INORGANIC CHEMISTRY

FRONTIERS

RESEARCH ARTICLE

Check for updates

Cite this: Inorg. Chem. Front., 2022, 9, 4111

Received 19th April 2022, Accepted 27th May 2022 DOI: 10.1039/d2qi00837h

rsc.li/frontiers-inorganic

Introduction

Scientific questions related to the origins of life involve different fields of science and represent an area of interdisciplinary endeavour. The persistent challenge of prebiotic chemistry is to identify the possible mechanisms of synthesis of canonical molecules in the transition process from non-life to life.¹ The "RNA world" hypothesis assumes RNA as the first biopolymer of life that rules both informational and catalytic

Deadlocks of adenine ribonucleotide synthesis: evaluation of adsorption and condensation reactions in a zeolite micropore space[†]

Francisco Rodrigues, (¹) *^{a,b} Thomas Georgelin,^c Baptiste Rigaud,^d Guanzheng Zhuang,^a Maria Gardennia Fonseca,^e Valentin Valtchev^f and Maguy Jaber ⁽¹) *^{a,g}

Herein, we report on adenine, D-ribose, and monophosphate adsorption/co-adsorption into the synthetic analog of the zeolite mineral mordenite followed by drying at 50 °C and thermal activation at 150 °C under an argon atmosphere. Adenine/mordenite samples were prepared first and then used to co-adsorb D-ribose (1:1 molar) and monophosphate (1:2 molar). The optimal conditions to charge adenine into mordenite were pH 3, contact time of 5 min and 1300 mg L⁻¹ of C_i , resulting in the maximum adsorption of 65.6 µg g⁻¹. The drying and thermal activation effects in the adsorbed/co-adsorbed confined species were followed by FTIR, TGA, ³¹P and ¹³C CP-MAS and solid-state NMR. From the results, the electrostatic interaction was proposed as the main mechanism for adenine adsorption into mordenite. The stabilization of α - and β -D-ribofuranose enantiomers and condensation reactions involving D-ribose and inorganic monophosphate were also evidenced even at 50 °C. Data suggested the mordenite micropore space may play a role in the origins of life both to adsorb and concentrate prebiotic molecules and to promote isomerization and condensation reactions to nucleotides from their building blocks.

functions in primitive cells. In this perspective, canonical nucleotides are considered indispensable precursors of RNA. Therefore, the chemical pathways and geochemical conditions on the early Earth leading to their formation should be elucidated.

Simple primitive organic compounds such as formamide and formaldehyde, likely present in the early oceans, are suggested as precursors of ribonucleotides. In the literature, some pathways are proposed mainly in aqueous systems and involve intermediate reactions to overcome thermodynamics and kinetics problems associated with hydrolysis reactions. Activated ribonucleotides have been considered as monomers to form RNA and some authors have stated in this perspective.^{2–5}

Powner and colleagues³ proposed one successful route to activated ribonucleotides from starting plausible prebiotic feedstock. They demonstrated nucleotides can be formed in a short sequence that bypasses free ribose and the nucleobases with some intermediates. On the other hand, it is perfectly reasonable to expect that ribonucleotides must have prebiotically assembled from three components: ribose, canonical nucleobases, and inorganic phosphates (see Fig. 1).

Free ribose produced in the formose-like reactions and nucleobases obtained from hydrogen cyanide or formamide are some probable chemical routes to these precursors.⁶ In turn, the phosphate content necessary to assemble the build-

View Article Online

View Journal | View Issue

^aSorbonne University, CNRS UMR 8220, Laboratoire d'Archéologie Moléculaire et Structurale, 4 place Jussieu, F-75005 Paris, France.

E-mail: maguy.jaber@sorbonne-universite.fr,

francisco.rodrigues@servidor.uepb.edu.br

^bState University of Paraíba, UEPB, Department of Chemistry, Campina Grande, Paraíba, Brazil

^cCentre de Biophysique Moléculaire, CNRS, Rue Charles Sadron, 45000 Orléans, France

^dCNRS Institut des Matériaux de Paris Centre (FR2482), 4 place jussieu, 75005 Paris, France

^eFederal University of Paraíba, UFPB, Department of Chemistry, João Pessoa, Paraíba, Brazil

^fNormandy University, Laboratoire Catalyse & Spectrochimie, ENSICAEN,

⁶ bl Maréchal Juin, 14050 Caen, France

^gInstitut Universitaire de France, France

[†]Electronic supplementary information (ESI) available: ³¹P MAS-NMR data and D-ribose ¹³C CP/MAS-NMR. See DOI: https://doi.org/10.1039/d2qi00837h



Fig. 1 Contributions of adenine, ribose and phosphate groups as building blocks to adenine ribonucleotides.

ing blocks to ribonucleotides could have been available in solution. Meanwhile, prebiotic chemistry seeks to elucidate an approach to select and concentrate constituent parts to promote self-assembling reactions.

From a "prebiotic soup" in a primitive ocean to anhydrous environment systems, the presence of minerals may play an important role in the origin of life. Investigations by Bernal (1949) and Oparin (1953) suggested minerals may rule both adsorption and catalytic functions such as polymerization in prebiotic systems.^{7,8} Indeed, different mineral surfaces have been studied to select and concentrate key monomers from dilute solutions and promote chemical reactions at their active sites. The adsorption of amino acids and formation of peptides,9,10 adsorption of nucleic acids, bases, and proteins,^{11–13} selective adsorption of nucleosides and nucleotides,^{14,15} and even adsorption of RNA and DNA¹⁶⁻¹⁹ are some studied mechanisms towards the concentration of primer molecules. Furthermore, catalytic applications leading to the assembly of elementary blocks to key precursor molecules have also been studied on mineral surfaces.²⁰⁻²³

In previous papers from our group, we demonstrated the onepot synthesis of adenosine monophosphate (AMP) on the mineral surface of amorphous silica²⁴ as well as the obtention of adenosine -mono, -di, and triphosphate into mordenite zeolite.²⁵ Here, we focus on the effect of adsorption and confinement of the adenine ribonucleotide building blocks, *i.e.* adenine, p-ribose, and monophosphate, into micropores of the synthetic mordenite.

