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Key steps from the "RNA World" to the "DNA World"

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Abstract. In the « RNA World » hypothesis of the origin of life, RNAs are assumed to be the central macromolecules able to self-replicate, conserve information and catalyze the reactions necessary for a primitive metabolism and many enzymatic cofactors may be regarded as molecular fossils of the "RNA World". In the key steps involved in the transition from the RNA World to the DNA World, two main steps can be distinguished: (i) the synthesis of 2'-deoxyribonucleotides from ribonucleotides catalyzed nowadays by the enzyme ribonucleotide reductase and (ii) the synthesis of thymine, a base specific for DNA, from uracil which is a base specific for RNA, catalyzed today by the enzyme thymidylate synthase. In regard to the chemistry of sulfur used by both enzymes for achieving their respective catalysis, we were interested in the search for simple sulfur reactions able to catalyze such transformations and report here on first results in an approach from thionucleosides to the catalysis involved in the conversion of uracil to thymine. In the RNA World, the recruitment of cofactors was crucial to expand the catalytic repertoire of RNA and we also describe interesting preliminary results obtained in the prebiotic synthesis of pyridoxal (vitamin B6) that is the precursor of the key coenzyme pyridoxal phosphate (PLP) able to catalyze nowadays seven different enzymatic reactions.

1 Introduction

In the « RNA World » hypothesis of the origin of life, RNAs are assumed to be the central macromolecules able to self-replicate by base pairing, conserve information and catalyze the reactions necessary for a primitive metabolism [1-6].

Many arguments lead to the idea that RNA predated DNA in Evolution and suggest that DNA is an RNA which has been modified to fit it efficient storage and repair of genetic information.
In the key steps involved in the transition from the RNA World to the DNA World, we can point out two key steps:

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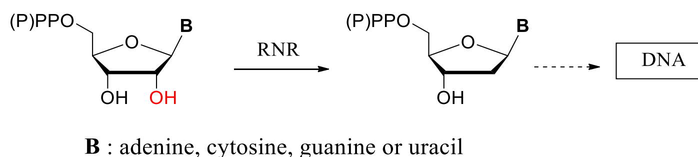
(i) the synthesis of 2'-deoxyribonucleotides from ribonucleotides (diphosphates or triphosphates catalyzed nowadays by the enzyme ribonucleotide reductase (RNR) (Figure 1) and

(ii) the synthesis of thymine, a base specific for DNA, from uracil which is a base specific for RNA, catalyzed today by the enzyme thymidylate synthase (TS) at the 5'-mononucleotide level (Figure 2).



B : adenine, cytosine, guanine or uracil

Fig. 1. Synthesis of 2'-deoxyribonucleotides from ribonucleotides catalyzed by RNR.

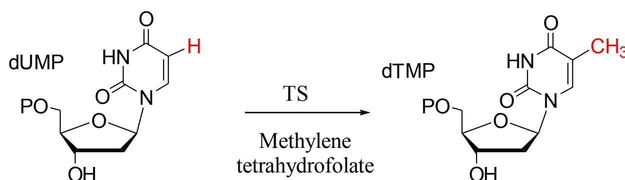


Fig. 2. Synthesis of thymine from uracil catalyzed by TS.

Both RNR and TS enzymes use the chemistry of sulfur for achieving their respective catalysis. Probably, the chemistry of sulfide and disulfide ions has played a major role in the chemical evolution at the origins of life. G. Wächtershaüser proposed a surface-based primordial autotrophic catalysis on the negatively charged pyrite surface [7,8] that could have emerged in the "black smokers" environment. In this regard, iron-sulfur redox proteins containing cage-like clusters formed by iron cations chelated by sulfide ions and cysteine residues of a peptidic chain are widely involved in our contemporary metabolism could be seen as pyrite derivatives.

