adenosine monophosphates (AMP) as final products. In fact, a very feasible synthesis of a ribonucleotide (adenosine monophosphates), from its three molecular components, a nucleobase (adenine), a sugar (D-ribose) and a phosphate (trimetaphosphate or P_4O_{10}), occurs giving adenosine monophosphates in good yields. The synthesis is made in aqueous solution and in a 'one-pot' manner at room temperature. In recent years we have postulated,[6] on the basis of experimental data, that the evolution of life should be governed or driven by a mechanism in which the formation of cyclic pentacoordinate phosphorus intermediates is more activated of 10^{6-8} fold with respect to other collateral processes.[7]

In other words, it is necessary to find primordial phosphorylating reagents containing the phosphate group belonging to a cycle in order to obtain very activated cyclic pentacoordinate phosphorus intermediates.[8] One of the primordial cyclic reagents was very likely P_4O_{10}. The discovery of Yamagata[9] demonstrating that P_4O_{10} and its derivatives as trimetaphosphate (TMP) are produced from volcano magma is, in this context, very important. TMP is a very stable cyclic compound containing three phosphate groups used for condensation and phosphorylation reactions.[10] For this reason we began to study the possibility to obtain a ribonucleotide (adenosine phosphates) by a simple reaction in aqueous solution of a mixture of D-ribose, adenine and a cyclic phosphate as TMP (Scheme 1), monitoring for 60 days two types of reactions: one with a highly concentrated mixture of the three components in water, and another at high dilution.

It should be noted that D-ribose in solution is in equilibrium between different forms (α-pyranose 20%, β-pyranose 60%, α-furanose 5%, β-furanose 15%).[11] Then, the formation of natural adenosine, adenine β-ribofuranoside, can be obtained



Scheme 1 One-pot generation of adenosine monophosphates in aqueous solution from adenine, D-ribose and sodium trimetaphosphate.