Unlike amorphous silica that presents a disordered silica framework, zeolites are microporous aluminosilicates with a crystalline structure. From a prebiotic perspective, zeolites may represent one of the oldest minerals on our planet derived from silica-rich volcanic glasses, which should have been abundant in the early Earth.^{26,27} The high hydration enthalpies of zeolites may provide the driving force for condensation or polymerization reactions to form more complex biomolecules such as nucleotides and RNA. Last but not least, mordenite is a high silica zeolite and thus exhibits a certain hydrophobicity which favors the adsorption of organic molecules. Its pore system consists of a main channel of 6.5×7.0 Å, which is connected by small pores of 2.6 \times 5.7 Å that form the so-called side pockets which are unavailable for bulk molecules. Additionally, the high silica content also makes mordenite stable under highly acid or basic conditions and at high temperatures. Although mordenite occurs readily in nature as a mineral, synthetic ones are preferable to academics and industrial processes in terms of purity and a controlled pore structure. Under acid conditions, an ion exchange between extra framework cations and hydronium ions takes place to generate strong Brønsted acid sites. The properties such as ionic exchange capacity, ordered pore systems, high specific area and flexible framework are highlighted and makes mordenite widely used as an adsorbent/catalyst worldwide and as a probable prebiotic nanoreactor.

Results and discussion

Adenine pre-adsorption into mordenite

In order to check the optimum conditions for adsorption of adenine into mordenite, the effects of pH, contact time and initial concentration were evaluated (see Fig. 2). Adenine adsorption was pH-dependent with the maximum uptake at around pH 3 (see Fig. 2a). Indeed, a cationic exchange between adeninium cations and sodium in zeolite channels under low pH conditions is expected. On the basis of experimental data and theoretical calculations, the most stable protonated adenine species is 1H-9H-A⁺(protonated at N₁) as presented in Fig. 3.^{28–30} The experimental pK_a of 4.15 (ref. 31 and 32) and calculated values around 3.99 (ref. 33) for protonated adenine



Fig. 2 Effect of (a) pH, (b) contact time and (c) initial concentration for adenine adsorption into Na/mordenite. Experimental conditions: 23 °C \pm 3 °C; 720 µg mL⁻¹ (pH and contact time) and 20 mg (dosage).



Fig. 3 Experimental pK_a values for some of the most stable adenine species under acidic, neutral and alkaline conditions. According to ref. 31 and 32.

at N_1 highlighted 1H-9H-A⁺ species at pH 3 and justified the highest load of adenine into mordenite under this pH condition.

ATR-FTIR analysis for adenine and the adenine-containing mordenite sample in the 1800 to 1250 cm⁻¹ region corroborates adenine protonation at the N_1 position (see Fig. 4). Both spectra presented the two most intense bands between 1700 and 1600 cm⁻¹ related to the scissoring mode of the -NH₂ group and to a skeletal stretching mode within the pyrimidine ring, respectively.³⁴⁻³⁶ Both bands at 1688 and 1598 cm⁻¹ in the bulk adenine spectrum were blue-shifted to 1697 and 1619 cm⁻¹ respectively, in the spectrum of A/MOR. The shift toward higher frequencies suggested that the force constant has increased the bond strength due to a neighboring positive charge on the NH_2 group (from protonation at N_1). Furthermore, the band at 1305 cm⁻¹ assigned to the stretching mode of N1-C2, C2-N3, C5-C6, and C5-N7 for the adenine sample³⁵ was also shifted to 1314 cm⁻¹ for the A/MOR sample, highlighting the protonation effect.

According to Krishnamurthy,³⁷ the simple relationship between pK_a and pH may state a general prediction about why nature chose the canonical nucleobases in the context of chemical evolution. The author postulated that the (pK_a -pH) < 2 conditions usually lead to catalytic functions coupled with



Fig. 4 ATR-FTIR spectra for bulk adenine and the mordenite sample containing adenine (A/MOR) before the thermal activation.

structural diversity. Once the pH of the early ocean was thought to lie more on the acidic side, and adsorption into mineral surfaces such as MOR is a plausible route to concentrate adenine under prebiotic conditions. Hence, pH 3 was set for the next steps of adenine adsorption study as well as for posterior co-adsorption of ribose and monophosphate.

Following the adsorption steps, the results of the kinetic study showed a fast profile for the adsorption of adenine into mordenite that reached equilibrium at 5 min (see Fig. 2b). Kinetic data were evaluated using the pseudo-first order $(PS1)^{38}$ and pseudo-second order $(PS2)^{39}$ models as well as the intra-particle diffusion (IPD) model. The results presented in Table 1 indicated an adsorption well-fitted to the PS2 model with a high value of the coefficient of determination (R^2) and close agreement between experimental and theoretical q_e values. Additionally, the PS2 model reached minor residual values compared to other models.

The strong electrostatic interactions between protonated adenine and the MOR structure are the driving force leading to the fast load equilibrium for this system. The IPD kinetics model gave further insight into the adenine sorption mechanism. Due to the multi-linear nature, the plot of q_t versus $t^{1/2}$ did not pass through the origin ($\theta_1 = 24.45$). One may anticipate that the pore diffusion is not the sole rate-controlling step. The plot showed two portions: an initial steep rather rapid portion and a second less steep phase. The first portion is related to a gradual sorption of adenine where intra-particle diffusion within the micropores is rate-limiting. The last steep phase represented that equilibrium had been achieved and the rate of the sorption and desorption is insignificant. The θ_1 (µg g⁻¹) value indicates the thickness of the boundary layer achieved around 72% of the micropore saturation on equili-

Table 1 Linear kinetic model data from adenine adsorption into Na/ mordenite. Experimental conditions: 23 °C \pm 3 °C, 720 μg mL^⁻¹, 20 mg and pH 3

