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FURTHER ^{31}P-NMR EVIDENCE FOR PHOSPHORAMIDE BONDING OF NUCLEOBASES BY MG^{2+} ENHANCED NUCLEOPHILIC ATTACK ON CYCLIC TRIPHOSPHATE

BY

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ABSTRACT

Further ^{31}P-NMR evidence for phosphoramide bonding of nucleobases by Mg^{2+} enhanced nucleophilic attack on cyclic triphosphate. - Mg^{2+} enhanced nucleophilic attack by nitrogen bases, phosphoramido-bonded on the opening cycle of trimetaphosphate detected by ^{31}P-NMR spectral changes, is extended from imidazole to purines and pyrimidines.

Key-words: NMR, Nucleobases, Imidazole, Cyclic and Linear Triphosphates

INTRODUCTION

We have recently interpreted the activation by either imidazolides or nucleobases of the carbodiimide (EDC)-catalyzed condensation of linear triphosphate to the cyclic trimetaphosphate as due to the simultaneous opening of the triphosphate cycle products, thereby displacing the chemical equilibrium in favor of further cyclic condensation (TURIAN *et al.*, 1998).

The opening of the triphosphate cycle was conceivably the result of the well known nucleophilic attack of the >NH containing azole bases on one of the phosphoanhydric bonds of the polyphosphate molecules (RABINOWITZ & HAMPAI, 1985). This proposal has led us to the present attempt to obtain direct evidence of phosphoramidic bonding with the nucleobases, all bearing in their molecule the same reactive >NH group (N1 in pyrimidines, N9 in purines).

Such a possible phosphoramide (N-P) bonding of nucleobases would be most interesting in the perspective that the presumed "prebiotic" synthesis of prenucleic polymers might have first short-circuited that of nucleotidic nucleic acids (TURIAN, 1996-1998).

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MATERIALS AND METHODS

"Prebiotic" chemicals were dissolved in 2 ml San Pellegrino mineral water (SP, Milano, Italy) chosen because of its equilibrated light mineral composition and pH (7.7):

10 (5) mM of trimetaphosphate (Sigma, grade III) was dissolved in this solution naturally containing 2.5 mM of Mg^{2+} or enriched with 5 (10) mM of $MgCl_2.6H_2O$ to benefit of the known increased sensitivity of the phosphorus bonds to the nucleophiles in the presence of cationic metals (CORBRIDGE, 1978). The solutions were incubated in the presence of each nucleobase (Sigma, 5 mM except 0.25 mM for guanine) or imidazole (Fluka, 5 mM) from 11 to 18 days in shaken Pyrex capped miniflasks at 25°C.

The percent trimetaphosphate decyclized to linear triphosphate was determined by ^{31}P-NMR at 81 MHz on a AC200F Bruker NMR spectrometer using H_3PO_4 as an external reference. Ratios of trimetaphosphate (singlet signalled at -21.5 ppm, VOGEL, 1984) and linear triphosphate (2 resonance peaks, doublet + triplet signals, CALLIS *et al.*, 1957; VAN WAZER, 1958) were measured by integration and normalized on the external reference (capillary containing H_3PO_4 solution).

The nucleophilic bases have been imidazole (efficient nucleophile, CHUNG *et al.*, 1971; RABINOWITZ & HAMPAI, 1978), and the imidazole-ring containing purines (adenine and guanine) or the pyrimidines (cytosine and uracil) also bearing the nucleophilic >NH group.

