Synthesis and Degradation of Nucleobases and Nucleic Acids by Formamide in the Presence of Montmorillonites

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We describe the role of formamide, a product of the hydrolysis of hydrogen cyanide, as precursor of several components of nucleic acids under prebiotic conditions. When formamide is heated in the presence of montmorillonites, the efficient one-pot synthesis of purine, adenine, cytosine, and uracil is obtained. Along with these nucleobases, several components of the inosine pathway are obtained: 5-aminoimidazole-4-carboxamide, 5-formamidoimidazole-4-carboxamide and hypoxanthine. This almost complete catalogue of nucleic acid precursors is accompanied by N^9-formylpurine, which, containing a masked glycosidic bond in its formyl moiety, is a plausible precursor of purine acyclonucleosides. In addition, montmorillonites differentially affect the rate of degradation of nucleobases when embedded in 2′-deoxyoligonucleotides; namely, montmorillonites protect adenine and guanine from the degradative action of formamide, while thymine degradation is enhanced. The oligonucleotide backbone reactivity to formamide is also affected; this shows that the interaction with montmorillonites modifies the rate of abstraction of the Hα and Hβ protons on the sugar moieties.

Introduction

Since Bernal's analysis of the physical basis of life,[1] several prebiotic scenarios invoking clays have been proposed.[2] Accordingly, both the surface and, possibly, the interlamellar spaces of clays might serve as favourable microenvironments (or microreactors) to concentrate organic building blocks for life-related syntheses such as the formation of peptides and polynucleotides.[3–5]

Montmorillonite is a naturally occurring clay characterized by the ability to swell and exchange ions.[6–8] One single montmorillonite crystal consists of about 15 multilayers of silicium (SiO_2) and aluminium (Al_2O_3) oxides. Each layer is split into three defined sheets built of two enclosing SiO_2 units encompassing one Al_2O_3 unit. Due to disorder in the crystal, the sheets carry a negative charge, compensated for by intercalated cations. The individual sheet may be readily separated by dilution and reassembled by removal of water, so that metal ions intercalated in the multilayered structure can be exchanged by other catalytically active metal ions or by H_3O^+ ions. Because of their favourable physical and chemical properties, such as high surface area, large adsorption and ion-exchanging capacity, montmorillonites are widely employed as catalysts in the synthesis of fine chemicals and drugs under mild experimental conditions.[9–17] Remarkably, montmorillonites catalyze the aldol condensations of formaldehyde into sugars ("formose reaction").[18,19] From these points of view montmorillonites can be considered as primitive enzymes. Their role in the synthesis of oligonucleotides up to the length of small ribozymes has been reported in a system consisting of the adsorption of a template on the mineral surface and repeated incubations with activated nucleotides.[20–22] It is also relevant that nucleobases such as thymine, which are not normally intercalated into montmorillonite, are taken up through specific molecular recognition processes based on hydrogen-bond pairing with partner molecules localized in the interlamellar region.[23]

Cairns-Smith has pointed out the possibility that imperfections in a growing crystal lattice are replicated upon crystal growth. The fact that biopolymers formed in the montmorillonite microenvironment could be determined by the information encoded in the charge distribution of the layers suggested a role for clays in a "rudimentary prebiological evolution process".[24] The template and the shielding properties proposed for clays are of additional relevance. It was shown that montmorillonite–nucleic acid complexes are resistant to biotic and abiotic degradation;[25–27] this maintains their biological activity.[28–36]

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Taken together, these data suggest that the formation of clay–nucleic acid complexes could have played an important role in the preservation of genetic material under prebiotic conditions.

Even though the potential role of montmorillonites in the oligomerization and evolution of nucleic acids is well studied, only a few data on the catalytic properties of these minerals are available that could be connected with the prebiotic synthesis of purines and pyrimidines, the building blocks for activated nucleotides.[37]

We have reported that formamide, a product of the hydrolysis of hydrogen cyanide present in the ocean of the primitive Earth,[38] is a compound active in both the synthesis of nucleobases[39,40] and in the selective degradation of DNA.[41,42] The condensation of formamide into nucleobases is catalyzed and modulated under catalytic conditions by different inorganic compounds ($CaCO_3$, silica, alumina, kaolin and zeolite).[43] We have also observed the unprecedented syntheses of thymine, 5-hydroxymethyluracil and purine acyclonucleosides from formamide in the presence of titanium dioxide.[44] The role of these catalysts is not limited to the improvement of the yield of reaction products, but also provides high selectivity. Since formamide is able to degrade DNA,[41,42] our experimental approach consists of the parallel study of the formamide-based syntheses and degradative pathways of nucleic acid components under comparable experimental conditions.

This coupled approach is necessary because in any physicochemical scenario dealing with the origin of life, *stability* of the precursor molecules is a major concern.[45,46] Thus, in order to evaluate the possible prebiotic role of a given synthetic reaction both the conditions that preferentially lead to synthesis and those leading to degradation must be jointly determined. Here we describe the role of montmorillonites in both the synthesis of purine and pyrimidine derivatives starting from formamide and in the selective degradation of 2′-deoxyoligonucleotides induced by montmorillonite clays.

