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SPECTRAL EVIDENCE FOR PHOSPHORAMIDE BONDINGS BETWEEN NUCLEOBASES AND TRIPOLYPHOSPHATE POSSIBLY GENERATIVE OF PRENUCLEIC POLYBASEPHOSPHATE CHAINS

BY

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ABSTRACT

Spectral evidence for phosphoramide bondings between nucleobases and tripolyphosphate possibly generative of prenucleic polybasephosphate chains. - Hypochromy of the UV spectra measured in slightly alkaline, aqueous mixtures of nucleobases and tripolyphosphate and its reversal by acidification have grounded evidence for their dehydrating, phosphoramidic condensation into acid-labile bimolecular base-pyrophosphate monomeric units of polybasephosphates, plausibly considered as riboseless prenucleic polymers of prebiotic evolution.

Key-words: Prenucleic polymers, Nucleobasephosphates, Hypochromicity.

INTRODUCTION

The evolutionary primacy of the «RNA world» has been questioned by many molecular evolution scientists (SHAPIRO, 1988; COHEN, 1996) because of serious difficulties in the primordial synthesis of its sugar D-ribose. As a tentative answer to this fundamental question, we have modellized an intermediate step of prenucleic polymers devoid of pentose sugar but already lining up the nucleobases on a polyphosphate backbone (TURIAN, 1996-97). Such a complex formation would have implicated phosphoramide bondings of the well-known type intervening in the production of creatine-phosphate or phosphagene, energetically driven by the splitting of inorganic pyrophosphate linkages instead of those of ATP.

To experimentally concretize this corner-stone of our model, we have developed a conceptual strategy founded on two physico-chemical tenets, both exploiting the possible changes in the ultra-violet spectral characteristics of the nucleobases resulting from the putative N-P bonding of their N_9H for purines or their N_1H for pyrimidines to

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the 2nd P of the tripolyphosphate (3P) chosen for its potential to polymerize into polyphosphates. Our 1st tenet relied on the principle of hypochromicity, according to which «the extinction coefficient of a nucleic acid increases significantly on degradation or hydrolysis, for the sum of the extinctions of the constituent nucleotides is greater than the extinction of the polynucleotide» (see DAVIDSON, 1957; MAHLER & CORDES, 1969). It could then be expected that, when compared with any of its free bases, the molecular complex nucleobase-pyrophosphate (BPPi) would show a UV spectral hypochromy in the same range as that of nucleosides-nucleotides. As for the 2nd tenet, it exploited the acid-lability of the putative complexes presumed from their analogy with that of creatine-phosphate. At low pH, this compound dissociates as such:

$$=\dot{C}\text{-}NH\text{-}P^{+}\text{-}O^{-} \rightarrow -\dot{C}=NH^{+} \leftrightarrow P^{+}\text{-}O^{-}$$, see MAHLER & CORDES (1969, p. 216)

Such acidification tests, complementary to the hypochromacities, were made at pH 4 or 2, lower than the p*Ka* (NH_3^+) of the bases (4.5 for C and 4.2 for A, 3.3 for G and 3.2 for U, see MAHLER & CORDES, 1969; DAWSON & al., 1969). They were expected to provide further evidence that the measured hypochromicity of each of the 4 possible base components in the incubated mixtures (A/G/C/U + tripolyphosphate) was really due to their condensation.

MATERIALS AND METHODS

All chemicals were first dissolved in 10 ml of bidistilled water. For purine and pyrimidine bases (Sigma), concentrations depended on their saturation solubility, namely 5 mM for adenine (A), 0.25 mM for guanine (G), 5 mM for both cytosine (C) and uracil (U). The anhydrous tripolyphosphate salt ($Na_5P_3O_{10}$, Sigma) was generally dissolved at 1 N, alone or in the presence of one of the bases, with a spontaneous pH of 7.8 adjusted with KOH 1 N to 8.0 as for the bases alone. All incubations have been made with liquid volumes of 5 ml in 10 ml pyrex tubes bilaterally shaken at low speed at 25° C. Samples of 1 ml were diluted 100x with distilled water before establishment of spectral curves or measurements of intensity of optical density (OD) at the specific maxima of each base with a spectrophotometer Uvikon 940 (Kontron).