RESULTS AND DISCUSSION

In the low magnesium controls, all nitrogen bases assayed have provoked a moderate opening of the trimetaphosphate (Table Ia, Fig. 1). By contrast, supplementary Mg^{2+}

TABLE I

Decyclization of trimetaphosphate (% - TriMP)

Nitrogen bases	Reaction times 11 d	18 d	Nitrogen bases	Reaction times 11 d	18 d
a) Controls	0*	0**	b) + Mg^{2+}	9	11
Imidazole	12	14	Imidazole	28	**35**
Adenine	8	10	Adenine	19	22
Guanine	5	7	Guanine°	11	15
Cytosine	7	11	Cytosine	15	17
Uracil	9	8	Uracil	25	21

Comparative efficiencies of TriMP (10 mM) decyclization by 5 mM nitrogen bases (imidazole or nucleobases) incubated in mineral water a) native (low Mg^{2+}, controls) or b) enriched in 5 (10) mM Mg^{2+}.

Results calculated from the lowerings of the ratio "height of the ^{31}P NMR peak of the TriMP singlet (-21.5 ppm) / height of the H_3PO_4 capillary reference", expressed as % of such lowerings averaged from at least 2 series of experiments.

*-** TriP cycles of controls have to be considered as 98% intact (Sigma, grade III) even though they and all decycling values presented have undergone additional 1-2% of further spontaneous cycloTriP openings during incubations.

alone produced additional cycle opening as evidenced by a significant decrease of the -21.5 ppm (Table Ib). This decyclization was presumably due to the straight hydrolysis of an anhydride bond of triphosphate as also occurring with ATP (MAHLER & CORDES, 1969) and polyphosphates (KORNBERG, 1995; KORNBERG *et al.*, 1999).

However, in our experimental conditions, the labilization of the phosphoanhydride bonding by Mg^{2+} ions led to the 2 peak linear triphosphate only (Fig. 1A,B,C), contrarily to the splitting into pyrophosphate + Pi reported by SHABAROVA (1970).