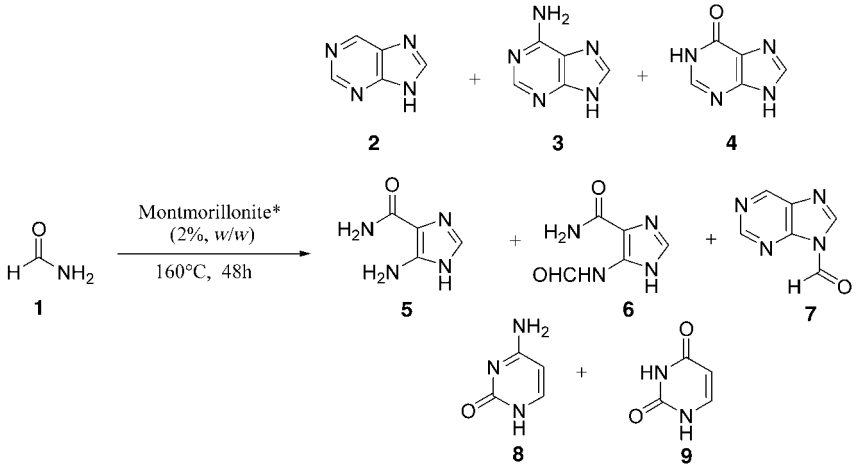
Table 1. *Physical and chemical properties of montmorillonites KP-10, K-30, KSF and Al-PILC.*

Clay	Micropore volume [mL g^{-1}]			Surface area [m^{2}g^{-1}]	pH[a]	CEC[b] [meq g^{-1}]
	0–80 nm	0–24 nm	0–14 nm			
KP-10	0.28	0.25	0.19	240	3.2	0.36
K-30	0.50	0.44	0.38	330	3.1	0.5
KSF	0.02	0.01	0.01	10	1.4	0.02
Al-PILC	n.d.[c]	n.d.	n.d.	280	4.5	0.13

[a] Determined on 10% filtered suspension. [b] CEC: Cation exchange capacity. [c] Data not available.

change capability (CEC) and acidity. KP-10 and KSF have high Brønsted acidity, while K-30 has mainly Lewis acidity. The Al-PILC, which is formed upon intercalation of polyaluminium-hydroxide derivatives and subsequent calcination, has a very narrow pore-size distribution in the micropore range combined with a highly specific surface area and high shape selectivity.[47,48]

Briefly, the syntheses were performed by heating pure formamide **1** (0.12 mol) at 160 °C for 48 h (Scheme 1)[43] in the presence of catalytic amounts of the appropriate montmorillonite (2.0% *w/w*). We focused on the characterization of the



Scheme 1. *Schematic drawing of montmorillonite-catalyzed purine and pyrimidine synthesis out of formamide. *Montmorillonite: hAL-PILC, KP-10, K-30, KSF and Al-PILC.*

Results

Syntheses

We have selected montmorillonites KP-10, K-30, KSF and aluminium-pillared clay (Al-PILC) as representative models of clay catalysts, based on the widespread availability of the calcium montmorillonites. The major physical and chemical properties of these clays are reported in Table 1. The various montmorillonites differ in pore size distribution, surface area, cation exchange capability (CEC) and acidity. KP-10 and KSF have high Brønsted acidity, while K-30 has mainly Lewis acidity. The Al-PILC, which is formed upon intercalation of polyaluminium-hydroxide derivatives and subsequent calcination, has a very narrow pore-size distribution in the micropore range combined with a highly specific surface area and high shape selectivity.[47,48]

Briefly, the syntheses were performed by heating pure formamide **1** (0.12 mol) at 160 °C for 48 h (Scheme 1)[43] in the presence of catalytic amounts of the appropriate montmorillonite (2.0% *w/w*). We focused on the characterization of the more abundant purine and pyrimidine derivatives by gas chromatography, mass spectrometry and nuclear magnetic resonance (^{1}H and ^{13}C NMR) analyses, and, when necessary, by comparison with authentic samples after chromatographic purifications.

We identified a large range of products. In accordance with data previously reported in the literature, the condensation of formamide performed in the absence of montmorillonite afforded **2** as the only recovered product (34.1 mg **2** per gram formamide, Table 2, entry 1).[44] The reaction was also per-

*Table 2. Quantitative profile of products **2–9** obtained from formamide/montmorillonite systems.[a]*

	Catalyst	Yield [mg g^{-1}][b]							
		2	**3**	**4**	**5**[c]	**6**[c]	**7**[c]	**8**[c]	**9**
1	–	34.1	–	–	–	–	–	–	–
2	CaAl-PILC	1.0	–	–	–	–	–	–	–
3	KP-10	21.0	6.0	0.8	4.3	5.8	14.0	16.0	1.1
4	KSF	27.6	13.5	1.2	4.5	6.2	–	18.0	2.0
5	Al-PILC	19.2	2.7	0.4	–	6.6	–	14.4	1.3
6	K-30	3.1	22.0	–	–	–	21.0	11.0	0.5

[a] Products were identified by comparison of their retention times and mass spectra with those of authentic samples. [b] Quantitative evaluations were performed by capillary gas-chromatographic analysis as described in the experimental section. Because of the uncertainty of the number of formamide molecules involved in the synthesis of the recovered products the yields were calculated as mg of product formed per gram of formamide. [c] Products isolated by flash chromatography and identified by ^{1}H and ^{13}C NMR spectra.

formed in the presence of homoionic calcium Al-PILC (CaAl-PILC) as a reference of a neutral clay. In this latter case, **2** was again obtained as the only recovered product (Table 2, entry 2). In a similar way, the condensation performed in the presence of homoionic neutral calcium K-30 (CaK-30) afforded **2** as the only recovered product. On the other hand, montmorillonites KP-10, K-30, KSF and Al-PILC yielded a complex mixture of reaction products. Among these, purine **2**, adenine (**3**), hypoxanthine (**4**), 5-aminoimidazole-4-carboxamide (**5**; AICA), 5-formamidoimidazole-4-carboxamide (**6**; (fAICA), N^9-formyl purine (**7**), cytosine (**8**) and uracil (**9**) were isolated in varying amounts depending on the experimental conditions (Table 2, entries 3–6). Selected mass spectrometry data for products **2–9** are reported in Table 3. In the presence of montmorillonites, the yield of **2** was always lower than in their absence, probably because of the competitive production of several other compounds (see above and Table 2). Purine derivatives **2**–**3** and **7**, and cytosine (**8**) were previously prepared from formamide by using different metal oxides.[43,44] The formation of hypoxanthine **4**, imidazole carboxamide derivatives **5**–**6** and uracil (**9**) from formamide alone has not previously been observed. Products **4**–**6** were obtained in the presence of KP-10, KSF and Al-PILC (Table 2, entries 3–5), while they were not obtained with K-30 and CaAl-PILC (Table 2, entries 2 and 6).