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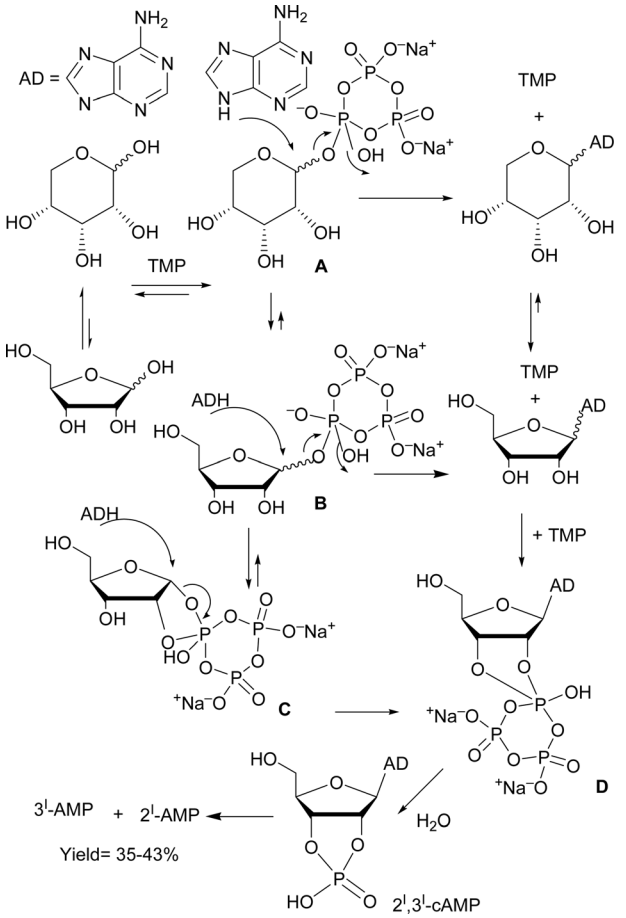
together with other isomeric forms derived from the reaction between adenine and other isomers of ribose. It should be noted that the four forms of adenine ribofuranosides and adenine ribopyranosides are in equilibrium.[12] Then, in our process it will be necessary to consider these equilibria, the different possible nucleosides,[3b,c] and to understand if, at the end of the reaction, a preferential formation of some isomers could occur. As first attempt we performed a reaction at high concentration ($\sim$0.1 M) of the reagents and at a pH value in the range 7.0–6.5. Trisodium salt of TMP (459 mg, 1.5 mmol), D-ribose (150 mg, 1.0 mmol) and adenine (135 mg, 1.0 mmol) were added in 10 mL of water (a small amount of sodium azide was added as an aseptic) in a 1.5 : 1 : 1 relative molar ratio (pH $\approx$ 7.0). At this concentration value adenine, being much less soluble with respect to the two other reagents, remains partially undissolved. The initial pH (pH $\approx$ 7.0) was that obtained after introduction of the three components in water. Variations of pH until the final value of 6.5 are due to the very slow hydrolysis of trimetaphosphate with formation of phosphoric acid which forms a salt with the remaining adenine. In these conditions, the final yield of adenosine monophosphates is very low, about 10%. Subsequently, we have carried out a reaction at high dilution in which all the reagents were completely dissolved and probably some eventual collateral reactions are minimized. The reaction was carried out in water solution (pH $\approx$ 7.0–6.5) with high dilution of the three reagents (1.85×10^{-4} M). Adenine (50 mg, 0.37 mmol), D-ribose (55 mg, 0.37 mmol), and trisodium salt of TMP (168 mg, 0.55 mmol) were dissolved in 2 L of aseptic water, in a 1 : 1 : 1.5 relative molar ratio. The reaction course was followed by HPLC and, after lyophilization, by ^{1}H and ^{31}P NMR spectroscopy (See Experimental section in ESI†). Very probably, the first step that occurs in the mixture is the attack of TMP on the anomeric OH group of ribose isomers giving unstable pentacoordinated intermediates (see intermediates A, B, and C in the mechanism). In the second step we have condensation of adenine with concomitant formation of AMPs and adenosine isomers. In fact, in the first time of the reaction (5 days) we observed by HPLC the formation of AMPs and adenosine isomers in a ratio of about 1 : 1. The adenosine isomers are adenosine β-ribofuranoside and adenosine α-ribofuranoside (in a relative ratio of about 6 : 1 calculated from ^{1}H NMR of a reaction mixture). They have been assigned on the basis of a comparison with the isomers obtained by keeping natural adenosine for several days in aqueous solution. In these conditions a slow isomerization of adenosine occurs and the two isomers, adenosine β-ribofuranoside and adenosine α-ribofuranoside, have been identified by comparison of their ^{1}H NMR data with the published[12] chemical shifts for the adenosine isomers. After about 25 days we have as major product AMPs in which the 3′-AMP and 2′-AMP are in a ratio of about 3 : 2. A small amount of cyclic 2′,3′-AMP (2′,3′-cAMP) was also observed. After about 45 days we have a total disappearance of 2′,3′-cAMP and a gradual decrease of the relative amount of the different adenosine monophosphates indicating a probable formation of short oligonucleotides that might arise from the cyclic phosphate and 5′-OH of 3′-AMP. The AMPs are separated by preparative HPLC. The structures have been assigned by ^{1}H NMR by comparison with authentic commercial samples (See experimental section in ESI†). The total yield, after about 30–40 days, in adenosine monophosphates, is of about 35%. No traces of ATP or 5′-AMP were detected by HPLC and ^{31}P NMR spectroscopy. Then, the global process is highly regio-, chemio-, and stereoselective because we found only AMP in β-furanose forms which are the natural nucleotides. It should be noted that in the literature[13] is reported a similar phosphorylation reaction with commercial samples of adenosine and TMP (or P_4O_{10})[9b] in different reaction conditions. The reported[13a,b] results are very close to our data with exclusive formation of 2′- and 3′-adenosine phosphates and exclusion of 5′-AMP and ATP. The reported[3b] very low yields (4%) of natural adenosine obtained by adenine and D-ribose with TMP at 100 °C for several hours could be explained by the concomitant formation of adenosine phosphates (or by the decomposition and isomerization[12] of adenosine at 100 °C) which were not considered by the authors. Our relatively low yield (35%) might be due to the lack of free adenine in the solution due to the formation, after about 30 days, of a small amount of phosphoric acid which forms a salt with the remaining adenine. In the light of these considerations, and in order to increase the yields of reaction products, we carried out the reaction with an excess of adenine. In particular, when we used adenine, D-ribose, and TMP in 1.2 : 1 : 1.5 relative molar ratio and in these conditions, after 35–40 days, the yields in adenosine monophosphates reached 43% with respect to the ribose. When the reaction is carried out with equimolar amounts of the three reagents we obtained after about 30 days formation of relevant amount of AMP isomers indicating that TMP is reformed after the first step or gives directly the AMPs without relevant formation of adenosine (see Scheme 2).