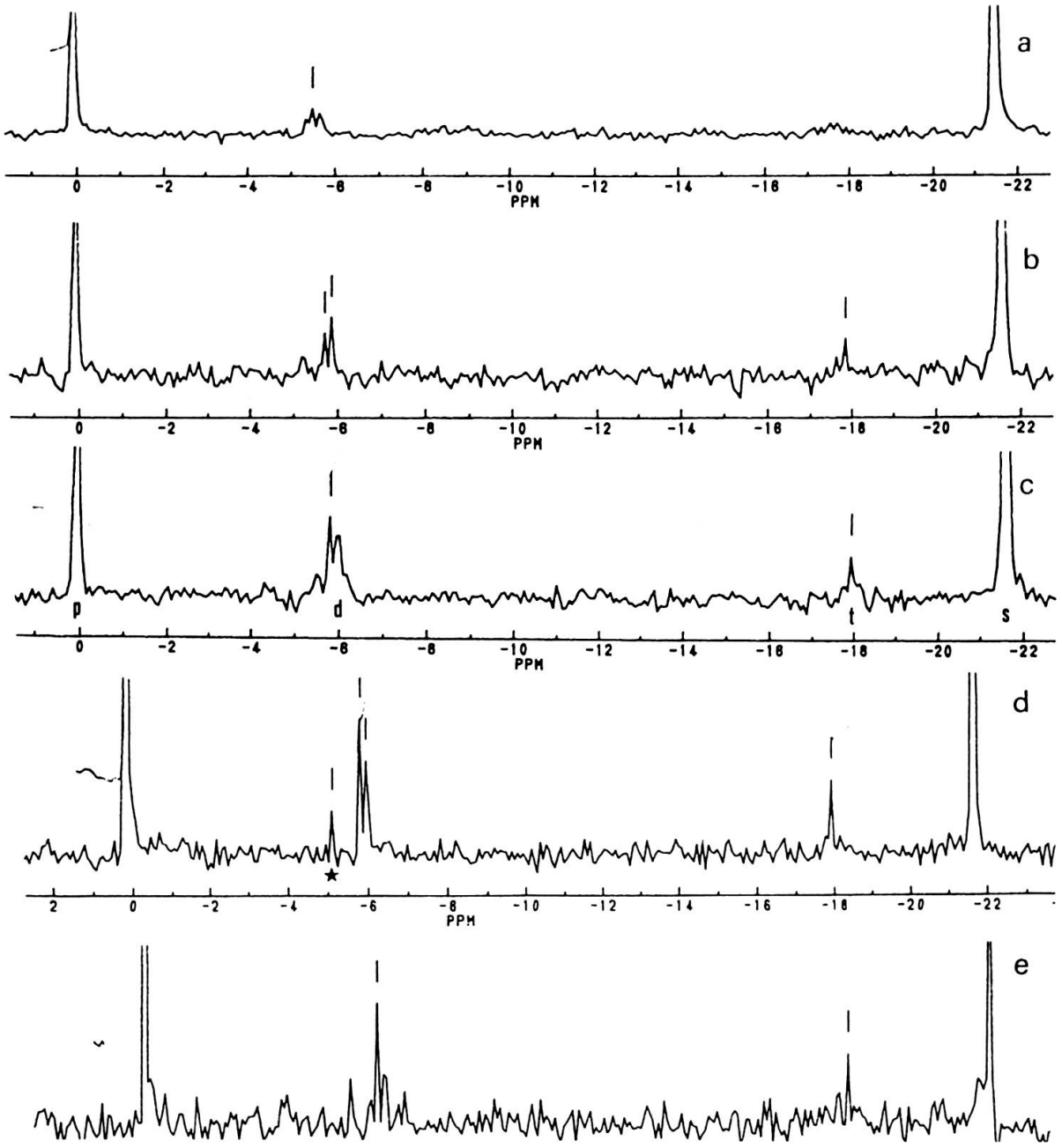

FIG. 1A. Comparative intensities of the ^{31}P NMR spectral signals of linear triphosphate (doublet = d + triplet = t) increasingly decyclized (18 days) from 10 mM trimetaphosphate (singlet = s) in mineral water (hydrolytic control a) by nucleophilic attack of the purine **adenine** (5 mM) alone (b) or doped by 5 mM Mg^{2+} ions (d) with hydrolytic control 5 mM Mg^{2+} ions alone (c) and with **imidazole** as optimal reference (e). * = oligophosphate peak ?