Table 3. Selected mass spectrometry data of compounds 2–9.[a]

Products	*m/z* (%)
Purine (**2**)	120 (100) [*M*], 93 (37) [*M*−HCN], 86 (19) [*M*−2HCN]
Adenine (**3**)	135 (100) [*M*], 108 (38) [*M*−HCN], 81 (18) [*M*−2HCN], 66 (21) [*M*−69], 54 (28) [*M*−3HCN]
Hypoxanthine (**4**)	136 (100) [*M*], 109 (3) [*M*−HCN], 81 (8) [*M*−HCN−CO], 54 (23) [*M*−2HCN−CO], 28 (45)
AICA (**5**)	126 (100) [*M*], 110 (58) [*M*−NH_2], 99 (37) [*M*−HCN], 82 (16) [*M*−NH_2CO]
fAICA (**6**)	154 (100) [*M*], 138 (41) [*M*−NH_2], 125 (36) [*M*−HCO], 110 (68) [*M*−HCONH]
N^9-formylpurine (**7**)	148 (100) [*M*], 121 (8) [*M*−HCN], 92 (18) [*M*−HCN−HCO], 66 (28) [*M*−2HCN−HCO]
Cytosine (**8**)	111 (100) [*M*], 95 (20) [*M*−NH_2], 83 (28) [*M*−CO], 69 (45) [*M*−NCO], 41 (58) [*M*−HNCO−HCN]
Uracil (**9**)	112 (100) [*M*], 69 (74) [*M*−HNCO], 42 (21) [*M*−HCNO−HCN]

[a] Mass spectrometry was performed with a Hewlett-Packard 5971 mass-selective detector on a Hewlett–Packard 5890 III gas chromatograph with FID detector. Samples were analyzed after treatment with *N*,*N*-bis-trimethylsilylacetamide and pyridine.

Steps on the route to inosine: AICA (**5**) is a well-known intermediate in the prebiotic synthesis of hypoxanthine by polymerization of HCN.[49–52] AICA is usually formed by hydrolysis of 5-aminoimidazole-4-carbonitrile (AICN), which serves as precursor for the synthesis of adenine. In the late steps of the biochemical pathway to inosine monophosphate (IMP), the eighth intermediate is AICA linked to ribose-5′-monophosphate (AICAR-5′-phosphate). AICAR-5′-phosphate is formylated by formyltransferase to give 5′-phosphoribosyl-4-carboxamide-5′-formamidoimidazole (fAICAR-5′-phosphate). In the next step, fAICAR-5′-phosphate is cyclized by IMP cyclohydrolase to IMP.

Based on this biochemical pathway, Zubay and co-workers showed that AICA can be efficiently converted into hypoxanthine by treatment with ammonium formate as a formyl donor.[53] In this case, fAICA (**6**) was suggested as a key intermediate, even though it was not isolated from the reaction mixture. Similarly, the authors proposed that in the AICN to adenine conversion, formate and an ammonium ion were used as reactants for the construction of the purine ring. In agreement, Hill and Orgel showed that adenine may be formed when the HCN tetramer is heated with ammonium formate at 110 °C.[54]

Since both AICA and fAICA were recovered in our reaction mixtures, the question arises for the origin of HCN and ammonium formate that are necessary for their formation. Formamide decomposes at 190 °C to give HCN, NH_3, CO and H_2O. This reaction is known to be catalyzed by the presence of metal oxides.[55] Moreover, even though formamide is hydrolyzed slowly to give ammonium formate ($K_w = 1.1 \times 10^{-10}$ s^{-1} corresponding to $t_{1/2} = 200$ y at 25 °C), the reaction can be efficiently accelerated under the acidic clay conditions.[56] Thus, it is reasonable to suggest that the decomposition of **1** to HCN and its partial hydrolysis to ammonium formate are side-processes responsible for the synthesis of **5**–**6** and then of hypoxanthine **4.** This hypothesis is confirmed by the low reactivity observed with CaAl-PILC and by the recovery of appreciable amount of formic acid in the reaction mixture (data not shown). The Brønsted acidity of KP-10 and KSF appears to be an important variable for the formation of **4**–**6.** In fact, in the case of K-30, which is characterized mainly by Lewis acidity, these products were not recovered in the reaction mixture. To the best of our knowledge, this is the first report dealing with the prebiotic synthesis of compounds that are part of the current biochemical pathway to IMP.

Adenine and purine: All montmorillonites catalyze the synthesis of **2** and **3**, K-30 being the best catalyst for the production of adenine (Table 2, entry 6). The reaction mechanism for the formation of purine by heating pure **1**, or by heating **1** in the presence of HCN, was previously studied by ^{13}C,^{15}N-coupling experiments by using doubly enriched HCN and formamide.[57] These studies indicated that the adenine ring is built up from three molecules of HCN and two molecules of **1**, while the purine scaffold is formed by two molecules of HCN and three of **1**. Probably, the formamide decomposition to HCN in the presence of montmorillonites was an important variable for the synthesis of **2** and **3**.