Model	Values
$q_{\rm e}$ (exp)/µg mg ⁻¹	33.8 ± 0.8
Pseudo-first order: $\log(q_e - q_t) = \log(q_e) - \frac{k_1}{2.202}t$	
$q_{\rm e}$ (theor.)/µg mg ⁻¹	14.8
k_1	0.86
R^2	0.89
Residuals	7.38×10^{-2}
Pseudo-second order: $\frac{t}{q_t} = \frac{1}{k_2 q_e^2} t + \frac{t}{q_e}$	
$q_{\rm e}$ (theor.)/µg mg ⁻¹	35.4
\hat{k}_2	0.10
R^2	1.00
Residuals	$1.05 imes 10^{-5}$
<i>Intraparticle diffusion:</i> $q_t = k_{id} \cdot t^{1/2} + \theta$	
k _{id1}	4.40
$\theta_1/\mu g m g^{-1}$	24.45
R^2_1	0.95
Residuals	7.3×10^{-1}
k _{id2}	0.37
$\theta_2/\mu g m g^{-1}$	35.0
R^2_2	0.98
Residuals	1.00×10^{-2}

Equilibrium sorption was achieved at 65.6 μ g mg⁻¹ using 1300 mg L⁻¹ of C_i (see Fig. 2c). Sorption data were evaluated by using the Langmuir⁴⁰ and Freündlich⁴¹ models as well as the Temkin⁴² model as shown in Fig. 5. The resulting parameters summarized in Table 2 showed a Freündlich model with higher R^2 , and close agreement between experimental and theoretical q_e values and minor residuals. Thus, the Freündlich model showed a good degree of reproducibility compared to others. According to this model, the A/MOR system assumes adsorption on a heterogeneous surface through a multilayer adsorption mechanism. Furthermore, the adsorbed amount of adenine increased with the increase of the initial concentration (C_i) until equilibrium (C_e). The 1/*n* parameter reached a value of 0.24 and represents sorption on



Fig. 5 Equilibrium isotherms for adenine adsorption into Na/mordenite. Experimental conditions: 23 °C \pm 3 °C, 20 mg, pH 3 and 15 min.

Table 2 Linear adsorption model data from adenine adsorption into Na/mordenite. Experimental conditions: 23 °C \pm 3 °C, 20 mg, pH 3 and 15 min

Model	Values						
Langmuir: $\frac{C_{\rm e}}{q_{\rm e}} = \frac{C_{\rm e}}{q_{\rm max}} + \frac{1}{bq_{\rm max}}$							
$q_{\rm e} ({\rm exp}) ({\rm \mu g \ m g^{-1}})$	65.6 ± 0.8						
$q_{\rm max} (\mu {\rm g \ mg^{-1}})$	66.1 ± 6.8						
$b (L \mu g^{-1}) R^2 \chi^2 $	0.05± 0.02 0.79 73.66						
Freündlich: $Lnq_e = LnK_f + -LnC_e$							
$\frac{K_{\rm f}}{n}$ $\frac{1}{R_{\rm c}^2}$	15.41 ± 1.66 4.24 ± 0.38 0.95						
χ^2 Temkin: ^a $a_c = A + BLnC_c$	17.01						
A_t	1.98 ± 1.03						
b_t	267.45 ± 32.06						
R^2	0.91						
χ^2	32.23						
$^{a}B = (RT/b_{t}); A = B\ln(A_{t}).$							

predominantly heterogeneous sorption sites which describes highly curved isotherms.

According to this adsorption study, the optimal conditions to charge the adenine into MOR were pH 3, contact time of 5 min, and 1300 mg L^{-1} of C_i . These conditions were employed to prepare A/MOR samples for posterior co-adsorption with p-ribose and monophosphate and thermal activation at 150 °C.

Heating and confinement effects in the adenine and/or ribose and/or monophosphate adsorbed species into mordenite

In order to follow the drying and thermal activation effects in the species adsorbed into mordenite, TGA analysis, ³¹P and ¹³C CP-MAS and solid-state NMR were applied. In this regard, P_i/MOR, R/MOR, (R + P_i)/MOR, and RA/MOR samples were analyzed before and after thermal activation. The results for (R + P_i)A/MOR samples were presented in a preliminary study.²⁵

Stabilization of D-ribose. The low stability of D-ribose is a big challenge to overcome in the prebiotic synthesis of ribonucleotides. Thus, the use of inorganic surfaces such as minerals to stabilize its structure is highly desired before considering condensation reactions. In a previous paper,⁴³ we showed that melting D-ribose around 90 °C induces chemical heterogeneity and that its stability was below 150 °C. We also showed adsorbed ribose into silica did not present any melting transition and was stable up to 150 °C during the analysis time, at which point ring opening was observed associated with minor oxidation to lactone. In the present study, ribose adsorbed into mordenite (R/MOR sample dried at 50 °C) presented similar thermal behavior to that adsorbed into silica with a three-step weight loss in the entire temperature region (see Fig. 6). However, an improvement of ribose stability can be noticed at 217 °C with an endothermic event between 180 °C and 250 °C mainly associated with ribose polymerization with water elimination. This way, it seems mordenite stabilizes ribose up to 180 °C as an effect of confinement. Once the experiments were run under an inert atmosphere (Ar), a temperature of 150 °C



Fig. 6 (a) TGA and (b) DTG curves for bulk D-ribose (R) and mordenite samples containing sodium (Na/MOR) and/or ribose (R-MOR-50).

seems quite safe to run condensation reactions experiments into MOR and avoid ribose oxidation.

