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PREBIOTIC PHOSPHORAMIDATION OF NUCLEOBASES BY Mg^{2+}-TRIGGERED DECYCLIZATION OF TRIMETAPHOSPHATE

BY

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ABSTRACT

Prebiotic phosphoramidation of nucleobases by Mg^{2+}-triggered decyclization of trimetaphosphate. – Nucleobases can be phosphoramide-bonded on trimetaphosphate decyclized by the nucleophilic attack of their reversibly tautomerized units in stringent synergy with Mg^{2+} asymmetrically shielding the repellent OH^- charges of trimetaphosphate. The produced linear triphosphates are thermopolymerizable, after splicing, into polybasediphosphates.

Key-words: phosphoramidation, nucleobases, trimetaphosphate, decyclization.

INTRODUCTION

In the realm of our interest in the prebiotic origin of the genetic code, we have postulated the prenucleic role of phosphoramidic (N~P) bonding of nucleobases recognizing amino acids of primal peptides (TURIAN, 1996-2000). This has incited us to experimentally check our hypothesis by ^{31}P NMR identification of decyclized products of trimetaphosphate (TMP), essentially linear triphosphate (TP), presumably freed by the nucleophilic attack of thereby P~N bonded nucleobases (TURIAN *et al.*, 1999). Such decyclizations were obtained in 2,5 mM Mg^{2+} – containing mineral water and found to be enhanced by complements of 5mM Mg^{2+} ions. It was therefore interesting to further study the possible stringency of Mg^{2+} ions in this decycling process by incubation of the mixture in plain H_2O as solvent enriched or not (controls) in Mg^{2+} ions.

MATERIALS AND METHODS

We have followed the recently described procedure (TURIAN *et al.*, 1999), but with the major change to 2 ml distilled H_2O (pH adjusted to 8.0 with molar NaOH) instead of mineral water (pH 7.7) as solvent containing 10 (5) mM of trimetaphosphate (TMP Sigma, grade III) enriched or not with 5 (10) mM of $MgCl_2.6H_2O$. The solutions (2 ml)

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were incubated in the presence of the chosen nucleobase (5 mM adenine or cytosine, Sigma products), from 18 to 23 days in shaken Pyrex capped miniflasks at 25°C.

The amounts of triphosphate (TP) decyclized from TMP have been compared by the intensity of their doublet peaks determined by ^{31}P-NMR at 81 MHz on a AC200F Bruker NMR spectrometer using H_3PO_4 as an external reference. These measures have been quantified by the increase of the ^{31}P NMR signal of TP (at –6.0 ppm according to van WAZER *et al.* (1956), CALLIS *et al.* (1957) et VOGEL (1984)).

To ascertain the amidation of one of the phosphates of linear TP, we have thermopolymerized (120° C, 1h) the incubated mixtures of presumed polyphosphates and centrifuged their white precipitates, washed with slightly alkaline (pH 8) H_2O to insure the robustness of the P~N bond and lyophilized into powder (1) checked for its polyphosphate nature by metachromatic staining with toluidine blue (BRACHET, 1956, KORNBERG *et al.*, 1999) and (2) tested for putative nucleobase bonding by the UV (254 mμ) quenching of the blue fluorescence of filter paper (SCHLEICHER & SCHÜLL) supports at the level of spots of wetted (distilled H_2O pH 8) powder .

RESULTS AND DISCUSSION

Decyclization of TMP by hydrolytic opening of one of its phosphorus anhydride bonds (P – O – P – ...) into a linear TP did not occur in plain distilled H_2O (controls Fig. 1a) but required the addition of 5 mM Mg^{2+} as evidenced by the NMR detection of the double peak at ~ -6 ppm characteristic of TP (Figs 1b and 2a).

Nucleobase (adenine or cytosine) alone could not decyclize TMP into linear TP but only hydrolytically split it into traces of pyrophosphate signaled at ~ -8 ppm (Fig. 1d). Both required complementation by Mg^{2+} ions while overpowering the efficiency of these ions alone (Figs 1c and 2b).