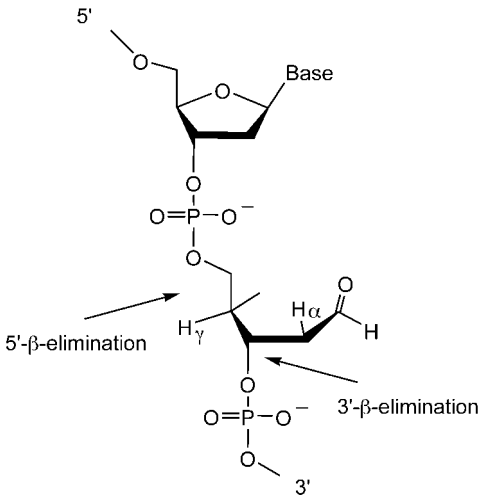
A hidden glycosidic bond: The presence of ammonium formate in the reaction mixture may be responsible for the recovery of N^9-formyl purine **7** supplying the formyl group at the N^9-position of the purine ring. As shown previously,[44] compound **7** may be an important precursor of purine acyclonucleosides under "formose reaction" conditions, mainly because it contains a masked glycosidic bond in the N^9-formyl moiety.

Pyrimidines: Cytosine **8** was always observed to be a significant product, KSF being the best catalyst (Table 2). Because the formation of a carbon–heteroatom bond is easier than that of a carbon–carbon bond, prebiotic syntheses of cytosine have usually been accomplished by condensation of three-carbon precursors with urea and urea-like derivatives.[58] The most prominent carbon fragments used for these transformations were cyanoacetylene and cyanoacetaldehyde.[59–62] However, these carbon fragments are not elemental precursors due to the presence of preformed carbon–carbon bonds and they can react with common nucleophiles more readily than forming **8.** These observations made the role of **8** in the origin of life controversial.[63] Thus, the synthesis of **8** directly from formamide is of special interest in the development of plausible prebiotic scenarios including cytosine. The formation of uracil (**9**) is in agreement with the well-known deamination process of cytosine.[64–65] This reaction is pH dependent, and shows its minimal rate in the pH range of 6–9. Given that acids catalyze the deamination process by protonation of the cytosine, the Lewis and Brønsted acidities of montmorillonites are presumably the major player in the formation of **9**.

Degradations

Different factors control the interaction of nucleic acids with clays, such as the type of clay,[66] the pH of the reaction medium,[34–36] the size and molecular structure of the nucleic acid[27,67] and the ionic strength.[66] In general, the decrease of the pH and the increase of the concentration of cations favour the adsorption process, probably due to the lowering of the electrostatic repulsion between the phosphate moiety and the clay surface. Purines are adsorbed more readily than pyrimidines in the presence of monovalent and divalent cations[68] even though double-stranded DNA molecules with different G+C content are adsorbed equally well.[28] There are no data on the tuning of the reactivity of polynucleotides with amides in the presence of montmorillonites. We have investigated the reaction of formamide with selected 2′-deoxyoligonucleotides, dubbed polyA, polyG, polyT and polyC, in the presence of montmorillonites.

The degradation pathway of purine[39] and pyrimidine[40] bases into oligonucleotides by formamide alone has previously been described. The decreasing order of sensitivity of the nucleobases is: purine, inosine, guanine > adenine > cytosine ≫ thymine, this last compound being quite resistant. The chemical mechanisms involved have been determined.[39,40] For purines, degradation occurs by nucleophilic attack at the C-8 position, leading to degradative ring opening of the imidazole ring. For pyrimidines, degradation occurs by nucleophilic attack at the C-6 or at both C-4 and C-6 positions.[43] Following degradation and removal of the heterocyclic purine or pyrimidine bases, the two reactive protons Hα and Hγ (as indicated in Scheme 2) are available for β-elimination reactions. This



Scheme 2. *The cleavage of the 3′- and 5′-phosphodiester bonds by formamide. Schematic representation of the degradation of the sugar moiety indicating the two different β-eliminations.*

leads to cleavage of 3′- and 5′-phosphodiester bonds, respectively. In the presence of a weak base, such as formamide, the cleavage of the 3′-phosphodiester bond occurs preferentially, based on known chemistry and on previous observations.[41,42] Accordingly, when a 5′-labelled DNA strand is treated with formamide, two bands are observed for each degraded base, corresponding to the cleavages of the couple of phosphodiester bonds occurring at the 5′- and the 3′-end. The 5′-β-elimination being less efficient than that at the 3′-end, the band produced by this cleavage can be clearly observed only on 5′-end-labelled DNA molecules, favoured by its occurrence in the label-proximal position. Alternatively, when the formamide cleavage reaction is performed on 3′-end-labelled DNA molecules, the cleavage at the 5′-end is label-distal and is masked by the robust band generated at the 3′-label-proximal side. Comparison of the effects of different amounts of montmorillonites on the polyA degradation by formamide under standard conditions revealed that Al-PILC and KSF have a similar reactivity, while KP-10 and K-30 were both essentially inert (data not shown). Thus, Al-PILC was selected as a model for reactive

montmorillonites and its effects on the reaction of formamide with 2′-deoxyoligonucleotides were studied in detail.

Degradation of polyA

Experiments were performed on a 46 bp oligomer made of a 30 bp polyA–polyT central segment and of mixed-sequence terminal segments (as described in the Experimental Section), labelled at the 5′- (Figure 1, panel A) or at the 3′-end (Figure 1, panel B) of the polyA strand. Treatment of the polyA–polyT oligomer with formamide in the presence of the amounts of Al-PILC indicated at the top of the lanes, shows the selective inhibition of the 3′-cleavage by the clay. At a concentration higher than 100 ng mL^{-1}, the 3′-cleavage is abolished, favouring the detection of the cleavage at the 5′-end (panel A).