On the route to evaluate D-ribose adsorption into mordenite, ¹³C CP-MAS NMR spectra in the solid-state were recorded for the R/MOR, (R + P_i)/MOR and RA/MOR samples before and after activation (see Fig. 7 and Table 3). The bulk D-ribose spectrum containing α - and β -D-riboenantiomer attributions is shown in Fig. SI-1.†

Data showed that mordenite stabilizes α - and β -p-ribofuranose enantiomers for most of the samples even after thermal activation. The exception was noticed for the RA/MOR-50 sample. Although it presented as 46% and 9% of α - and β -pribofuranose, respectively, 45% of ribose remained as β -p-ribopyranose. This fact may relate to the inaccessibility of p-ribose molecules to the zeolite active sites due to adenine pre-adsorp-



Fig. 7 13 C CP-MAS NMR spectra of mordenite samples with adenine (A) and/or inorganic monophosphates (P_i) and/or D-ribose (R) dried at (a) 50 °C in air or (b) activated at 150 °C under an argon atmosphere.

			Chemical shift (ppm)				
Sample	Form	%Form ^a	C1	C2	C3	C4	C5
Bulk ribose	αp	35	92.3	69.5	68.3	66.3	63.4
	βp	51	94.6	70.8	68.8	68.2	64.4
	αf	14	95.4	72.4	71.4	_	55.7
	βf	_	—	_	_	_	_
R/MOR-50	αp	_	—	_	_	_	_
	βp	_	—	_	_	_	_
	αf	30	95.5	71.7	68.3	84.1	58.6
	βf	70	102.1	71.7	68.3	81.5	63.5
R/MOR-150	αp	_	—	_	_	_	_
	βp	_		_	_	_	_
	αf	31	98.5	71.7	68.5	83.9	63.4
	βf	69	102.9	71.7	68.5	80.0	65.2
$(R + P_i)/MOR-50$	αp	_		_	_	_	_
	βp	_	—	_	_	_	_
	αf	94	95.7	71.2	68.9	82.0	64.5
	βf	06	102.4	71.2	68.9	82.0	64.5
$(R + P_i)/MOR-150$	αp	_		_	_	_	_
	βp	_		_	_	_	_
	αf	43	97.1	71.4	69.0	80.8	64.2
	βf	57	102.4	71.4	69.0	80.8	64.2
RA/MOR-50	αp	_		_	_	_	_
	βp	45	94.8	70.9	68.4	68.4	64.0
	αf	46	95.7	71.8	68.4	82.1	58.6
	βf	09	102.4	72.7	68.4	82.1	64.0
RA/MOR-150	αp	_		_	_	_	_
	βp	_		_	_	_	_
	αf	35	96.3	71.1	68.6	80.8	64.0
	βf	65	102.5	72.3	68.6	80.8	64.0

Table 3 ¹³C CP-MAS NMR data based on the ribose part for R/MOR,

 $(R + P_i)/MOR$ and RA/MOR dried samples before and after thermal acti-

vation at 150 °C

^{*a*} Calculated from integrated area data of the C₁ signals.

tion that makes the isomerization difficult. Nevertheless, it seems the increase of temperature from 50 °C to 150 °C may overcome the diffusion problem into the zeolite channels and lead to ribofuranose species. Dass and coworkers⁴⁴ showed that the high temperature affects the thermodynamic isomerization equilibrium between D-ribofuranose and D-ribopyranose in favor of the β -furanose isoform. Since the R/MOR-50 and R/MOR-150 samples presented a similar ratio for α - and β -furanose isoform, it seems the mordenite catalyzes its isomerization to a thermodynamic equilibrium achieved even at 50 °C. Thus, the rise of temperature to 150 °C for RA/MOR and (R + P_i)/MOR samples may favor β -furanose isomerization due to improved diffusion.

After the drying process and thermal activation, other carbon environments appeared in the ¹³C NMR spectra of bulk p-ribose. The new signals at 105.2 ppm for R/MOR-50 and R/MOR-150 samples and at 100.5 ppm for the R/MOR-150 sample may also be related to the C₁ position of β -p-xylulofuranose and α -p-arabinofuranose, respectively, due to ribose isomerization.⁴⁵ The reaction network for the formation of isomeric pentoses from p-ribose into mordenite may follow the pathway described in Scheme 1.^{44,45} Each acyclic isomer may present a cyclization reaction to the α - and β -p-forms. On its turn, rotomutation equilibria may occur between its anomeric



Scheme 1 Possible D-ribose isomerization network into mordenite. According to ref. 44 and 45.

pairs. Mechanistic effects and intermediate formation are beyond the objectives of the present study.

Spectra of samples after activation showed new signals around 182 ppm, 152 ppm, 129 ppm, and 114 ppm compared to dried samples. They are associated with ribose dehydration to furfural at 26%, 24% and 13% for RA/MOR-150, $(R + P_i)/$ MOR-150 and R/MOR-150 samples (calculated from C=O and C1 signals). Contrary to what TGA results have shown, mordenite seems to start degrading ribose even at 150 °C, though to a lesser extent than in presence of phosphates or adenine. Additionally, the signals at 145 ppm, 152 ppm, 76 ppm, 65 ppm and 72 ppm may also be assigned to C1–C5 carbon environments for 1-pentene-1,2-diol, respectively. It seems to make evident the furfural production through 1,2-enediol as the intermediate. Scheme 2 shows a possible p-ribose dehydration network to furfural into mordenite.^{45–48} The signals from 10 to 50 ppm may be associated with aliphatic compounds such as solid humins and may highlight furfural decomposition as side products.

Phosphorylation reactions. The influence of phosphorylation reactions into mordenite followed by ³¹P NMR is shown in Fig. 8. The dried P_i/MOR-50 sample presented a spectrum suggesting different phosphate environments: one Q⁰, four Q¹, and four Q². The Q⁰ peak at +0.3 ppm (*ca.* 5.2%) is characteristic of orthophosphate groups (monomeric phosphates) adsorbed into the mordenite surface. The signal of solid KH₂PO₄ at +4.1 ppm (ref. 49) was not observed here. Q¹ signals at -3.2 (4.1%), -6.3 (10.6%), -8.8 (12.7%) and -11.4 (16%) ppm are related to terminal phosphates in linear phosphates adsorbed into different MOR adsorption sites. The peak at -8.8 ppm indicated a significant amount of phosphate has dimerized to diphosphate (PP_i). Q² signals at -13.8 (16.8%),



Scheme 2 Possible D-ribose dehydration pathways to furfural into mordenite. According to ref. 45-48.