In the experimental conditions of complete solubility of the bases tested, the extinctions E measured (peaks at 245 nm for guanine, 257 nm for uracil, 260 nm for adenine and 270 nm for cytosine) were expressed in the ordinates of the graphs as OD units of the base tested. In the supersaturated conditions, the E were simply presented as units (x1000) measured in the supernatants. All acidifications dissociating the putative base-diphosphates formed were checked with special microelectrodes. The tripolyphosphate provided for incubation in excess of any base tested could be precipitated in the presence of 50 per cent ethanol solution. From the thereby separated putative condensate base-3P, each of its two components could be identified after acid (HC1)-splitting: the

base by specific E(UV), the pyrophosphate (see Fig. 4) by its precipitation by ethanol or by the classical molybdate reagent (yellow reaction).

RESULTS AND DISCUSSION

To control the parallelism between intensity of hypochromy and molecular complexity in the structural units of ribonucleic acid, we compared on a molar basis the UV absorptions of the three terms of their series, namely nucleobase-nucleoside-nucleotide. The hypochromicities were significantly progressive from the free bases to the nucleosides, with the exception of the pyrimidine ribosides which, for some unclear reason (alkaline tautomery?), rather presented a slight hyperchromy. Nevertheless, the four nucleobases, when presumably bonded to pyrophosphate bimolecular groups (P + P), presented a sharp UV hypochromy when compared to their homologous free bases (Fig. 1).

To further ascertain the link between hypochromy and the real formation of a complex nucleobase-diphosphate, we have applied the acid-lability test to a 1st series of experiments involving nucleobases at concentrations (4 mM) imposed by their low solubility in slightly alkaline water (pH 8.0). All bases except cytosine (2 mM) have produced a noticeable hypochromy when mixed with tripolyphosphate (1 N) followed, after 24 hours incubation, by a close to full recovery of their specific UV absorption when acid-freed from their putative base(N)-pyrophosphate(P) complex (Fig. 2 featuring adenine and uracil).

A 2nd series of experiments was devoted to test the possible active uptake of the tripolyphosphate on the slowly solubilized bases in their saturated solutions. Such conditions provided a potentially increased availability of bases for the presumed «predatory» effect of tripolyphosphate as evidenced by the frequent excess of UV extinction after acid-splitting compared to the controls devoid of tripolyphosphate (Fig. 3). In such saturated conditions of incubation, and to our surprise, we have found cytosine (5-10 mM) an as efficient molecular partner as the three other bases in the hypochromicity, possibly by the concentration effect compensating the hyperchromic tautomery (see above).

The «predation» of bases by tripolyphosphate could be reenforced in the presence of Mg^{2+} or of Ca^{2+} ions which would precipitate the first PO_4^{3-} hydrolyzed in the coupling process anhydrically generating the N-P bond (Fig. 5), thereby shifting the condensation equilibrium toward further hydrolyses or exerting a catalytic effect as known in the synthesis of ATP.

Now that spectral criteria in favor of a phosphoramidic bonding between a nucleobase and tripolyphosphate have been obtained, it remained to isolate the monomeric unit base-pyrophosphate formed (see Fig. 5). According to preliminary experiments, the excess of tripolyphosphate initially added (2 mM) could be precipitated by ethanol 50% out of the alkaline incubation solution, leaving in the supernatant an ethanol-soluble but acid-splittable adenyl-phosphate complex. This putative