The cleavage pattern of the 3′-end labelled oligomer (panel B) confirms this interpretation. With this terminal labelling, the decreased intensity of the 3′-cleavage at Al-PILC concentrations higher than 100 ng mL^{-1} allows the detection of the otherwise hidden 5′-cleavage. This cleavage is still observed at high Al-PILC concentrations. Moreover, the reactivity towards formamide of the adenine base itself appears to decrease as a function of the increase of Al-PILC (arrowed in Figure 1, panel B, top right). In conclusion, montmorillonite modifies the β′-elimination reactions occurring at both the 5′- and 3′-ends of a degraded adenosine residue by markedly and selectively inhibiting the 3′-β-elimination and exerting little effect on the cleavage of the 5′-phosphodiester bond.

Degradation of PolyG

The strong reactivity of guanines to formamide is well established.[39,41] Accordingly, the oligomer containing the 30 bp polyG stretch (Figure 2, panel A) is rapidly degraded in shorter reaction times (see legend). At clay concentrations higher than 100 ng mL^{-1}, aggregates form that are difficult to dissolve and do not migrate into the gel (data not shown). Both the high reactivity and the consequent fast elimination of both 5′- and 3′-phosphodiester bonds,[39,41] and the tendency to form aggregates with clays prevent an analysis for polyG that is as informative as the one that can be performed on polyA. Consequently, the effect of Al-PILC on the polyG-containing DNA oligomer was not studied in further detail. Anyhow, the protection of the G residues by Al-PILC towards degradation by formamide can be clearly observed on the G residues when embedded in the mixed sequences polymer present in the polyT construct (see Figure 2, panel B).

Degradation of PolyT

Thymine residues are highly resistant to formamide.[39,41] Analysis of the polyT-containing oligomer (Figure 2, panel B) shows that, at high Al-PILC concentration, both 5′- and 3′-β-eliminations take place with equivalent intensity, due to an appreciable and unprecedented[39,40] degradation of the T-residues. The upper part of the gel (above the arrowhead, showing the mixed-sequence component of the oligomer, as indicated) was exposed a third of the time relative to the polyT-containing lower part of the gel in order to facilitate its quantitative evaluation.

The data reported in the previous sections for polyA and polyG, and for polyC below, are confirmed in their general trend in this mixed-sequence tract analysis. However, it should be noted that the intensity of the bands in a mixed-sequence tract is influenced by the reactivities of the bases that are in a more label-proximal position. The resulting information is therefore biased by a context effect. This fact is mentioned here in order to point to the necessity of the use of homogeneous polymers in this type of assays and to the

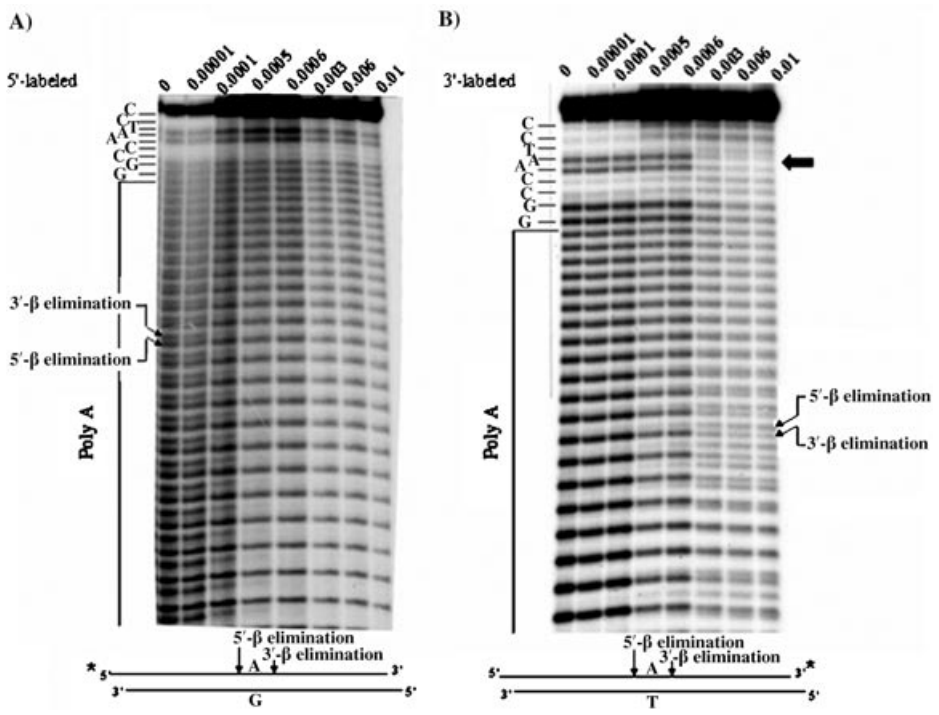


Figure 1. *Degradation of a 46 bp oligonucleotide containing an homogeneous polyA stretch by formamide in the presence of increasing amounts of Al-PILC montmorillonite (mg mL^{-1}). Panel A) 5′-end labelled. Panel B) 3′-end labelled. The reactions were performed in the presence of the amount of montmorillonite indicated on top of each lane. The sequence of the heterogeneous tract present at the 5′-end is indicated. The order of the DNA sequence sensitivity to formamide is G > A > C > T.[39] One couple of bands resulting from both the 5′- and the 3′-β-eliminations relative to a given degraded base (see text) is indicated. In the lowest part of the gel in panel B, allowing the highest resolution, the bands resulting from the 5′- and 3′-cleavages for each given base tend to mingle due to relative differential migrations; this leads to difficult local assignments. This problem is irrelevant for the overall interpretation of the analysis.*