The reduction of the four ribonucleoside diphosphates (ADP, CDP, GDP and UDP) in *Escherichia coli* under aerobic conditions is catalyzed by ribonucleoside diphosphate reductase (RDPR, class I enzymes) through a mechanism involving free radicals generated on the sugar ring of the four natural substrates (Figures 1 and 3) [9-15]. A transient cysteinyl radical formed at the active site of the enzyme abstracts the 3'-hydrogen atom of the ribose and then a cascade of reactions involving two other cystein residues led to the reduction products, the 2'-deoxynuleotides dNDP (Figure 3) [14]. A diferric oxygen linked iron center and a stable tyrosyl radical generates during the catalytic reaction the cysteinyl radical by intramolecular electron transfer. Class II enzymes also generate a transient cysteinyl radical but employ adenosylcobalamin (coenzyme B12) for this purpose and have a simpler structure than class I and class III reductases. A specific ribonucleoside triphosphate reductase is induced in anaerobic *E. coli* (Class III reductases). Class III enzymes contain an iron-sulfur center and, in their active form, a glycyl radical. This radical is generated from *S*-adenosylmethionine by interaction of the reductase with a complex enzyme system present in *E. coli*.

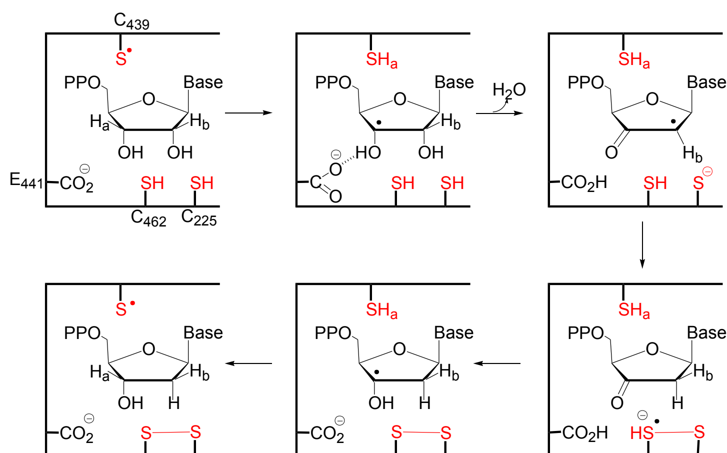


Fig. 3. Proposed mechanism for ribonucleotide reduction by RNRs. A transient cysteinyl radical initiates the nucleotide reduction process and two cysteine residues achieved the reduction [14].

Clearly, the chemistry of sulfur is involved in the reduction performed by all enzymatic class of ribonucleotide reductases.

RNRs allow the synthesis of three of the four 2'-deoxyribonucleoside triphosphates necessary for the synthesis of DNA i.e. dATP, dCTP, dGTP. The synthesis of the fourth triphosphate dTTP necessary to DNA synthesis is achieved from dUDP or dUTP, produced by RNRs, through dephosphorylation and phosphorylation processes and a key methylation step of dUMP catalyzed by the enzyme thymidylate synthase (TS) leading to dTMP. Three classes of TS have been identified and, in the main class, the enzymes called classical thymidylate synthases use the coenzyme N^5,N^{10}-methylene-5,6,7,8-tetrahydrofolate (CH_2H_4folate) and dUMP to produce dihydrofolate (H_2folate) and dTMP (Figures 2 and 4) [16, 17].

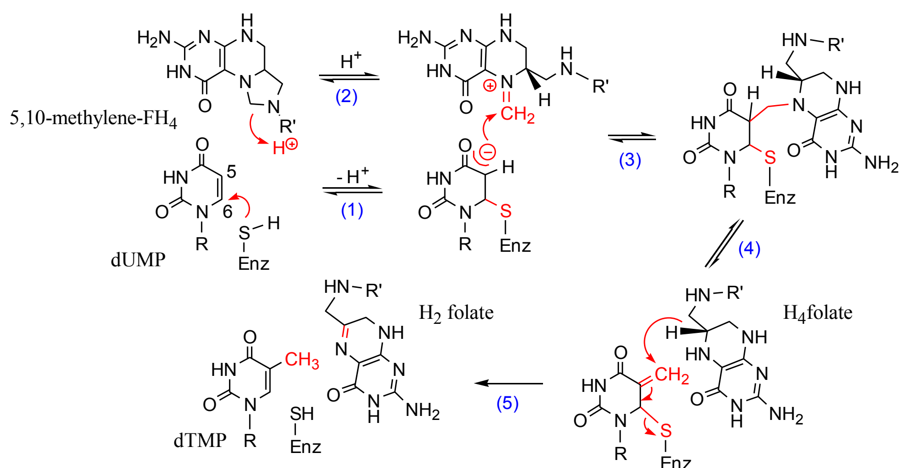


Fig. 4. Mechanism for methylation of dUMP by thymidylate synthase (TS) [17].