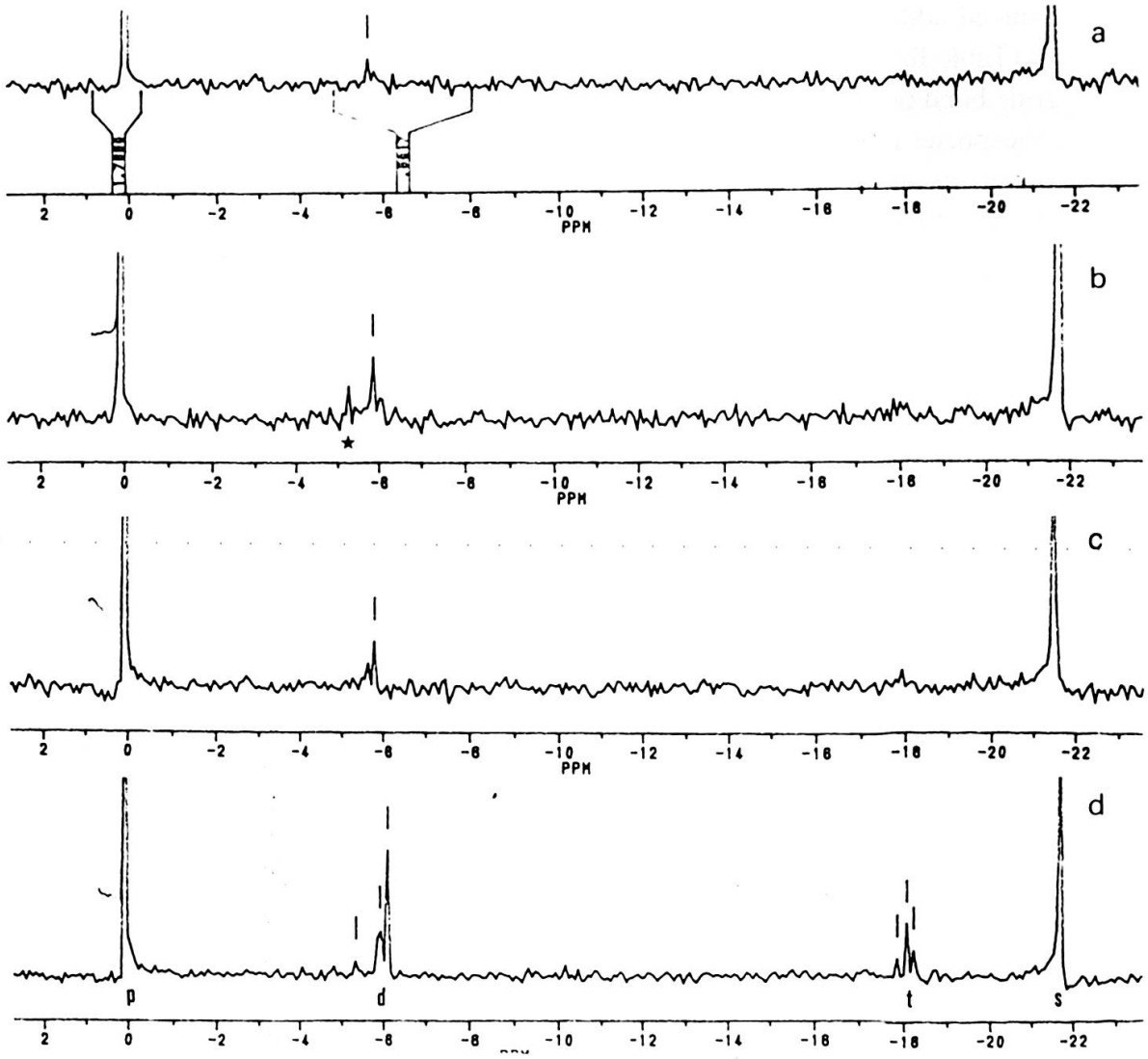

FIG. 1B. Comparative intensities of the ^{31}P NMR spectral signals of linear triphosphate (doublet = d + triplet = t) decyclized (18 days) from 10 mM trimetaphosphate (singlet = s) in mineral water (hydrolytic control a) by nucleophilic attack of the pyrimidine **cytosine** (5 mM) alone (b) or doped by 5 mM Mg^{2+} ions (d) with hydrolytic control 5 mM Mg^{2+} ions alone (c).

The most significant decrease of the trimetaphosphate signal was measured in the high Mg2+ solutions incubated with each of the 4 nucleobases and, optimally, with imidazole (Table Ib, Fig. 1A,B). When doped with Mg^{2+} ions, pyrimidines were as effective decycling molecules (Fig. 1B,C) as the imidazolide purines (Fig. 1A). Uracil even showed the sharpest differential when tested with supplementary Mg^{2+} (10 mM).

As for the enhancing effect of Mg^{2+} ions on the cycle opening by all nitrogen bases, it could rather be ascribed to their shielding effect of the charged groups of cyclic phosphate favoring (Westheimer, 1987) - and thereby revealing - their nucleophilic attack by the nitrogen bases (Fig. 2).