Theoretically, the stringent role of Mg^{2+} in the process of TMP decyclization could be explained by their asymmetric shielding of the OH^- charges of the 3P atoms (Fig. 3a): the two positive charges of one Mg^{2+} would balance the OH^- charges of two P atoms while a second Mg^{2+} ion could only half devote one of its 2 positive charges to balance the charge of the third P, keeping its second positive charge free to ionize H_2O to H^+ + OH^-. This would thus provide an hydroxyl nucleophile (see WESTHEIMER, 1987) as "opener" of the cyclic bond of TMP while maintaining the OH^- group completing the fifth valence of the P atom at the tip of the now linear triphosphate (Fig. 3a). The additional presence of a tautomerized nucleobase endowed with an "aggressive" nucleophilic N group would overpower that of the nucleophile OH^- of H_2O while benefiting of the Mg^{2+} shield (Fig. 3b). This protonic tautomerization of the nucleobases by the low pH locally produced by H^+ ions from ionized H_2O is thus a stringent condition for the transition of the NH group (1' in pyrimidines, 9' in purines) to the NH^+ provider of the pair of free electrons nucleophilically attacking one of the electrophilic P atoms of TMP.

The N~P bonding of a nucleobase on decyclized TMP requiring the prototropic tautomerization of the base is only the initial step of the process. It must then be secondly enforced by an alkaline (pH>4) – induced tautomeric "back-shift" of its double bond from

