Short Communication

High-performance liquid chromatographic and ^{31}P NMR spectroscopic evidence for novel analogue of triphosphate formed by phosphonylation of orthophosphate with diphosphonate

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Abstract

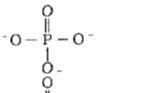
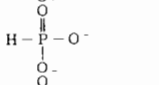
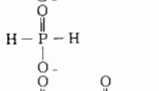
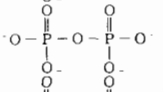
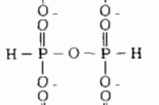
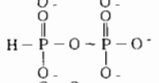
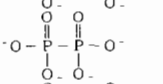
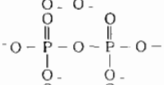
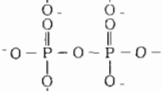
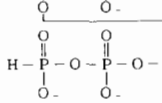
Formation of a dimer and a trimer by stepwise phosphonylation of orthophosphate with diphosphonate, a dimeric oxoacid of phosphorus(III), in aqueous solution is described. The trimer, a novel analogue of triphosphate (tripolyphosphate), has been confirmed by ^{31}P NMR spectroscopy and high-performance liquid chromatography to be composed of a central phosphorus(V) group and two terminal phosphorus(III) groups. A triplet ^{31}P NMR signal at -21 ppm assignable to the central group is of key importance to determine its structural analogy with the well-known triphosphate. The results suggest that the spontaneous growth of orthophosphate to give diphosphate, triphosphate, and/or ATP, one of the possible scenarios in the ancient process of chemical evolution, might be achieved by the contribution of oxoacids of phosphorus(III) in precursor formation.

Introduction

As shown in Table 1 a number of inorganic phosphorus compounds with complicated structures are known or being synthesized. Much attention has been focussed on the reaction mechanisms by which these compounds are formed or converted. This paper deals with the formation of a novel trimeric compound. Since no systematic nonenclature has been given for these compounds [1] the abbreviations denoting oxidation numbers of phosphorus (Table 1) are used in this paper as well as in previous papers [2, 3].

All polymers of P^V (phosphate or phosphoryl) groups and/or P^{III} (phosphonate, phosphonyl) groups are so unstable thermodynamically in aqueous solutions [1, 2] that the conversion of monomers to trimers is believed to be very difficult or impossible. Nevertheless, we have been interested in obtaining the formation of such unstable polymers from orthophosphate in aqueous and non-catalytic media [3–6], with a view to achieving the spontaneous growth of orthophosphate (P^V) to diphosphate (P^VP^V), triphosphate ($P^VP^VP^V$) and ATP, one of the possible scenarios in the ancient process of

TABLE 1. Phosphorus compounds used for ^{31}P NMR measurements

Abbreviations[a]	Structural formula	Nomenclature[b]
P^V		orthophosphate
P^{III}		phosphonate phosphite
P^I		phosphinate hypophosphite
P^VP^V		diphosphate pyrophosphate
$P^{III}P^{III}$		diphosphonate pyrophosphite diphosphite
$P^{III}P^V$		isohypophosphate
$P^{IV}P^{IV}$		hypophosphate
$P^VP^VP^V$		triphosphate tripolyphosphate
$cP^VP^VP^V$		cyclo triphosphate trimetaphosphate
$P^{III}P^VP^{III}$		?

[a]Denote oxidation numbers of phosphorus. [b]Includes trivial names.

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chemical evolution and in the metabolic process of current organisms [2, 7–11]. This paper describes the formation of precursors, a dimer, $P^{III}P^{V}$, and a trimer, $P^{III}P^{V}P^{III}$, by stepwise phosphonylation of P^{V} with diphosphonate, $P^{III}P^{III}$, in an aqueous solution [3]. The trimeric product, a novel analogue of triphosphate, has been confirmed by ^{31}P NMR spectroscopy and high-performance liquid chromatography to be composed of a central P^{V} group and two terminal P^{III} groups. A triplet ^{31}P NMR signal at -21 ppm assignable to the central group of $P^{III}P^{V}P^{III}$ is of key importance in determining its structural analogy with $P^{V}P^{V}P^{V}$.

Experimental

Disodium diphosphonate ($P^{III}P^{III}$), $Na_2P_2H_2O_5$, was prepared according to a previous paper [7]. Other chemical were JIS-S grade from Wako (Osaka, Japan).

Chromatographic separation using an HPLC system with an anion-exchange column [2, 3, 12] was the same as that previously reported [3]. ^{31}P NMR spectra were recorded by using 10 mm NMR tubes on a JEOL-GX-400 spectrometer operating at the frequency of 162 MHz. Chemical shifts, δ values in ppm, were presented with respect to an external reference of 85% phosphoric acid, with positive values being downfield of the reference.