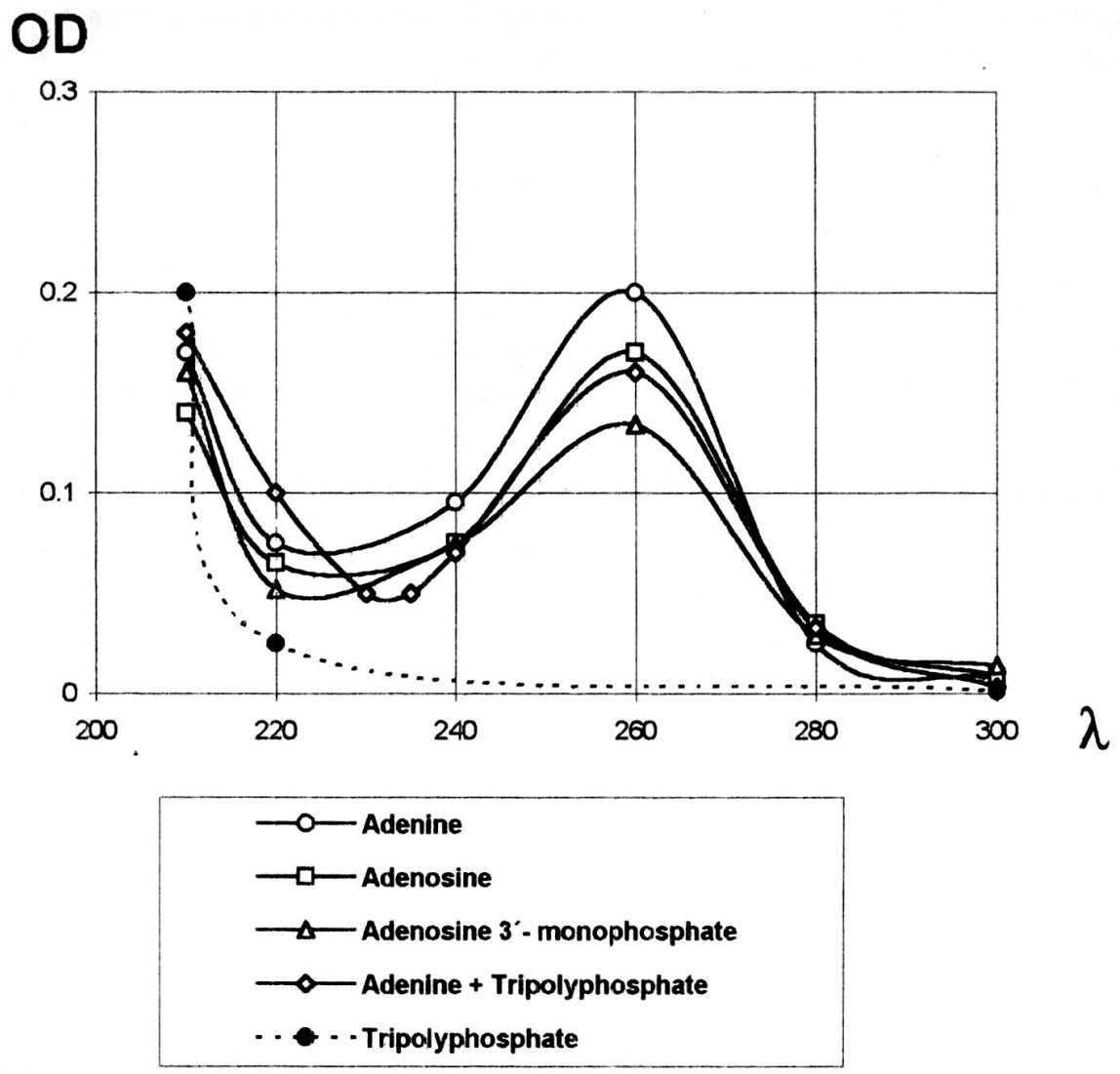

FIG. 1.