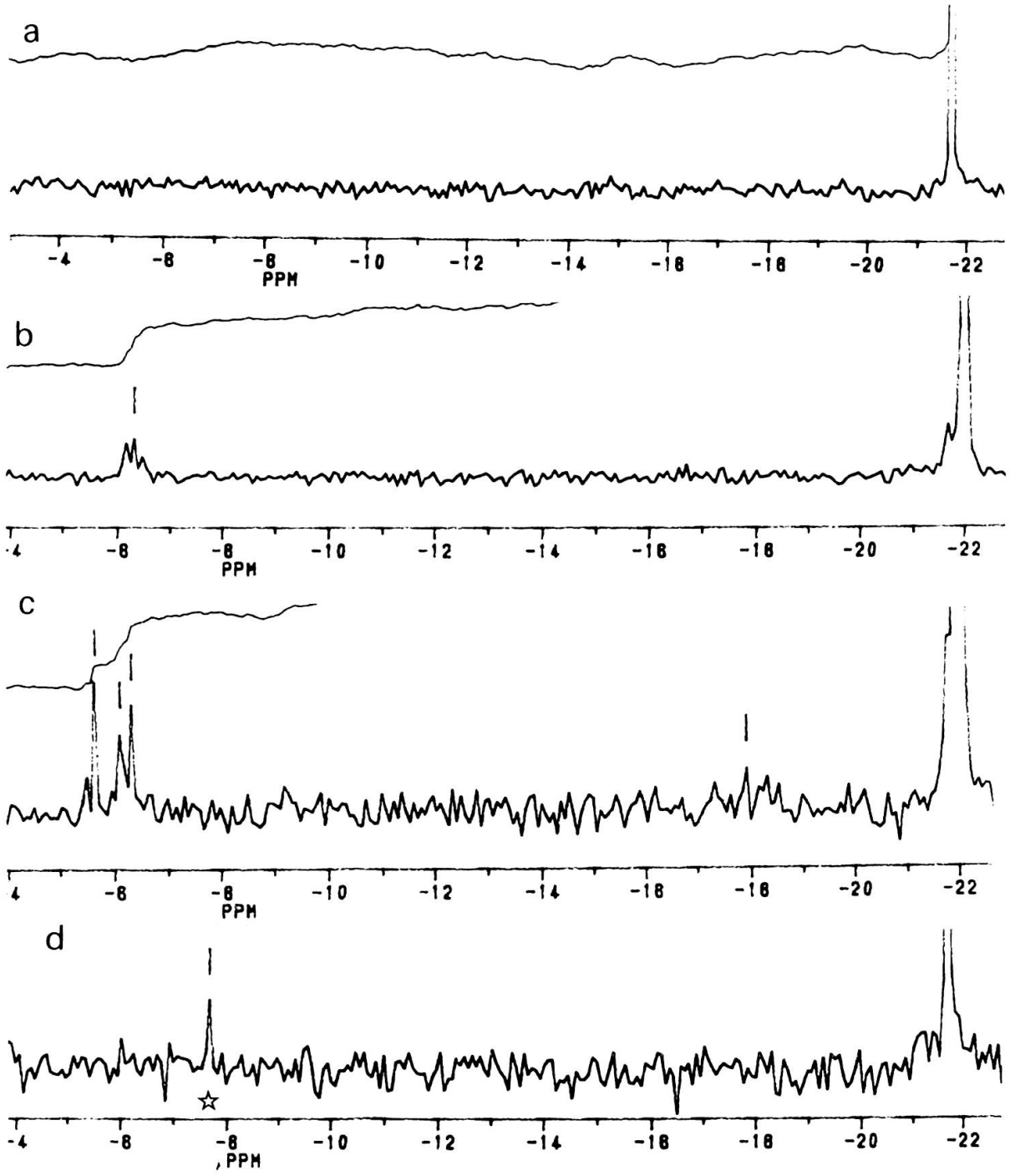

FIG. 1.

^{31}P – NMR spectra from 18-day incubation mixtures compared for doublet signals at ~ -6 ppm (at -7.8 for the pyrophosphate singulet*) of triphosphate (TP) decyclized from a trimetaphosphate (TMP): a) TMP alone; b) TMP + Mg^{2+}; c) TMP + Mg^{2+} + the nucleobase adenine; d) TMP + nucleobase alone.

the N1 atom to position 6-5 (pyrimidines) or from N9 to N8-7 (purines). Such shifts as proposed by Vogel (1984) for imidazole can thus also concern nucleobases to produce adenyl/cytosine triphosphates.

To experimentally check such locking of the nucleobases on the TPs, we separately thermopolymerized Mg^{2+}-TP and Mg^{2+} nucleobase triphosphates. To confirm their poly-

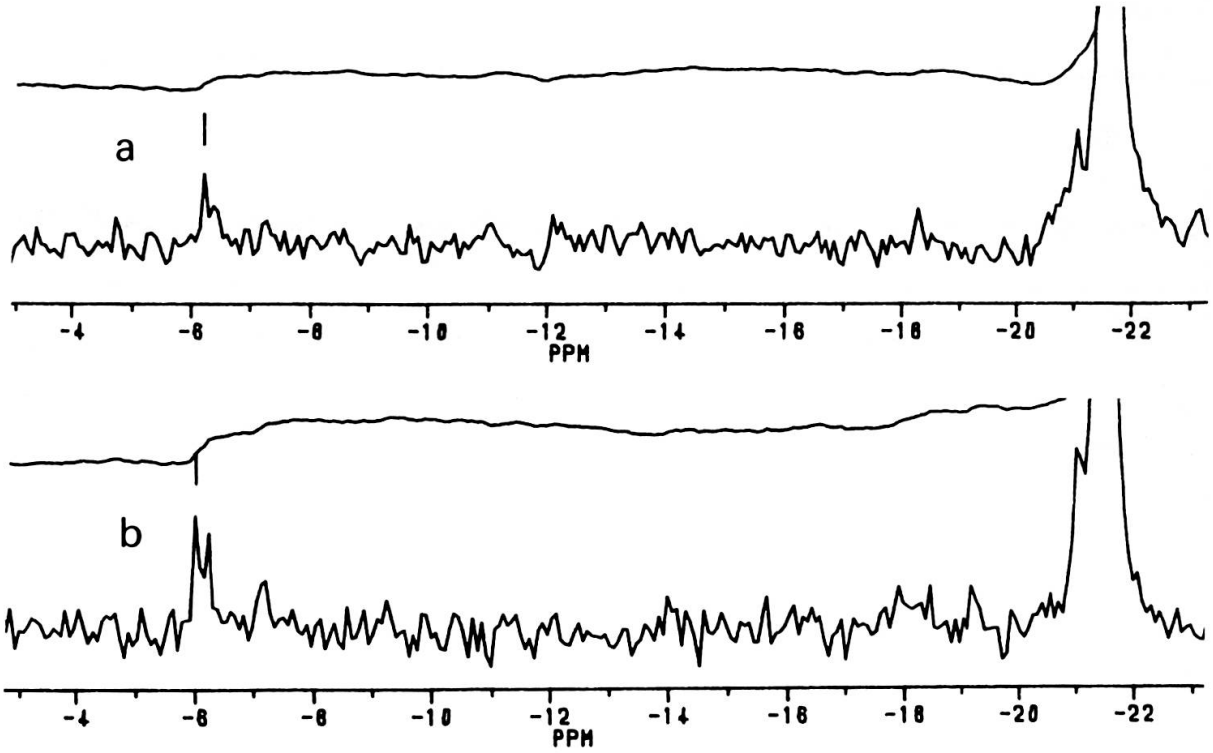

FIG. 2.
Idem to Fig. 1 (23-day incubation): a) TMP + Mg^{2+}; b) TMP + Mg^{2+} + the nucleobase cytosine.