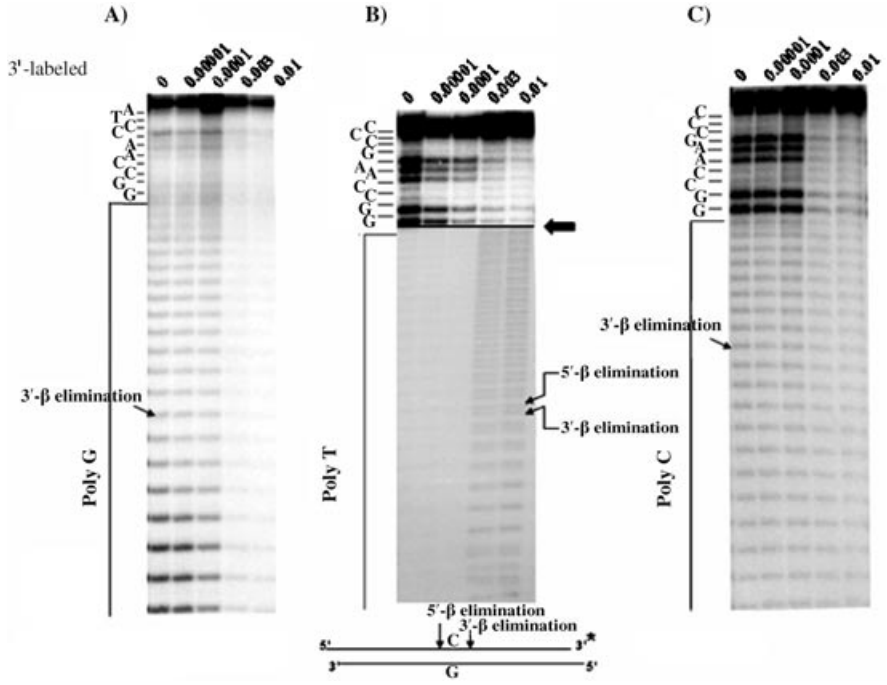


Figure 2. *Degradation of the 46 bp oligomers containing 3′-labelled polyG (panel A), polyT (panel B) and polyC (panel C) stretches. Experimental conditions as in Figure 1, except for the reaction time with polyG: 10 min. For polyT: two different autoradiographic exposures of the gel are shown (see text).*

risk of using a mixed-sequence analysis as an analytical shortcut.

Degradation of PolyC

The effect of montmorillonite on the polyC-containing oligomer is minor (Figure 2, panel C). The intensity of the homogeneous stretch is essentially unaffected, while that on the mixed-sequence tract confirms the described inhibition of the formamide reaction on As and Gs.

Discussion

The data reported here show that montmorillonites KP-10, K-30, KSF and Al-PILC are selective catalysts for an unprecedented one-pot synthesis of purine and pyrimidine nucleobases starting from a one-carbon atom fragment as simple as formamide.

Among the main four nucleobases found in RNA the two pyrimidines and one purine, adenine, were contemporarily obtained in yields that are quite high for a prebiotic process. Montmorillonite K-30, characterized mainly by Lewis acidity, was the best catalyst for the synthesis of both adenine and cytosine, while the highest yield of uracil was obtained with KSF. CaAl-PILC, which is a neutral clay, was only able to catalyze the synthesis of purine; this showed the relevant role played by the acidity of montmorillonite on the reaction pathway. On the basis of data previously reported, HCN and NH_3 (which are formed by decomposition of formamide) were essential reagents for the construction of the purine and pyrimidine scaffolds. Uracil was probably obtained from cytosine by a deamination process.

Three compounds (AICA, fAICA and hypoxanthine) that were not previously obtained from formamide alone, were recovered in acceptable yields. Ribosyl derivatives of AICA and fAICA are intermediates in the late steps of the biosynthesis of inosine monophosphate.

N^9-formylpurine was also recovered when the condensation of formamide was performed in the presence of KP-10 and K-30. In these latter cases, ammonium formate, a product of hydrolysis of formamide, was the donor of the formyl moiety, in agreement with data previously reported.[53] N^9-formylpurine is of great relevance in the prebiotic synthesis of nucleoside derivatives because it contains a masked glycosidic bond. In fact, we have previously shown that formyl purines react with formaldehyde (generated in situ from formamide and TiO_2) at the formyl moiety to give purine acyclonucleosides.[44] The formation of the glycosidic bond between nucleobases and sugars is a difficult synthetic step, and major problems arose in demonstrating this condensation under prebiotic experimental conditions.[51,69] Apart from the synthesis of cytosine arabinonucleoside, no prebiotic condensation of uracil or cytosine with ribose is known.[70] The synthesis of acyclonucleosides reported here opens novel scenarios for the formation of nucleic acids on primitive Earth.

We also observe that montmorillonites tune the reactivity of formamide with 2′-deoxyoligonucleotides. Two major models of interaction between oligonucleotides with clays have been suggested. In the first, the oligonucleotides are partially adsorbed and bound to the mineral surface, a part of the molecule interacting on the edges of the clay.[71] The second model claims that one end of the oligonucleotide is bound to the clay surface, while the other part remains unbound.[72] At the nucleobase level, purines interact with the clay surface more efficiently than pyrimidines. In the specific case of adenine or adenyl moieties of the GAAA RNA hairpin tetraloop, the anchorage site on the electronegative montmorillonite surface was suggested to be the electropositive C-6 amino group.[73] Moreover, the role of inorganic cations in the binding of oligonucleotides on clays has been described (the so called "cation-bridge model").[36] As shown in Table 1, the pH value of the filtered suspension for Al-PILC is 4.5. At this pH value DNA is efficiently adsorbed on montmorillonite.[74] In agreement with this observation, the oligonucleotides used for the formamide reactions remained bound to the Al-PILC surface, and treatments

with defined amounts of sodium pyrophosphate were necessary to desorb the reaction products and analyze the samples. It should be noted that, under these experimental conditions, the oligonucleotides were not in equilibrium with the solution, as evaluated by repeated washings with doubly distilled H_2O. Thus, the reactivity of oligonucleotide/Al-PILC complexes with formamide can be conveniently used as a probe for the specific interactions between nucleobases and the mineral surface. Irrespective of considering the generally accepted model, our observations imply that relevant modifications of the conformation of nucleobases and of their electronic distributions occur upon the interaction of the oligonucleotides with clays, as described below.