Preliminary results show that a similar reaction occurs with guanidine obtaining guanosine phosphates (see ESI†).

Finally, it has to be noted that the use of P_4O_{10} instead of TMP gave a similar behaviour but with minor yields in AMP because of its major instability in water that gives rise, after several days, to phosphoric acid which forms a salt with adenine.

It should be noted that the process is activated when in the solution Mg^{2+} ions are present.[3,10] Actually, in this case the reactions reached the end after only 20 days (yield in adenosine monophosphates is of about 45%) thus suggesting that the magnesium ions act as catalysts for all the process (both condensation and phosphorylation).

Very likely, the first step of this reaction is the preferential phosphorylation (by TMP) of the hydroxy anomeric group of the ribose, which is the most reactive, to give ribose-1-TMP as pentacoordinated phosphorus intermediates A and B (Scheme 2). The TMP group is a very good leaving group and could activate a second step, involving the subsequent nucleophilic attack of adenine from the opposite side with reformation of TMP thus producing adenosines (probably[14] in furanose forms). However, it is also possible that a bicyclic pentacoordinate phosphorus intermediate C is preferred[8] over A and B for its two cycles around the P atom. In this case, the attack of adenine is now obliged to go in the opposite side of the O-group giving the new intermediate D which can be



Scheme 2 Proposed reaction mechanism.

formed also by direct attack of TMP on β-adenosyl furanoside. The hydrolysis of intermediate D gives 2′,3′-cAMP which by subsequent hydrolysis gives 2′-AMP and 3′-AMP. The attack of TMP on adenosines to give phosphorylated adenosines is probably driven both by the position of the adenylic moiety and by the presence of the two OH groups in the *cis* relative position of the ribose moiety in the adenosine ribofuranoside form. In the adenosine ribopyranoside form the position of the two OH groups is disfavored to form a cyclic phosphate. It is reported[14] that the transformation of ribose into its cyclo-phosphates belongs to the functionalizations of the ribose molecule which selects the furanose form from the sugar's furanose/pyranose equilibrium. For this reason we found only adenosine β-ribofuranoside monophosphates, determined by their ^{1}H NMR. These factors conduct to the preferential formation of 2′,3′-cAMP that in aqueous solution can be hydrolyzed to adenosine-2′-phosphate and adenosine-3′-phosphate (Scheme 2). In this manner the 5′-hydroxy group remains free, thus explaining why we could detect neither 5′-AMP nor ATP. It is reported[13a] that when deoxyadenosine is reacted with TMP, only 3′- and 5′-monophosphates are obtained in very low yield (2%) suggesting a disfavored formation of a 3′,5′-cyclic phosphate. This is in good accord with our mechanism which is also in agreement with reported data in which it has been observed[3b] that when α- and β-D-ribofuranose-1-phosphate or any phosphate-containing products were heated with adenine no formation of adenosines was observed. This is in accord with the formation of unstable intermediates such as A, B, and C. It is reported[15] that in adenine the 9-N is the prevalent position of a nucleophilic attack.

In conclusion, our findings demonstrate that it is very simple to put together the three components to generate spontaneously in a one-pot reaction and with high chemio-, regio-, and stereoselectivities, adenosine monophosphate, a ribonucleotide that is one of the building blocks of RNA. This process might explain the spontaneous generation of pre-RNA molecules in the primordial Earth.

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