Fig. 8 Solid-state ^{31}P NMR spectra of mordenite samples with inorganic monophosphates (P_i) and/or D-ribose (R) dried at 50 °C in air and/or activated at 150 °C under an argon atmosphere.

-16.1 (13.6%), -18.4 (12.8%) and -21.5 (8.2%) ppm are attributed to middle groups from long chain linear or non-linear condensed phosphates such as polyphosphates or cyclic metaphosphates. These results indicated that concentration and drying conditions are sufficient to prepare condensed phosphate precursors from activated phosphoric anhydride (P–O–P) groups by P-OH condensation.⁴⁹ Indeed, the high hydration enthalpy of the zeolite allied to the dry confinement environment may provide the driving force for redox and condensation reactions even at low temperatures.

After thermal activation at 150 °C, deconvolution data for the P_i/MOR -150 sample suggested some ³¹P signals: one Q⁰, three Q¹, and six Q² (see Table SI-1†). The component at -0.3 ppm (*ca.* 4.1%) lies in the same range as monomeric phosphates with a broader signal than the dried sample that corresponds to a nonmobile species interacting with MOR surface groups. In comparison with the P_i/MOR -50 sample, data showed terminal phosphates in polyphosphate contributions decreased from 43% to 25% Q¹ while middle phosphates in polyphosphates increased from 51% to 71% (Fig. 9). The results suggested MOR may favor polymerization to a higher condensed phosphate yields at 150 °C.

Only four ³¹P environments were attributed to a dried sample containing ribose and P_i into mordenite (R/MOR-50): one Q⁰ and three Q¹ (see Table SI-1†). The contribution at +0.7 ppm (55.6%) is much broader than the P_i/MOR-50 sample and is also attributed to monophosphates species adsorbed into MOR. The absence of a peak from 2.9 to 3.9 ppm suggests that the phosphoryl group on the 5' position of ribose (R5P) is not presented or is not detectable.²⁴ Thus, the signals at -6.1 (18.8%), -6.3 (17.9%) and -9.6 (7.6%) are strictly related to



Fig. 9 Phosphate distribution calculated from Q^0 , Q^1 and Q^2 area of ³¹P MAS-NMR spectra of mordenite samples with inorganic monophosphates (P_i) and/or D-ribose (R) dried at 50 °C in air and/or activated at 150 °C under an argon atmosphere.

terminal phosphates in diphosphates. Furthermore, the peaks at -6.3 and -9.6 ppm suggested most mobile phosphate species than the P_i/MOR-50 sample. The higher content of monophosphates indicates condensation reactions are unfavored at 70 °C with uncompleted phosphate polymerization. Additionally, the absence of Q² signals suggested the presence of ribose into mordenite, limiting the condensation to diphosphates. This fact may relate to the following reasons: (i) the diffusion problem of phosphate species into the zeolite channels; (ii) the non-accessibility of phosphate groups to some adsorption/catalytic sites of zeolite pre-covered by ribose; or (iii) even to trapped water molecules into zeolite channels that make the environment thermodynamically unfavorable to phosphate condensation reactions or to ribose phosphorylation.

One should further note that these problems evoked before seemed overtaken after thermal activation. The (R + Pi)/ MOR-150 sample presented two Q^0 , three Q^1 , and four Q^2 phosphor environments. One new peak at +2.9 ppm (2.5%) appears and falls in the chemical shift range of monophosphates. This signal suggests the presence of the phosphoryl group on the 5' position of ribose,²⁴ as in R5'P or PRPP (phosphoribosyl-pyrophosphate). Furthermore, Q¹ contributions at -4.7 ppm (6.7%) and -10.2 ppm (13.1%) highlight phosphorylated ribose at $-1\alpha P$ and $1-\beta P$ positions of PRPP. This molecule is considered as an activated intermediate in the abiotic synthesis of nucleotides.²⁴ The peak at -4.7 ppm may also be attributed to di-phosphorylated ribose (RPP). The last Q¹ signal at -7.6 ppm (17.2%) is suggestive of adsorbed diphosphates into the zeolite surface. Signals around -13.3 to -27.9 ppm were assigned to middle groups from long chain linear or non-linear condensed phosphates.

Concluding remarks

The synthetic counterpart of a natural zeolite was used as a mineral substrate to adsorb canonical nucleobases such as adenine. From the adsorption study, the optimal conditions to concentrate adenine into MOR were pH 3, contact time of 5 min, and 1300 mg L^{-1} of C_i . Although the pseudo-second order model better described adsorption kinetic data, the intra-particle diffusion model may also play some role in the sorption rate mechanism. From equilibrium isotherms, the maximum adenine uptake was 65.6 µg g⁻¹. Adenine-containing samples were subjected to posterior co-adsorption with D-ribose (1 : 1 molar) and/or monophosphate (1 : 2 molar) and thermal activation at 150 °C.

The samples were characterized by FTIR and TGA analysis as well as ^{31}P and ^{13}C CP-MAS and solid-state NMR. Adsorption and FTIR data suggested electrostatic interactions as the main mechanism between adenine and mordenite. TGA data showed mordenite stabilized D-ribose until 150 °C. ^{13}C CP-MAS NMR analysis showed stabilized α - and β -D-ribofuranose enantiomers for most of all adsorbed/co-adsorbed samples in mordenite pores, even after thermal activation. Although the stabilization of β -D-ribofuranose is evidenced, the analysis of ribonucleosides was not conclusive and additional characterization studies are necessary to figure out glycosylation reactions.