When treating oligonucleotides with formamide, two different reaction pathways have to be taken into account: a) the reactivity of the single nucleic bases, and b) the reactivity of the polynucleotide backbone (as measured by the 3′- and the 5′-β-elimination processes).

a) Data reported in Figures 1 and 2 show that the degradation of the purines in polyA and polyG molecules is inhibited by high concentrations of Al-PILC, as indicated by the decrease of the band intensities both in homopolymeric stretches and in the mixed sequence segment. As for pyrimidines, cytosine is unaffected, whereas degradation of thymine is moderately activated. The differential behaviour of purines and pyrimidines towards formamide in the presence of Al-PILC is possibly due to different sites of complexation of the base with the negatively charged mineral surface or with cation bridges. The fact that different metal binding sites exist in nucleobases is well documented. Endocyclic atoms in guanine (N-7), adenosine (N-7 and N-1), cytosine (N-3), and thymine (N-3) are the major binding sites for metal ions.[75] Exocyclic NH_2 groups of adenine and cytosine are poor ligands even when deprotonated.[75] As previously reported, adenine and guanine react selectively with formamide by nucleophilic addition to the C-8 position of the purine ring.[39,41] Thus, the decreased reactivity of purine nucleobases into polyA and polyG upon increased concentration of Al-PILC might be explained by the formation of complexes at the N-7 position and/or at the exocyclic NH_2 group. In both cases the electronic distribution and the steric hindrance of the adjacent C-8 position of the bases is highly modified, thus justifying a novel pattern of reactivity. In the case of pyrimidines, this effect is less pronounced, probably because of the known minor efficiency of pyrimidines in the complexation with clay.
b) The effect of Al-PILC on the polynucleotide backbone reactivity is unprecedented and selective. In the polyA context, the predominant 3′-β-elimination is strongly inhibited (Figure 1, panels A and B), while that at the 5′-end decreases only at much higher concentrations. In the polyC context (Figure 2) on the other hand, the 3′-β-elimination is moderately affected only at high concentration of the clay. The 5′-β-eliminations are observed neither for polyG nor for polyC. An interesting, different behaviour is shown by the polyT homopolymer: both 3′- and 5′-cleavages occur with the same efficiency, and only at high clay concentration. Given that the cleavage of the phosphodiester bonds occurs *after* the degradation of the corresponding base (that is, in its absence), these effects are probably explained by sequence-related chemical context effects on the sugar moiety. It is known that the binding of nucleotides to metal ions may distort the geometry of the molecule. Unfortunately, the determination of the effects of metal ions on the conformation of nucleotides in the presence of coordination to the nucleobase or to the sugar alone is still lacking.[76] Thus, we can only hypothesize that the interaction of the oligonucleotide with montmorillonites modifies the sugar packaging modes. This would favour or disfavour a specific β-elimination process (i.e., 3′- versus 5′-cleavage) depending on the nature of the nucleobases proximal to the reactive site and on the type of interaction with metal ions on the clay surface.

Conclusion

In conclusion, a whole set of nucleic acid precursors has been obtained by condensation from a one-carbon prebiotic precursor in the presence of montmorillonites. The physicochemical conditions used in our catalytic system (temperature up to 160 °C and acid clay minerals) are consistent with a volcanic prebiotic Earth scenario. The molecules produced are unstable in the very conditions that lead to their synthesis: moderately high temperatures and continued presence of formamide. Framed in an origin-of-life perspective, these syntheses would therefore be part of an ideal and genetically futile cycle of synthesis and degradation unless provided with a way-out towards stabilization. We hypothesize that polymerization itself—obtained in an as yet undefined physicochemical scenario—could provide such a way-out.

The pioneering observations that the rate of hydrolysis of N-glycosyl bonds in deoxynucleosides is higher than that in deoxynucleotides,[80–82] and that the rate of hydrolysis of free deoxynucleosides[77,78] is 10–50 times higher relative to the rate of cleavage of N-glycosyl bonds in single-stranded DNA[79] support this hypothesis.

Experimental Section

Materials: Formamide (Fluka, >99%), montmorillonites K-10, KSF, Al-PILC, K-30 (Fluka) and 6-methoxypurine (Aldrich) were used without further purification. Homoionic calcium Al-PILC (CaAl-PILC) was made by following the procedure previously reported.[36] Gas chromatography and mass spectrometry was performed by the use of a HP5890*II* gas chromatograph and by a Shimadzu GC-MS QP5050A spectrometer equipped with an Alltech® AT-20 column (0.25 mm, 30 m). ^1H and ^{13}C NMR spectra were recorded on a Bruker (200 MHz) spectrometer, and chemical shifts are reported in ppm. Microanalyses were performed with a C. Erba 1106 analyzer. Chromatographic purifications were performed on columns packed with Merck silica gel, 230–400 mesh for flash technique. TLC was carried out by using Merck Platten Kieselgel 60 F_{254}.