Thus, the chemistry of sulfur is also involved in the methylation step of dUMP necessary for the DNA synthesis catalyzed by most of the TS enzymes. A remarkable Michael addition of a cysteine residue at the 5 position of the uracil base is necessary to the catalysis (Figure 4). The enzymes activate the poorly reactive C5-pyrimidine position of dUMP through the formation of a transient

covalent linkage between the thiol side chain of an active site cysteine residue and the C6 atom of the targeted base for C5-methylation [16, 17]. A similar cysteine-dependent nucleophilic mechanism is also used by *S*-adenosylmethionine-dependent m5C DNA methyltransferases and RNA methyltransferases that methylate the C5 atom of pyrimidine [18].

In regard to the critical role of the sulfur chemistry in the DNA synthesis, possible simple sulfur reactions could have participated to the emergence of the natural deoxynucleotides found nowadays in cells. In our first works, we were interested in the possible prebiotic sulfur chemistry able to catalyze the conversion of uracil to thymine in aqueous solution from a thionucleoside model and we report on the first results obtained.

On another hand, many enzymatic cofactors may be regarded as molecular fossils of the "RNA World" [2, 19] and we also developed experiments in order to obtain under prebiotic conditions pyridoxal (vitamin B6) that is used today as precursor in the biosynthesis of the key coenzyme pyridoxal phosphate (PLP) able to catalyze seven different enzymatic reactions [20, 21]. In this regard, the paradox of the emergence of this key coenzyme has been discussed extensively in the book "The RNA World" [22]. We also report here on our first results in this field.

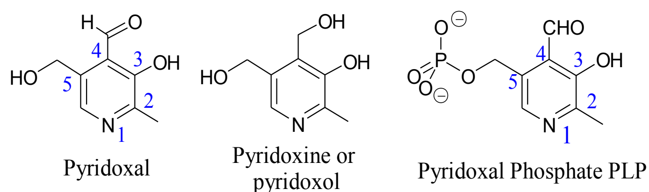


Fig. 5. Structure of pyridoxal, of one of this other vitaminic forms and of the coenzyme PLP.

2 A model for the first catalytic step performed by thymidylate synthase in the methylation of the uracil base

The nucleoside **1** (Figure 6, A) carrying a mixed disulfide function at the 3'-position was synthesised in six steps from uridine [23]. Such methyl disulfide can be reduced easily by dithiothreitol and/or tributylphosphine to lead to the corresponding thiol. Treatment of compound **1** with tributylphosphine (2 equiv.) at room temperature in the 90:10 water-methanol mixture resulted rapidly in 15 min in the quantitative formation of the thiol **2** detected by HPLC through its UV absorption (Figure 6A). To the solution obtained was added different aqueous buffer solution at pH 4, 7 and 9 and the resulting solutions were analyzed by HPLC. Compound **1** remained stable at pH 4 over more than 9 h at room temperature. At pH 7 and 9, the formation of a new compound was observed and was achieved after 2 h at pH 7. This compound was isolated in 52% yield and characterized as compound **3** (Figure 6A). Therefore, a remarkable intramolecular cycloaddition of the nucleoside **2** thiol function on the 6-position of the uracil ring occurs easily and can be considered as a model reaction of the first addition step of a TS cysteine residue on the dUMP substrate (Figure 4).