Comparative absorption spectra of the adenine family: optical density (OD) measured in H_2O solutions adjusted at pH 8.0. Maximum at the peak of adenine (A, 0.02 mM) but hypochromizing by molecular complexification from the bimolecular adenosine (A - ribose, 0.02 mM) and the putative complex A - pyrophosphate (from A 0.02 mM + tripolyphosphate 0.01 N) to the trimolecular adenylic acid (Adenosine 3'-phosphate 0.02 mM). Base line for free tripolyphosphate (0.01 N).

monomer could be identified by its hypochromy at 260 nm (pH 8) and, after acidification (pH 4) either by the ethanol 50 per cent precipitation of its acid-split pyrophosphate group or by its classical precipitation as yellow phosphomolybdate (Fig. 4). Such remaining presence of molybdate-precipitable phosphate paralleling the presence of the base adenine in the ethanol-HCl supernatant is thus an additional evidence for the formation of a monomeric adenine-organic phosphate component available for a further, pregenetically significant, polymerization into base-bearing polyphosphate chains.

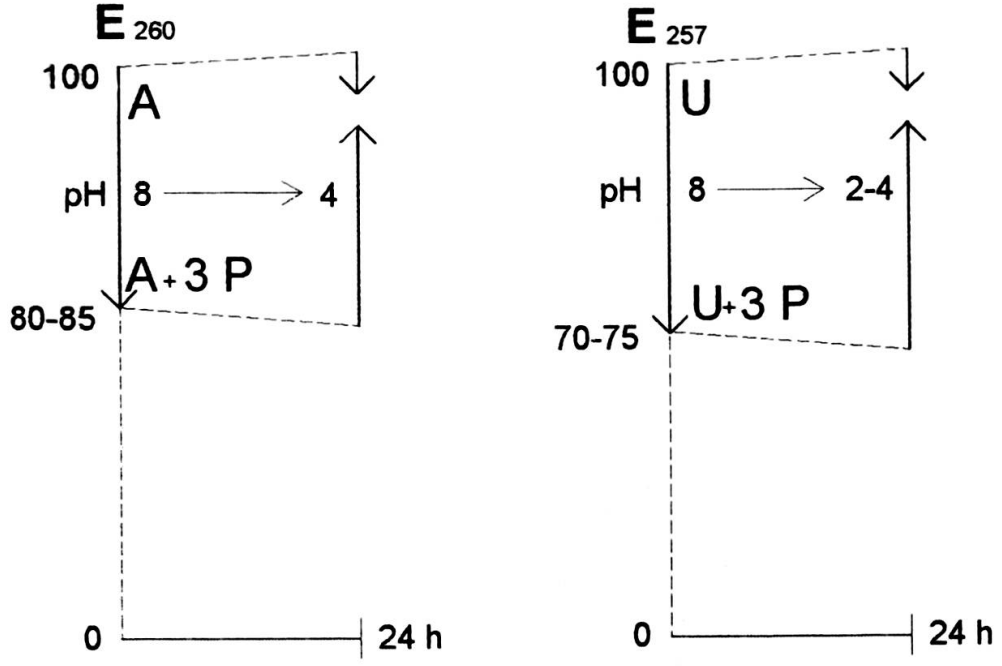

FIG. 2.

Hypochromicity (pH 8) conferred to adenine (A) or to uracil (U) by their condensation with tripolyphosphate (3P) and their original E value, equated to 100, nearly fully recovered after 24 hours by acidic dissociation (pH 4 for A, average 2-4 for U) of the putative base-pyrophosphate complex (see Fig. 5). The slightly low E values of A and U in acidic conditions might result of their prototropic tautomerization.