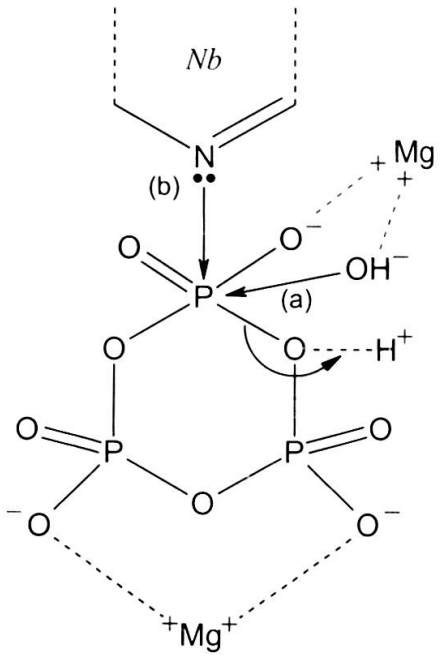

FIG. 3.
Declyclization of TMP permitted by Mg^{2+} shielding of PO^- groups allowing nucleophilic attack of one of the three P-O-P anhydride bonds by (a) Mg^{2+} – ionized H_2O synergetically overpowered by (b) the ≥ HN: group of any P~N bonded nucleobase (*Nb*).

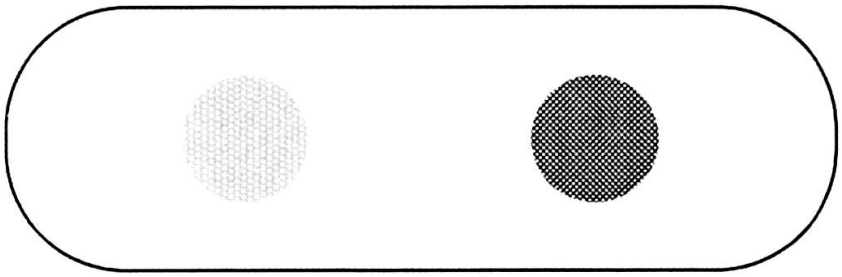

FIG. 4.
Slices of filter paper with its UV excited blue fluorescence: left, shadowed by nude polyphosphate; right, quenched by adenylpolyphosphate.

phosphate nature, we positively tested the switching of their toluidine blue color into pink. In this thermoprocess, the 3P must have been spliced into 2P as revealed by the accumulation of P_{inorg} (signal at ~0 ppm) and PP (signal at -7 – -8 ppm) in the supernatant of the precipitated polyphosphates. Additionally, their adenine N~bonding was ascertained by the UV (254 mμ absorption test) showing a strong quenching of fluorescence restricted to the powder produced from the original nucleobase-TP (Fig. 4).

These preliminary results then lead us to consider the phosphoramide linkage of adenine into adenyldiphosphate and, presumably, of the other nucleobase cytosine on diphosphates as nucleophosphate units of riboseless prenucleic polymers. A sequential bonding of nucleobases lined up on a polyphosphate backbone might well be relevant for pregenetic coding while highlighting Kornberg's (1995) prediction of a vicariant role of polyphosphates in the prebiotic evolution.

RÉSUMÉ

Phosphoramidation prébiotique de nucléobases par décyclisation du trimétaphosphate activée par les ions Mg^{2+}. - Les nucléobases peuvent être phosphoramidoliées sur des trimétaphosphates décyclisés par l'attaque nucléophile de leurs unités réversiblement tautomérisées en synergie obligatoire avec des ions Mg^{2+} protégeant les charges répulsives OH^- des trimétaphosphates. Les triphosphates linéaires produits sont thermopolymérisables, après épissure, en polybasediphosphates.

Mots-clés: phosphoramidation, nucléobases, trimétaphosphate, décyclisation.

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