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Oxidative Conversion of Diphosphonate to Diphosphate and Isohypophosphate by Hydrogen Peroxide

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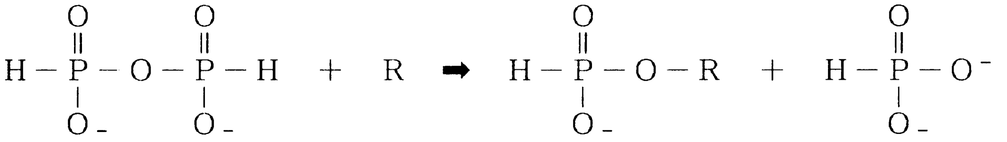
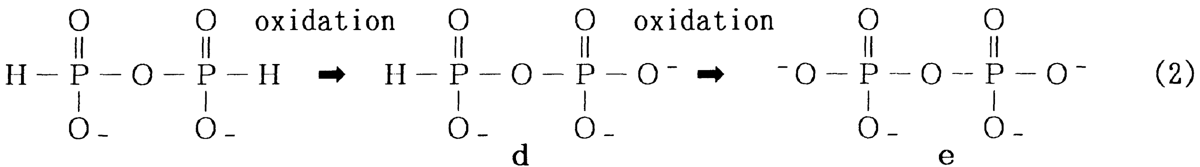
Diphosphonate (oxidation number of phosphorus +3) could be oxidized by hydrogen peroxide without P-O-P bond cleavage. The formation of dimeric compounds; diphosphate (oxidation number +5) and isohypophosphate (oxidation numbers +3 and +5), was characterized by HPLC and ^{31}P NMR.

Diphosphonate (a), a dimeric form of phosphonate (c; phosphite; oxidation number of phosphorus +3),[1] has been demonstrated to be very reactive as an acceptor of nucleophiles in S_N2 reactions and versatile for phosphonylating inorganic polyphosphates and various biomolecules such as nucleotides and nucleosides (R) according to Eq. 1.[2-4] For convenience each species in Eq. 1, as well as in Eqs. 2 and 3, was expressed in a dissociated form.



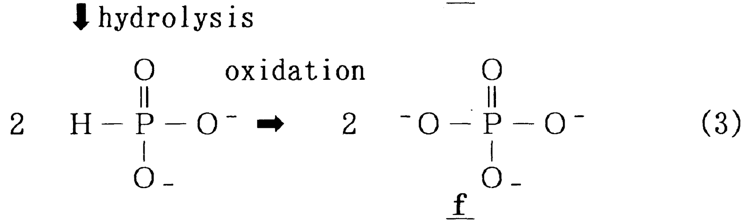
$$\underset{a}{H-\overset{\overset{\displaystyle O}{\|}}{\underset{\underset{\displaystyle O^-}{|}}{P}}-O-\overset{\overset{\displaystyle O}{\|}}{\underset{\underset{\displaystyle O^-}{|}}{P}}-H} + R \rightarrow \underset{b}{H-\overset{\overset{\displaystyle O}{\|}}{\underset{\underset{\displaystyle O^-}{|}}{P}}-O-R} + \underset{c}{H-\overset{\overset{\displaystyle O}{\|}}{\underset{\underset{\displaystyle O^-}{|}}{P}}-O^-} \qquad (1)$$

From the viewpont of synthetic interest it is of importance to find experimental conditions where oxidative conversion of the transfered phosphonyl group on b, such as an ATP analogue having a P-H bond, to phosphoryl group with a higher oxidation number +5 proceeds effectively and P-O-R bond cleavage can be minimized. This work was undertaken to get basic information about oxidation and bond cleavage by using diphosphonate as a related model compound. Main possible pathways during the oxidation of diphosphonate are shown in Eqs. 2 and 3.