Results and discussion

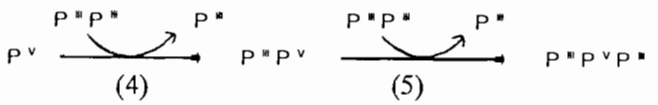
No positive evidence for the formation of polymers in eqns. (1)–(3) has been obtained because of extremely low equilibrium concentrations of the polymers, less than 1μM when each monomer concentration is 0.1 M [2, 11].

$$P^{V} + P^{V} \rightleftarrows P^{V}P^{V} \quad (1)$$

$$P^{III} + P^{III} \rightleftarrows P^{III}P^{III} \quad (2)$$

$$P^{III} + P^{V} \rightleftarrows P^{III}P^{V} \quad (3)$$

On the other hand; the stepwise formation of $P^{III}P^{V}$ and $P^{III}P^{V}P^{III}$ according to the reaction schemes in eqns. (4) and (5) has been clearly evidenced by high-performance liquid chromatography (HPLC) and ^{31}P NMR.



(4) (5)

These reactions can be explained in terms of S_N2 substitution mechanisms [3, 7]. Both P^{V} and the first product, $P^{III}P^{V}$, act as nucleophiles to attack $P^{III}P^{III}$ that behaves as a good P^{V} group acceptor or, in other words, as a P^{III} group donor.

In a previous paper [3] we have described in detail the formation of $P^{III}P^{V}$ that is known as isohypophosphate [1]. The novel trimeric $P^{III}P^{V}P^{III}$ has been of particular interest [3–6] because such a type of trimer with a central P^{V} group decorated with two P^{III} groups may be the first example in inorganic phosphorus chemistry [1]. This paper deals with the structural diagnosis of $P^{III}P^{V}P^{III}$ by ^{31}P NMR spectroscopy. Prior to the NMR experiments the formation of the dimer and trimer in an aqueous solution under mild and non-catalytic conditions was confirmed by chromatographic separation and compositional analysis [3–6]. A mixed solution of 0.1 M P^{V}, Na_2HPO_4, and 1.0 M $P^{III}P^{III}$, $Na_2P_2H_2O_5$, was incubated at 50 °C for 5 h. The reaction products were analyzed by HPLC using an anion-exchange column (TSKgel SAX) and an eluent, 0.25 M KCl–0.1% EDTA(4Na) solution. As shown in Fig. 1, P^{V}, $P^{III}P^{V}$ and $P^{III}P^{V}P^{III}$ having P^{V} groups could be detected with the P^{V}-selective Mo(V)–Mo(VI) reagent used for post-column detection, while P^{III} and $P^{III}P^{III}$ were insensitive [3, 7]. The compositions (P^{III}:P^{V}) of the $P^{III}P^{V}$ and $P^{III}P^{V}P^{III}$ in Fig. 1. were determined by the differential analysis of P^{III} and P^{V} groups to be 1:1 and 2:1, respectively [3].

From the HPLC profile in Fig. 1 the relative amount in molar concentration of $P^{III}P^{V}P^{III}$ is calculated to be 10% of the three compounds, corresponding to 0.01 M $P^{III}P^{V}P^{III}$. It is noted that both $P^{III}P^{III}$ and P^{III} are also included as the major components in the analyzed sample, but these compounds are not detectable with the molybdenum reagent used [6]. The ^{31}P NMR spectra in Fig. 2 were obtained with the same sample solution (pH 5.6) as used in Fig. 1. The results can be analyzed by consulting the ^{31}P NMR data of known compounds in Table 1 (not shown) and by considering the reaction schemes in eqns. (4) and (5). The peaks A_1 and A_2 for P^{III} (doublet), B for P^{V} (singlet) and C_1 and C_2 for $P^{III}P^{III}$ (double triplet), respectively, are observed as expected [3]. Unsymmetrical $P^{III}P^{V}$ exhibits a double doublet, X_1 and X_2, assignable to the P^{III} group and a doublet, X_3, to the P^{V} group, consistent with the detailed analysis of NMR spectrum for $P^{III}P^{V}$ in the

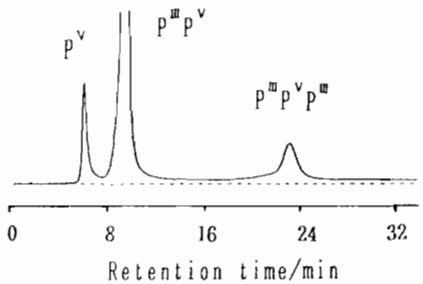


Fig. 1. HPLC profile for the reaction products of P^{V} and $P^{III}P^{III}$. Sample: a mixed solution of 0.1 M P^{V} and 1.0 M $P^{III}P^{III}$ incubated at 50 °C for 5 h; 0.1 ml injection.