Polyphosphate polymers have been advocated by KORNBERG (1995) as plausible precursors to nucleic acids following previous findings of their role as energy carriers in primitive synthesis of ATP (KORNBERG *et al.*, 1956; LIPMANN, 1965; BALTSCHEFFSKY & BALTSCHEFFSKY, 1992; WOOD, 1985). Going one step further, our results provide experimental evidence for our preceding proposal that the progressive linear polymerization of the base-bearing pyrophosphates into polyphosphate chains would then have determined the lining up of the N-P bonded bases (Fig. 5). Such polybasephosphate chains presumably formed in prebiotic conditions would have insured the necessary N-P stabilization of the primordial doublets of bases encoded by the 1-letter amino acids of the first formed peptides (see TURIAN, 1995). As prenucleic polymers, they would have thus provided the first evolutionary link between such 1-letter peptide code, by retrotranslation (TURIAN, 1997) of say glycine by cytosine + cytosine, *b*-alanine by cytosine + guanine, etc., and the «modern» 3-letter code of the «RNA world» ensuring the anterotranslation of increasingly sophisticated proteins.

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Incubated molecules	$E_{260\ nm}$					
	0 h - 1/2 h		24 h		36 h	
	pH 8	pH 4	pH 8	pH4	pH 8	pH 4
A 5 mM in H_2O + (KOH / HCl)	812	741	810	733	809	654
A 5 mM in H_2O + 3 P + (KOH / HCl)	550	865	605	835	620	671

Incubated molecules	$E_{245\ nm}$					
	0 h - 1/2 h		24 h		48 h	
	pH 8	pH 2	pH 8	pH 2	pH 8	pH 2
G 0,25 mM in H_2O + (KOH / HCl)	414		405	612	337	694
G 0,25 mM in H_2O + 3P + (KOH / HCl)	302		271	612	273	1065

Incubated molecules	$E_{270\ nm}$					
	0 h - 1/2 h		24 h		54 h	
	pH 8	pH 4	pH 8	pH 4	pH 8	pH 4
C 5 mM in H_2O + (KOH / HCl)	780	724	852	977	1248	1380
C 5 mM in H_2O + 3 P + (KOH / HCl)	347	416	563	655	1169	1380

Incubated molecules	$E_{257\ nm}$					
	0 h - 1/2 h		24 h		54 h	
	pH 8	pH 2	pH 8	pH 2	pH 8	pH 2
U 5 mM in H_2O + (KOH / HCl)	514	-	762	602	797	697
U 5 mM in H_2O + 3 P + (KOH / HCl)	417	-	608	732	660	755

FIG. 3.

Comparative UV absorbtivities (E units = OD x 10^3) of the 4 nucleobases incubated in H_2O for 24-54 hours at 25°C at their saturated concentrations (A 5 mM, G 0.25 mM, C 5 mM, U 5 mM) alone or in the presence of 3P (tripolyphosphate 1 N), before final acidification to pH 4.0 for A and C or pH 2.0 for G and U. Among the close to normal E values recovered by acid (HCl N)-dissociated bases, a few exceeded those at 0 time, suggesting an active sequestration of bases by 3P overstepping their low solubility (especially noticeable with the poorly soluble G). Results sampled as average out of 3 experiments.

Isolation in ethanol 50 per cent of the putative complex Adenine - 2 P(1)

Physico-chemical states	A	A + 3P	
	2 mM	2 + 2 mM	2 + 4 mM
Precipitate *			
$E_{260\ nm}$	0	5	10
Phosphomolybdate [2]	0	+	++
Supernatant **			
$E_{260\ nm}$	215	207	225
Phosphomolybdate [2]	0	+	+

FIG. 4.

1) N-P bond extends to the pyrophosphate component of the complex (see Fig. 5) the ethanol solubility of adenine.

2) Reagent: ammonium MoO3 – HC1 4N.

3) + mainly Pi released from equimillimolar complex formation, ++ for 2 mM excess 3 P, + for each 2P + Pi released from acid-split, equal A (2 mM)-containing complexes.

* Free 3P + released Pi. ** Ethanol 50% - soluble adenine-pyrophosphate complex.