$$\mathrm{H-\overset{\overset{O}{\|}}{\underset{\underset{O^-}{|}}{P}}-O-\overset{\overset{O}{\|}}{\underset{\underset{O^-}{|}}{P}}-H \xrightarrow{\text{oxidation}} H-\overset{\overset{O}{\|}}{\underset{\underset{O^-}{|}}{P}}-O-\overset{\overset{O}{\|}}{\underset{\underset{O^-}{|}}{P}}-O^- \xrightarrow{\text{oxidation}} {}^-O-\overset{\overset{O}{\|}}{\underset{\underset{O^-}{|}}{P}}-O-\overset{\overset{O}{\|}}{\underset{\underset{O^-}{|}}{P}}-O^-} \qquad (2)$$

d e



$$\downarrow \text{hydrolysis}$$

$$\mathrm{2\ H-\overset{\overset{O}{\|}}{\underset{\underset{O^-}{|}}{P}}-O^- \xrightarrow{\text{oxidation}} 2\ {}^-O-\overset{\overset{O}{\|}}{\underset{\underset{O^-}{|}}{P}}-O^-} \qquad (3)$$

f

The stepwise oxidation of two phosphonyl groups on diphosphonate is expected to give isohypophosphate(d ; oxidation numbers +3 and +5) and diphosphate (e ; pyrophosphate; oxidation number +5) according to Eq.2. Another pathway is the hydrolysis of diphosphonate followed by the subsequent oxidation to orthophosphate (f) in Eq.3.

In preliminary experiments we tried to oxidize diphosphonate by using various metal ions and halogens. All attempts to obtain diphosphate through the oxidative pathway in Eq. 2 were unsuccessful. Hydrolytic pathway to give monomeric phosphonate and orthophosphate proceeded preferentially even in a neutral medium, due probably to the catalytic hydrolysis by metal ions,halogens and/or halide ions. On the other hand hydrogen peroxide employed successfully in this work was effective to promote the oxidative pathway in Eq.2 and was clean in such a sense that the residual hydogen peroxide could be easily removed by catalytic decomposition in the presence of metallic platinum.

Disodium diphosphonate ($Na_2P_2H_2O_5$)[3] was dissolved in 1% (wt/wt) hydrogen peroxide to prepare a 1 mM diphosphonate solution (ca.pH 6; M = mol dm^{-3}). The oxidative conversion of diphosphonate was monitored by high-performance liquid chromatography (HPLC). A post-column reaction system using a Mo(V)-Mo(VI) reagent was able to differentiate various phosphorus compounds with different oxidation numbers.[2-6] The HPLC system employed for obtaining Fig.1 was designed to permit the selective detection of P(V)-units in the presence of P(Ⅲ)-units. A standard mixed solution of orthophosphate, diphosphate, and triphosphate (tripolyphosphate) with P(V)-units indicated three peaks as shown in Fig.1a. Diphosphonate composed of P(Ⅲ) units, as well as phosphonate, did not show HPLC peaks. In the presence of hydrogen peroxide, however, the diphosphonate sample solution gave three peaks corresponding to the oxidation products in Eqs.2 and 3; isohypophosphate, diphosphate and orthophosphate. Three peaks grew slowly with time to indicate HPLC peaks after two weeks as in

Fig.1b. Isohypophosphate to be used for standardizing its retention time was prepared according to the method in a previous paper.$^{2)}$ The retention times of isohypophosphate and diphosphate varied with different dependence on eluent compositions. With 0.13 M KCl, instead of 0.23 M KCl in Fig.1, isohypophosphate appeared in the reversed order before diphosphate to give improved resolution between isohypophosphate and diphosphate. Two peaks overlapped each other in such medium chloride concentration as 0.17 M KCl. Similar complicated chromatographic behavior was also observed for the oxidation products of diphosphonate, providing more confirmative evidence for the formation of isohypophosphate and diphosphate.

The residual hydrogen peroxide in the sample solution used for oxidation interfered with the spectrophotometric detection of phosphorus compounds, due probably to the oxidation of Mo(V) in the reagent. Careful removal of hydrogen peroxide was required to permit the quantitative detection of orthophosphate because insufficient removal of hydrogen peroxide, eluted near the retention time of orthophosphate, resulted in lowering the base-line level as shown in Fig.1b.