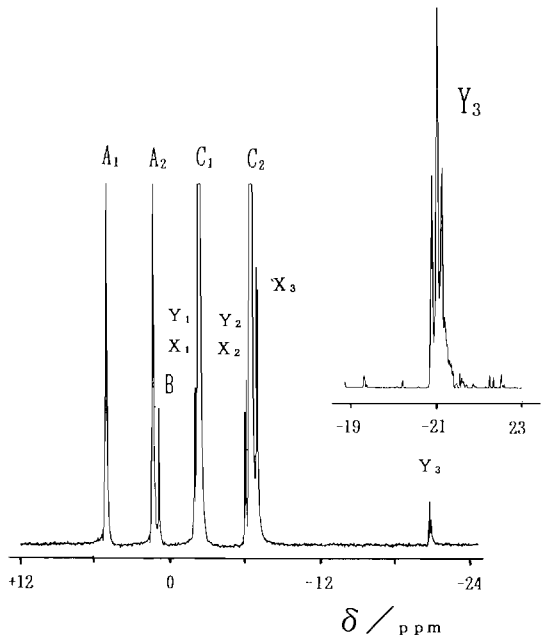


Fig. 2. ^{31}P NMR spectra for the reaction products of P^V and $P^{III}P^{III}$. The sample was the same as in Fig. 1 (pH 5.6). NMR measurement: 162 MHz; 85% H_3PO_4 reference (ref. 3). Peak assignment: see text. Inset: ten-fold amplified figure of the triplet Y_3.

previous paper [3]. This conclusion was also confirmed by proton-decoupling experiments [3].

The most significant finding is the triplet Y_3 that appears in the δ region between -19 and -23 ppm where the middle group of $P^VP^VP^V$ gives a triplet and *cyclo* triphosphate, $cP^VP^VP^V$, gives a singlet. The ^{31}P NMR signals for the monomers and dimers in Table 1 and for the terminal P^V groups of $P^VP^VP^V$ have been confirmed to appear downfield below -11 ppm [3, 14]. The Y_3 signal provides the NMR parameters, $\delta = -21.1$ ppm and $J = 18.3$–22.0 Hz, and is assignable to the middle P^V group of $P^{III}P^VP^{III}$ by close analogy with the triplet signal for the middle group of well-known $P^VP^VP^V$ [14]. The significant points are: (i) the compositional analysis for the third $P^{III}P^VP^{III}$ species in Fig. 1 is P^{III}:$P^V = 2$:1; (ii) the intensity of the triplet Y_3 in Fig. 2 increases with increasing reaction time of P^V and $P^{III}P^{III}$ (eqns. (4) and (5)) [3] or is proportional to the intensity of the $P^{III}P^VP^{III}$ peak in Fig. 1; (iii) the triplet Y_3 appears in the δ region where the middle P^V group of $P^VP^VP^V$ gives a triplet and all (middle) groups of $cP^VP^VP^V$ give a singlet; (iv) the Y_3 signal is not simplified by proton decoupling, excluding the contribution of the P–H bond; (v) the J values or $^2J_{PP}$ values of the triplet Y_3 are similar to the $^2J_{PP}$ values of the middle group of $P^VP^VP^V$ (18.5–22.2 Hz).

A double doublet, Y_1 and Y_2, for the end P^{III} groups of $P^{III}P^VP^{III}$ is expected to appear in the end P^{III} region between -2 and -7 ppm [3]. However, the region is so crowded that it is difficult to discriminate such small P^{III} signals of $P^{III}P^VP^{III}$ from the signals of major components, $P^{III}P^{III}$ and $P^{III}P^V$.

Under improved conditions as much as 78 and 14% of P^V can be converted, gradually in a few hours, to $P^{III}P^V$ and $P^{III}P^VP^{III}$, respectively [3]. Further attempts to oxidize $P^{III}P^VP^{III}$ by hydrogen peroxide [4] to obtain $P^{III}P^VP^V$ and $P^VP^VP^V$ are in progress. More detailed and comprehensive scenarios to obtain the spontaneous growth of orthophosphate to polymers under thermodynamically unfavorable conditions will be presented elsewhere.

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