Formamide condensation: Formamide (5.7 g, 5 mL, 0.12 mmol) was heated at 160 °C for 48 h in the presence of the appropriate montmorillonite (2% *w/w*). The reaction mixture was allowed to cool, filtered to remove the catalyst and evaporated under high vacuum.

Gas chromatography and mass spectrometry of the crude reaction mixture were performed by using an isothermal temperature profile of 100 °C for the first 2 min, followed by a 10 °C min^{-1} temperature gradient to 280 °C and finally an isothermal period at 280 °C for 40 min. The injector temperature was 280 °C. Chromatography grade helium was used as carrier gas. The fragmentation patterns were compared with those of authentic samples. 6-methoxypurine was used as an internal standard. When necessary, the crude reaction was purified by flash chromatography ($CHCl_3/CH_3OH$ 9:1), and the structures of isolated products were confirmed by spectroscopic techniques (^1H and ^{13}C NMR) and by comparison with authentic commercial samples.

Selected data for compounds **5**–**7**:

5-aminoimidazole-4-carboxamide (**5**): m.p. 242–244 °C (lit. 250–252 °C); ^1H NMR (200 MHz, $CDCl_3$): $\delta = 7.05$ (brs, 2H; NH_2), 7.39 (s, 1H; H_2), 7.86 (brs, 3H; NH and NH_2); ^{13}C NMR (200 MHz, $CDCl_3$): $\delta = 118.95$ (C), 137.87 (CH), 138.68 (C), 163.18 (C); for mass spectrometry data see Table 3.

5-formamidoimidazole-4-carboxamide (**6**): ^1H NMR (200 MHz, $CDCl_3$): $\delta = 8.45$ (s, 1H; CHO), 7.5–8.0 (brs, 4H; NH_2), 6.71 (s, 1H; CH); ^{13}C NMR (200 MHz, $CDCl_3$): $\delta = 163.56$ (C), 156.97 (C), 140.04 (CH), 139.47 (C), 102.26 (C); for mass spectrometry data see Table 3.

N^9-Formyl-purine (**7**): ^1H NMR (200 MHz, $CDCl_3$): $\delta = 5.11$ (brs, 2H; NH), 5.89 (s, 2H; NH_2), 6.55 (s, 1H; H_2), 6.67 (brs, 2H; NH_2), 11.56 (brs, 1H; NH); ^{13}C NMR (200 MHz, $CDCl_3$): $\delta = 106.66$ (C), 133.50 (C), 140.34 (CH), 150.76 (C), 171.22 (C); for mass spectrometry data see Table 3.

DNA substrates: The differential effects of montmorillonites on the degradation of polynucleotides was studied on homogeneous or mixed-sequence polymers. The overall approach consisted of the analysis of the degradation products of the following synthetic 2′-deoxyoligonucleotides: a) Homogeneous segments: two short mixed-sequence tails (10 and 6 bases, respectively) and a central 30-base homogeneous stretch of Gs, As, Cs or Ts. b) Mixed-sequence segment: a heterogeneous 40-base sequence.

Oligonucleotides used in the degradation of homogeneous sequences were:

Oli1: 5′-ACCTAACCGG$[G]_{30}$CCGGTT-3′

Oli2: 5′-ACCTAACCGG$[A]_{30}$CCGGTT-3′

Oli3: 5′-CCCGAACCGG$[C]_{30}$CCGGTT-3′

Oli4: 5′-CCCGAACCGG$[T]_{30}$CCGGTT-3′

These oligonucleotides were designed to be pair-wise complementary (Oli1 with Oli3 and Oli2 with Oli4). Upon annealing, 4-nucleotide-long 5′ protruding tails remained at both ends that could be used for selective labelling, as described.[44]

The mixed sequence used was made of Oli5 (40 bases) and Oli6 (44 bases) and was analyzed as described above:

Oli5: 5′-GTAACTCGGTGTTAGAGCCTGTAACTCGGTGTTAGAGCCT-3′

Oli6: 5′-CCGAAGGCTCTAACACCGAGTTACAGGCTCTAACACCGAGTTAC-3′

For both homo- and heterogeneous sequence oligonucleotides, the degradation conditions used in this assay generally cause less than one hit per molecule, as shown by the regularity of the cleavage patterns and by the presence of a substantial amount of unreacted molecules.

Degradations of oligonucleotides by formamide and montmorillonite: Each oligonucleotide (2 μg) was annealed with the same amount of the complementary oligomer and labelled with [γ-^{32}P]dATP (Oli3 and Oli4) or with [γ-^{32}P]dCTP (Oli1 and Oli2). Labelling was performed by using the T7 Sequenase (USBC-Amersham Biosciences), the labelled oligomer was purified on a 10% denaturing acrylamide gel (acrylamide/bisacrylamide 19:1). The polyacrylamide was removed by a NuncTrap Probe Purification Column (Stratagene), 2 pmol (typically 30000 counts per minute) of DNA were processed for each sample. The DNA was precipitated with ethanol and resuspended in formamide (5 μL, Fluka). 97% formamide (10 μL) containing the indicated amounts of Al-PILC montmorillonite were added. After 20 min at 110 °C, a solution of tetrasodium pyrophosphate (5×10^{-4} M, final concentration, Sigma) dissolved in water was added to a final volume of 50 μL. The samples were vortexed for 1 min, then centrifuged at 13000 rpm for 20 min. The wash was combined, precipitated with ethanol, resuspended in formamide buffer (5 μL), heated for 2 min at 95 °C and loaded on a 16% denaturing polyacrylamide gel (acrylamide/bisacrylamide 19:1). For the analysis of the effect of formamide–montmorillonite on the heterogeneous sequence, the oligonucleotides indicated above were labelled with [γ-^{32}P]dCTP.

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