Regarding phosphorylation, condensation reactions were followed by ³¹P MAS NMR, showing that the mordenite promoted the formation of condensed phosphates even at 50 °C. The data also showed ribose presence may limit condensed reactions to diphosphates. After thermal activation, phosphorylation produces long chains of linear or non-linear condensed phosphates. Phosphorylated ribose is suggested by the presence of the phosphoryl group assigned to the 5' position of ribose (R5'P) or phosphoribosyl-pyrophosphate (PRPP) at 150 °C. These new results suggest potential chemical pathways to abiotic synthesis in the field of origins of life.

Experimental

Materials and methods

Adenine (6-aminopurine; $\geq 99\%$; CAS 73-24-5), ribose (D-(-)-ribose; $\geq 98\%$; CAS 50-69-1), and potassium phosphate monobasic (KH₂PO₄; $\geq 99\%$; CAS 7778-77-0) were purchased from Sigma-Aldrich and were applied without any previous purification. Parent Na-mordenite (Si/Al = 6; S_{BET} = 367 m² g⁻¹) was purchased from Zeolyst.

Adenine pre-adsorption into MOR

Initially, the influence of pH on the sorption of the adenine (A) was investigated by using the batch method as described in Ref. 11. A quantity of 20 mg of mordenite was placed in an Eppendorf tube (2.0 mL) and then 1.0 mL of deionized water was added with or without 720 μ g mL⁻¹ of adenine. For standardization, the solutions were adjusted to pH 2–10 by adding 0.5 mol L⁻¹ HCl or NaOH and were kept under magnetic stirring for 24 h at room temperature. The system was then centrifuged, the solid was separated, and the supernatant was collected to determine the residual adenine concentration. Absorbance (Abs) was determined using an Ocean Optics

UV-Vis spectrophotometer set to 260 nm wavelength. The adsorbed amount of the adenine in the zeolite was calculated from eqn (1).

$$C_{\text{adsorbed}\,(\mu g \, g^{-1})} = (C_{\text{initial}} - C_{\text{sample}}),$$
where : $C_{\text{sample}} = \left[C_{\text{i}} \left(\frac{Abs_{\text{sample}}}{Abs_{\text{initial}}} \right) \right]$
(1)

The same procedure was adopted for the kinetic sorption study with contact times until 30 min at the optimal pH as determined in the previous stage. For the equilibrium isotherm, concentrations were varied from 20 to 2000 μ g mL⁻¹ of adenine, under optimum pH and contact time conditions. The solid sample obtained from optimized conditions was dried at 50 °C for 24 h and then designated A/MOR-50.

Ribose and monophosphate co-adsorption into MOR

According to the results of the adsorption study for adenine, 65.6 μ g of adenine is adsorbed per 1 mg of MOR at pH 3. Thus, the next steps concerning D-ribose (R) and monophosphate (P_i) co-adsorption were designed to follow 1 and 2 molar equivalents of the adenine content, respectively.

Initially, 1.5 mL of a solution containing D-ribose (R) or inorganic monophosphate (P_i) was added to 500 mg of MOR. The suspensions were dried at 50 °C in an oven under room humidity for 24 h and then were stored in a desiccator containing silica before analysis. The samples were designated R/ MOR-50 and P_i /MOR-50, respectively.

A similar procedure using 500 mg of the A/MOR-50 preadsorbed sample was taken to prepare the RA/MOR-50 sample. The co-adsorption method was applied in the preparation of the $(R + P_i)A/MOR-50$ sample by impregnation of both ribose and monophosphate into 500 mg of the A/MOR-50 preadsorbed sample.

Thermal activation

The dried samples were subjected to thermal activation using a silicone oil bath at 150 °C for 2 h under an argon atmosphere and then renamed to A/MOR-150, R/MOR-150, Pi/MOR-150, RA/MOR-150 and RPi/MOR-150.

Characterization

FTIR. Infrared analyses were performed on an Agilent Cary 630 FTIR spectrometer using the Attenuated Total Reflectance (ATR) mode with a diamond crystal detector and the spectral resolution of 2 cm⁻¹ and 30 accumulation scans. The spectra were recorded using Microlab FTIR Software (Agilent Technologies) between 4000 and 600 cm⁻¹.

Thermogravimetric analyses. TGA analyses were performed using a TA Instrument SDT Q600 analyzer with a heating rate of 5 °C min⁻¹ from 25 °C to 900 °C, under a dry airflow of 10 mL min⁻¹ using an alumina pan.

³¹**P** MAS-NMR. Solid-state ³¹**P** MAS-NMR spectra were recorded on a Bruker Avance 500 spectrometer using a 4 mm MAS probe and a spinning rate of 14 kHz with a field of 11.7 T at room temperature. The major experimental parameters

Inorganic Chemistry Frontiers

were: simple 1-pulse $\pi/2$ with a proton decoupling sequence with a pulse length of 5.20 µs, recycle delay of 20 s, and acquisition time of 40 ms. For spectral reference, an external standard of phosphoric acid was set to 0 ppm.

¹³C CP-MAS-NMR. Solid-state ¹³C spectra were obtained on a Bruker Advance 500 spectrometer operating at a frequency of 125.88 MHz. Cross-polarization of protons (CP-MAS) with spinal64 proton decoupling was applied with a contact time of 1 ms. The samples were rotated at the magic angle at a frequency of 14 kHz. The pulse length of ¹H was 3.40 µs (close to $\pi/2$) with a recycle delay of 1 s and acquisition time of 40 ms.

Author contributions

Francisco Rodrigues: conceptualization, methodology, validation, formal analysis, investigation, data curation, writing – original draft. Thomas Georgelin: conceptualization, validation, investigation. Baptiste Rigaud: investigation, formal analysis. Guanzheng Zhuang: validation, software. Maria Gardênnia da Fonseca: funding acquisition, validation, investigation. Valentin Valtchev: validation, investigation. Maguy Jaber: conceptualization, supervision, funding acquisition, project administration.

Conflicts of interest

There are no conflicts of interest to declare.

Acknowledgements

Dr F. Rodrigues thanks the Brazilian Government for the postdoc scholarship under the CAPES/COFECUB program (Project No. 835/15). The authors thank Île-de-France and CNRS for funding.