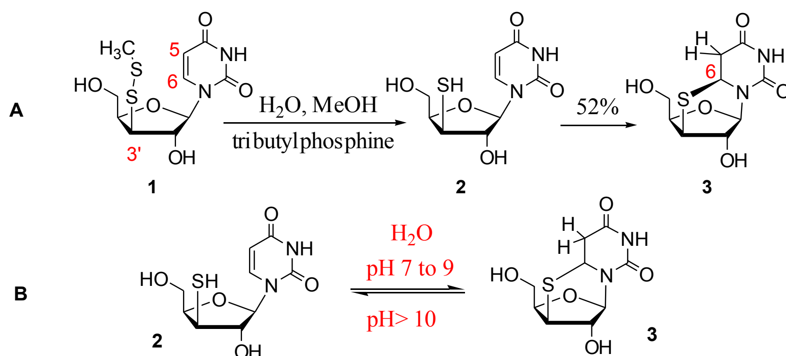


Fig. 6. A possible model for the first step in the conversion of dUMP to dTMP catalyzed by thymidylate synthase: (A) intramolecular cycloaddition of a thiol function generated at the 3'-position from an uracil nucleoside disulfide **1** leading to the intramolecular cycloadduct **3**, (B) reversibility of the intramolecular cycloaddition as a function of pH.

In order to confirm the cycloaddition reversibility, to an aqueous solution of compound **3** obtained quantitatively at pH 7, aqueous NaOH was added to reach pH 10. HPLC analysis showed that rapidly compound **3** led quantitatively under these conditions to compound **2** (Figure 6B). Two cycles of ring-opening and intramolecular cycloaddition were achieved quantitatively through modifications of the pH from 7 to 10 and inversely (HPLC).

Therefore, compound **2** appeared to be a possible model for studying the alkylation step at the 5-position of the uracil base assisted by a thiol addition at the 6-position as in the catalysis performed by TS and methylase enzymes. Deprotonation of the thiol function in compound **2** is probably necessary for achieving the intramolecular cycloaddition as shown by the major importance of the pH. The addition of formaldehyde concomitant to the thiolate addition on the uracil ring will be studied from **2** under various conditions.

Preliminary attempts of reaction of 2'-deoxyuridine with hydrogenosulfide ions (sodium hydrogenosulfide) were unsuccessful in water at room temperature.

3 Prebiotic synthesis of pyridoxal (vitamin B6) and related compounds

In the catalysis performed with the PLP coenzyme (Figure 5), the presence of the aldehyde function at the 4-position of the pyridine ring is essential for the PLP covalent binding to the enzyme or to the substrate through the formation of a Schiff base, from the amine function of an enzymatic lysine residue or of an amino acid substrate. The presence of a 3-hydroxyl function on the pyridine ring is also essential for the stabilization of the Schiff base through intramolecular hydrogen bond and for the complexation of metal ions able to orientate and accelerate the catalytic process. The presence of a 2-methyl group on the pyridine ring hinders the 3'-hydroxyl function decreasing its nucleophilic reactivity as well as that of the 1-nitrogen atom. The attachment position of this hydroxyl group is crucial in the catalysis process, when attached to the 2- or 4-position, a tautemoric equilibrium leads mainly to the corresponding pyridone (location of the hydrogen atom on *N*-1 nitrogen atom). The phosphorylation of the hydroxymethyl group present at the 5-position in pyridoxal allows additional electrostatic interactions of the PLP cofactor with the enzyme [20, 21, 24].

The main prebiotic synthesis of pyridoxal related derivatives has been reported by Austin and Waddell [25] from glycoaldehyde and ammonia in water at 110 °C at pH 7.6. Some interesting pyridine derivatives (Figure 7) were detected by liquid chromatography coupled to mass

spectrometry (LC/MS) after long times of reaction (50 h at 110 °C intermittently over 50 days) but were not isolated for identification.

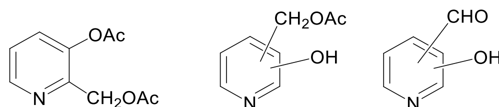


Fig. 7. Some pyridoxal analogues detected by reaction of ammonia and glycoaldehyde in water at 110 °C [25].

We have previously reported a remarkable stereoselective reaction of methylglyoxal (MG, pyruvaldehyde) with α-aminoazaheterocycles, as 2-aminopyridine, the nucleic base adenine and adenine nucleosides, leading in high yield to cyclic adducts in water under mild conditions at 50 to 70° C (Figure 8) [26-27].