Interestingly, a small but sharp peak at ~-4.5 ppm, visible downstream of the ~-6 ppm doublets (Fig. 1*) which increased after 18 days incubation, might be identical to

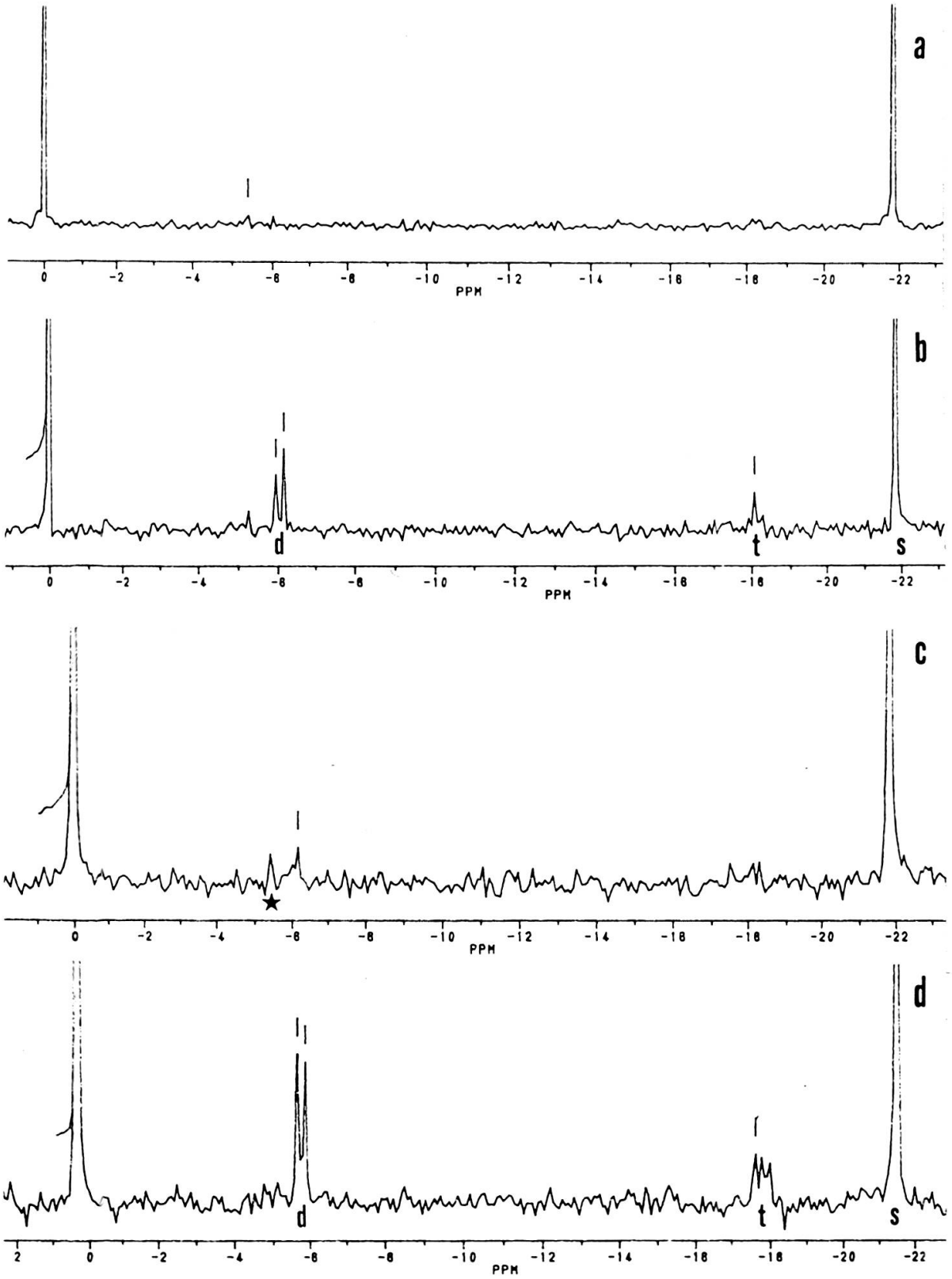

FIG. 1C. Comparative intensities of the ^{31}P NMR spectral signals of linear triphosphate (doublet = d + triplet = t) increasingly decyclized (11-18 days) from 5 mM trimetaphosphate (singlet = s) by 10 mM Mg^{2+} ions alone (a,c) and decyclized by nucleophilic attack of the pyrimidine **uracil** (5 mM) doped by 10 mM Mg^{2+} ions (b,d).