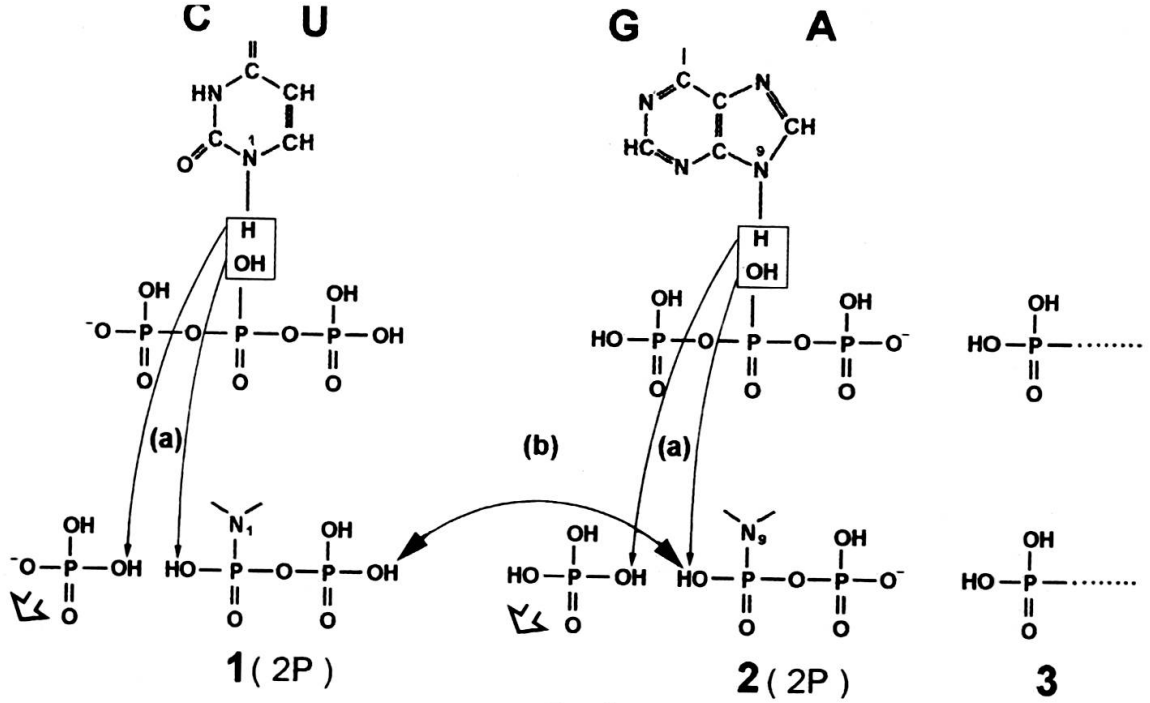

FIG. 5.

Modellization of the phosphoramide N-P condensation between a pyrimidine (C or U) or a purine (A or G) base and the intermediate phosphate group of tripolyphosphate involving coupling processes by interphosphate dehydrations, the 1st (a) releasing the terminal phosphate group, the 2nd (b) condensing the base bearing pyrophosphate to the next, when also bearer of a base, according to the following polyphosphate chain polymerization process: P(b)P-P(b)P-P(b)P-...

RÉSUMÉ

EVIDENCE SPECTRALE DE LIAISONS PHOSPHORAMIDES ENTRE NUCLÉOBASES ET TRIPOLYPHOSPHATE, GÉNÉRATRICES PRÉSUMÉES DE CHAÎNES POLYBASEPHOSPHATES PRÉNUCLÉIQUES

L'hypochromie des spectres UV mesurée dans des mélanges aqueux légèrement alcalins de nucléobases - tripolyphosphate et sa réversion par acidification plaident en faveur de leur condensation anhydrisante, par liaison phosphoamide, en monomères bimoléculaires acido-sensibles base-pyrophosphate de polybasephosphates considérés comme polymères prénucléiques, dépourvus de ribose, de l'évolution prébiotique.

Mots-clés: Polymères prénucléiques, Nucléobasephosphates, Hypochromie.

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