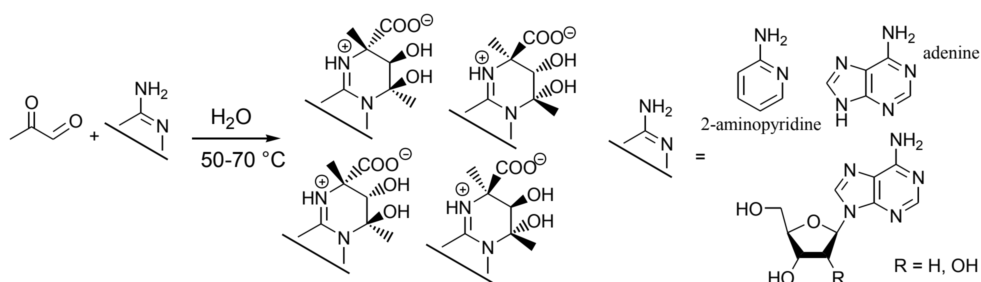


Fig. 8. Stereoselective reactions of MG with α-aminoazaheterocycles [27].

On another hand, the reactions of MG with the guanidinine function of arginine residues in proteins are considered to be involved in diabete complications because of the MG production by degradation of glucose *in vivo* [28-30]. The formations of fluorescent 3-hydroxypyrimidine biomarkers called Argpyrimidines and of MG adducts from arginine residues have been independently demonstrated *in vivo* and/or *in vitro* from the corresponding amino acid derivatives (Figure 9) but were not related [31-33].

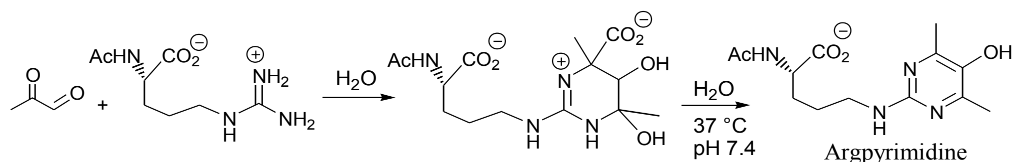


Fig. 9. Formation of Argpyrimidine from MG under physiological conditions [34].

More recently, we have shown that the MG adducts to an arginine derivative can be converted under physiological conditions to the corresponding Argpyrimidine in water at pH 7.4 and 37 °C (Figure 9) [34]. Such a conversion results from three very different one-pot cascade reactions, decarboxylation, dehydratation and oxidization. This easy transformation drew our attention regarding the structure of the final product that is a 3-hydroxypyrimidine and we decide to investigate the reactions of MG with ammonia in water in order to obtain pyridoxal under prebiotic conditions. The analysis of the chemical composition of pyridoxal suggests that it can result from the condensation of one molecule of MG and three molecules of ammonia followed by decarboxylation, dehydration and oxidization (cyclization and oxidization) processes.

According to the process considered at the prebiotic origin of sugars called Sugar Model, aldol condensation of formaldehyde and glycoaldehyde gives trioses and tetroses (Formose reaction) [35]. Dehydration of these sugars generates some α-ketoaldehydes such as pyruvaldehyde (MG) [36]. On another hand, the oxidized form of MG, pyruvic acid can be formed in an iron-, sulfur- and CO-rich environment [37].

Thus, we investigated the reactions of MG and ammonia in ratio 1:3, first in concentrated aqueous solution. The reactions were conducted at pH 3 (addition of phosphoric acid), pH 7 or 9 (addition of NaOH) at 35 °C during 12 to 24 h. Under these conditions, numerous products were formed and an additional heating at 60 °C for 12 h was done in order to select stable compounds under these conditions. Some stable products were detected by HPLC and one of them appeared to have a retention time and an absorption spectrum close to the ones of pyridoxal. It was detected at pH 3 under argon atmosphere or in the presence of dioxygen, upon UV irradiation (365 nm) or not. After this interesting result, we have now to isolate this compound for a complete characterization.

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