References

- 1 N. Kitadai and S. Maruyama, Origins of building blocks of life: A review, *Geosci. Front.*, 2018, **9**, 1117–1153.
- 2 S. R. Vogel, C. Deck and C. Richert, Accelerating chemical replication steps of RNA involving activated ribonucleotides and downstream-binding elements, *Chem. Commun.*, 2005, 4922.
- 3 M. W. Powner, B. Gerland and J. D. Sutherland, Synthesis of activated pyrimidine ribonucleotides in prebiotically plausible conditions, *Nature*, 2009, **459**, 239–242.
- 4 R. Yi, Y. Hongo and A. C. Fahrenbach, Synthesis of imidazole-activated ribonucleotides using cyanogen chloride, *Chem. Commun.*, 2018, 54, 511–514.
- 5 N. Zhang, S. Zhang and J. W. Szostak, Activated Ribonucleotides Undergo a Sugar Pucker Switch upon Binding to a Single-Stranded RNA Template, *J. Am. Chem. Soc.*, 2012, **134**, 3691–3694.

- 6 M. Yadav, R. Kumar and R. Krishnamurthy, Chemistry of Abiotic Nucleotide Synthesis, *Chem. Rev.*, 2020, **120**, 4766–4805.
- 7 J. D. Bernal, The Physical Basis of Life, *Proc. Phys. Soc., London, Sect. A*, 1949, **62**, 537.
- 8 A. I. Oparin, *The Origin of Life*, Dover Pubns, New York, 1953.
- 9 D. A. M. Zaia, A review of adsorption of amino acids on minerals: Was it important for origin of life?, *Amino Acids*, 2004, 27, 113–118.
- V. Erastova, M. T. Degiacomi, D. G. Fraser and H. C. Greenwell, Mineral surface chemistry control for origin of prebiotic peptides, *Nat. Commun.*, 2017, 8, 2033.
- 11 P. R. Anizelli, J. P. T. Baú, D. F. Valezi, L. C. Canton, C. E. A. Carneiro, E. Di Mauro, A. C. S. da Costa, D. Galante, A. H. Braga, F. Rodrigues, J. Coronas, C. Casado-Coterillo, C. T. B. V. Zaia and D. A. M. Zaia, Adenine interaction with and adsorption on Fe-ZSM-5 zeolites: A prebiotic chemistry study using different techniques, *Microporous Mesoporous Mater.*, 2016, 226, 493–504.
- 12 W. H. Yu, N. Li, D. S. Tong, C. H. Zhou, C. X. (Cynthia) Lin and C. Y. Xu, Adsorption of proteins and nucleic acids on clay minerals and their interactions: A review, *Appl. Clay Sci.*, 2013, **80–81**, 443–452.
- 13 D. Winter and G. Zubay, Binding of adenine and adeninerelated compounds to the clay montmorillonite and the mineral hydroxylapatite, *Origins Life Evol. Biospheres*, 1995, 25, 61–81.
- 14 U. Pedreira-Segade, C. Feuillie, M. Pelletier, L. J. Michot and I. Daniel, Adsorption of nucleotides onto ferromagnesian phyllosilicates: Significance for the origin of life, *Geochim. Cosmochim. Acta*, 2016, 176, 81–95.
- 15 K. Fisher and K. Huddersman, Separation of nucleosides and nucleotides using cation-exchanged zeolites, *New J. Chem.*, 2002, **26**, 1698–1701.
- 16 A. Gujjari, B. V. Rodriguez, J. Pescador, C. Maeder, G. W. Beall and L. K. Lewis, Factors affecting the association of single- and double-stranded RNAs with montmorillonite nanoclays, *Int. J. Biol. Macromol.*, 2018, **109**, 551–559.
- 17 E. Biondi, Y. Furukawa, J. Kawai and S. A. Benner, Adsorption of RNA on mineral surfaces and mineral precipitates, *Beilstein J. Org. Chem.*, 2017, **13**, 393–404.
- 18 J. H. Cleaves II, E. Crapster-Pregont, C. M. Jonsson, C. L. Jonsson, D. A. Sverjensky and R. A. Hazen, The adsorption of short single-stranded DNA oligomers to mineral surfaces, *Chemosphere*, 2011, 83, 1560–1567.
- 19 D. H. Taylor, The Adsorption of Yeast RNA by Allophane, *Clays Clay Miner.*, 1979, **27**, 261–268.
- 20 M. M. Hanczyc, S. M. Fujikawa and J. W. Szostak, Experimental Models of Primitive Cellular Compartments: Encapsulation, Growth, and Division, *Science*, 2003, **302**, 618–622.
- 21 T. L. Porter, M. P. Eastman, R. Whitehorse, E. Bain and K. Manygoats, The interaction of biological molecules with clay minerals: A scanning force microscopy study, *Scanning*, 2006, **22**, 1–5.