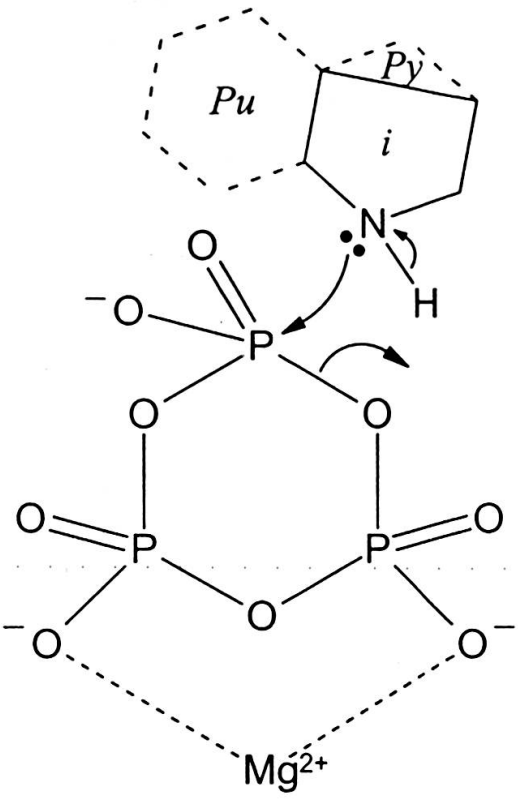

FIG. 2. Nucleophilic attack by the >NH group of nitrogenous bases (*i* = imidazole; *Pu* = purines; *Py* = pyrimidines) on an electrophilic P atom of trimetaphosphate (TriMP), resulting in an open chain or linear triphosphate. Mg^{2+} ions shield the $^-$ charges of the TriMP, thereby favoring its decyclization.

that ascribed by GILLIES *et al.* (1982) to the terminal phosphate of oligo-polyphosphates. Such peak could be considered as the polymerization product -hexo-nono-dodeca-polyphosphate- precedingly modellized (Fig. 1 in TURIAN, 1998) as base-bearing riboseless anhydride phosphate polymers which might have been precursors of the phosphoester bonded polyribonucleotides and thereby have assumed the function of some primitive type of replicators during prebiotic evolution.

In conclusion, our experimental results provide a first clue to the elusive problem of the precedence of the "modern" N-C-P, glycosidic-phosphodiester bonds with possible C precursors such as C_2-C_3 hydroxylated carbon compounds leading to the standard C_5 pyranoses finalized as furanoriboses (ESCHENMOSER, 1999). Such anteriority by direct N-P bondings of nucleobases lined up on polytriphosphates as primal amino acid coding units (TURIAN, 1996) would have been an advantage of simplicity at the onset of pregenetic molecular evolution: it might have provided, by catalytic acylation-phosphorylation entailing peptide bond formation from imidazole triphosphate (RABINOWITZ & HAMPAI, 1985; YAMANAKA *et al.*, 1988) or similarly from our putative nucleobase-triphosphates (adenyl-P, cytidyl-P, etc.), a self-entrained coupling mechanism of primary translation into more of the primally coded peptides.

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RÉSUMÉ

NOUVELLE ÉVIDENCE PAR ^{31}P-RMN D'UNE LIAISON PHOSPHORAMIDE DES NUCLÉOBASES PAR ATTAQUE NUCLÉOPHILE DU TRIPHOSPHATE CYCLIQUE

L'attaque nucléophile, dopée par les ions Mg^{2+}, des bases azotées phosphoramido-liées sur le cycle ouvert du trimétaphosphate a été détectée par les changements de spectres ^{31}P-RMN et généralisée de l'imidazole aux purines et pyrimidines.

Mots-clés: RMN, Nucléobases, Imidazole, Triphosphates cycliques et linéaires.

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