- 22 B. T. Burcar, M. Jawed, H. Shah and L. B. McGown, In Situ Imidazole Activation of Ribonucleotides for Abiotic RNA Oligomerization Reactions, *Origins Life Evol. Biospheres*, 2015, **45**, 31–40.
- 23 G. Ertem and J. P. Ferris, Template-Directed Synthesis Using the Heterogeneous Templates Produced by Montmorillonite Catalysis. A Possible Bridge Between the Prebiotic and RNA Worlds, *J. Am. Chem. Soc.*, 1997, **119**, 7197–7201.
- 24 M. Akouche, M. Jaber, M.-C. Maurel, J.-F. Lambert and T. Georgelin, Phosphoribosyl Pyrophosphate: A Molecular Vestige of the Origin of Life on Minerals, *Angew. Chem., Int. Ed.*, 2017, **56**, 7920–7923.
- 25 F. Rodrigues, T. Georgelin, G. Gabant, B. Rigaud, F. Gaslain, G. Zhuang, M. G. Fonseca, V. Valtchev, D. Touboul and M. Jaber, Confinement and Time Immemorial: Prebiotic Synthesis of Nucleotides on a Porous Mineral Nanoreactor, *J. Phys. Chem. Lett.*, 2019, **10**, 4192–4196.
- 26 W. A. Deer, R. A. Howie, W. S. Wise and J. Zussman, Rock-Forming Minerals - Framework silicates: silica minerals, feldspathoids and the zeolites, Geological Society, London, 2nd edn, vol. 4B, 2004.
- 27 J. V. Smith, Biochemical evolution. I. Polymerization on internal, organophilic silica surfaces of dealuminated zeolites and feldspars, *Proc. Natl. Acad. Sci. U. S. A.*, 1998, 95, 3370–3375.
- 28 V. Verdolino, R. Cammi, B. H. Munk and H. B. Schlegel, Calculation of p K a Values of Nucleobases and the Guanine Oxidation Products Guanidinohydantoin and Spiroiminodihydantoin using Density Functional Theory and a Polarizable Continuum Model, *J. Phys. Chem. B*, 2008, **112**, 16860–16873.
- 29 C. Marian, D. Nolting and R. Weinkauf, The electronic spectrum of protonated adenine: Theory and experiment, *Phys. Chem. Chem. Phys.*, 2005, 7, 3306.
- 30 F. Turecček and X. Chen, Protonated adenine: Tautomers, solvated clusters, and dissociation mechanisms, *J. Am. Soc. Mass Spectrom.*, 2005, 16, 1713–1726.
- 31 P. O. P. Ts'o, in *Basic Principles in Nucleic Acid Chemistry, Bases, nucleotides, and nucleotides,* Academic Press, New York, 1974, ch. 6, pp. 453–584.
- 32 H. A. Sober, G. D. Fasman and C. R. Company, *Handbook of biochemistry and molecular biology*, CRC Press, Cleveland, Ohio, 1968.
- 33 D. M. Close, Calculated p K a 's of the DNA Base Radical Ions, *J. Phys. Chem. A*, 2013, **117**, 473–480.
- 34 A. R. Klein, S. E. Bone, E. Bakker, Z. Chang and L. Aristilde, Abiotic phosphorus recycling from adsorbed ribonucleotides on a ferrihydrite-type mineral: Probing solution and surface species, *J. Colloid Interface Sci.*, 2019, 547, 171–182.
- 35 J. Kundu, O. Neumann, B. G. Janesko, D. Zhang, S. Lal, A. Barhoumi, G. E. Scuseria and N. J. Halas, Adenine– and

Adenosine Monophosphate (AMP)–Gold Binding Interactions Studied by Surface-Enhanced Raman and Infrared Spectroscopies, *J. Phys. Chem. C*, 2009, **113**, 14390– 14397.

- 36 M. Rueda, F. Prieto, A. Rodes and J. M. Delgado, In situ infrared study of adenine adsorption on gold electrodes in acid media, *Electrochim. Acta*, 2012, **82**, 534–542.
- 37 R. Krishnamurthy, Role of pKa of Nucleobases in the Origins of Chemical Evolution, *Acc. Chem. Res.*, 2012, 45, 2035–2044.
- 38 B. K. Lagergren and S. Svenska, On the theory of so-called adsorption of materials, *R. Swed. Acad. Sci. Doc.*, 1898, 24, 1–13.
- 39 Y. S. Ho and G. McKay, Pseudo-second order model for sorption processes, *Process Biochem.*, 1999, 34, 451– 465.
- 40 I. Langmuir, The adsorption of gases on plane surfaces of glass, mica and platinum, *J. Am. Chem. Soc.*, 1918, **40**, 1361–1403.
- 41 H. M. F. Freundlich, Over the Adsorption in Solution, *J. Phys. Chem.*, 1906, 57, 385–471.
- 42 M. I. Temkin and V. Pyzhev, Kinetics of ammonia synthesis on promoted iron catalyst, *Acta USSR*, 1940, **12**, 327–356.
- 43 M. Akouche, M. Jaber, E. Zins, M. Maurel, J. Lambert and T. Georgelin, Thermal Behavior of d -Ribose Adsorbed on Silica: Effect of Inorganic Salt Coadsorption and Significance for Prebiotic Chemistry, *Chem. – Eur. J.*, 2016, 22, 15834–15846.
- 44 A. V. Dass, T. Georgelin, F. Westall, F. Foucher, P. De Los Rios, D. M. Busiello, S. Liang and F. Piazza, Equilibrium and non-equilibrium furanose selection in the ribose isomerisation network, *Nat. Commun.*, 2021, **12**, 2749.
- 45 I. Delidovich, M. S. Gyngazova, N. Sánchez-Bastardo, J. P. Wohland, C. Hoppe and P. Drabo, Production of ketopentoses via isomerization of aldo-pentoses catalyzed by phosphates and recovery of products by anionic extraction, *Green Chem.*, 2018, **20**, 724–734.
- 46 H. Li, Q. Zhang, P. Bhadury and S. Yang, Furan-type Compounds from Carbohydrates via Heterogeneous Catalysis, *Curr. Org. Chem.*, 2014, **18**, 547–597.
- 47 I. Agirrezabal-Telleria, C. García-Sancho, P. Maireles-Torres and P. L. Arias, Dehydration of xylose to furfural using a Lewis or Brønsted acid catalyst and N2 stripping, *Chin. J. Catal.*, 2013, **34**, 1402–1406.
- 48 T. Istasse and A. Richel, Mechanistic aspects of saccharide dehydration to furan derivatives for reaction media design, *RSC Adv.*, 2020, **10**, 23720–23742.
- 49 T. Georgelin, M. Jaber, T. Onfroy, A.-A. Hargrove, F. Costa-Torro and J.-F. Lambert, Inorganic Phosphate and Nucleotides on Silica Surface: Condensation, Dismutation, and Phosphorylation, *J. Phys. Chem. C*, 2013, **117**, 12579– 12590.