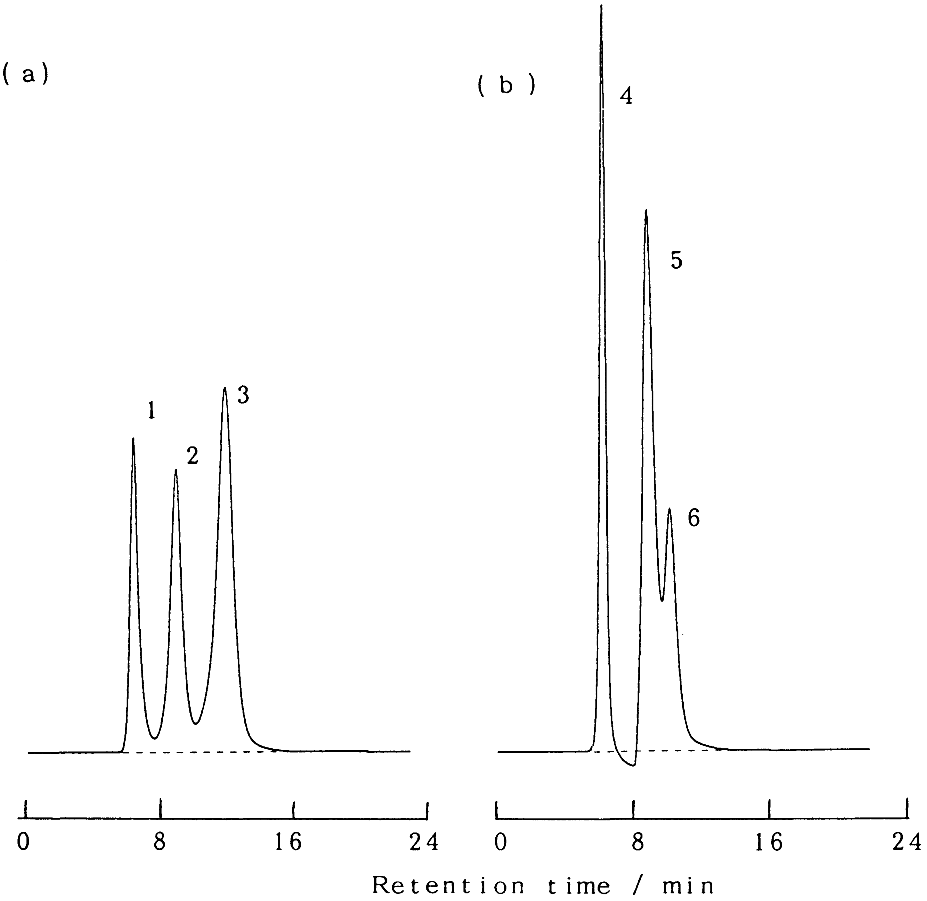


Fig.1. HPLC profiles for standard samples (a) and oxidation products of diphosphonate (b). <Peaks> 1 and 4, orthophosphate; 2 and 5, diphosphate; 3, triphosphate; 6, isohypophosphate. <Eluent> 0.23 M KCl + 0.1% EDTA (4Na). <Samples> 0.1 mM for each standard sample and 1 mM for diphosphonate.

Hydrogen peroxide, however, did not interfere with chromatographic detection of other phosphorus compounds with higher retention times when the eluent concentration was less than 0.23 M. The residual hydrogen peroxide, if it was unfavorable from analytical and preparative purposes, could be easily decomposed by allowing the sample solution to stand in contact with a platinum or platinum (palladium) wire.

The results in Fig. 1 indicate that hydrogen peroxide is effective to produce, according to Eq. 2, dimeric isohypophosphate and pyrophosphate that amount to more than 60% of the total oxidized phosphorus. Orthophosphate, the peak 4 in Fig. 1, may be mainly caused by the hydrolysis-oxidation pathway in Eq. 3, though its formation by the hydrolysis of isohypophosphate, not of stable pyrophosphate, is not completely excluded.

To obtain further evidence for the formation of dimeric compounds ^{31}P NMR measurements (161.8 MHz) were made with a sample solution of 0.1 M disodium diphosphonate - 3% hydrogen peroxide (around pH 4.5). The NMR peak for the starting diphosphonate (chemical shift δ = -4.4 ppm) decreased with increasing incubation time during two weeks to give growing NMR peaks that corresponded to isohypophosphate (δ = -4.4; -9.6 ppm), pyrophosphate (δ = -10.1 ppm), phosphonate (δ = +3.4 ppm) and orthophosphate (δ = +0.7 ppm). In addition to the five NMR peaks that were expected from Eqs. 2 and 3 an unidentified NMR peak (δ = +13.4 ppm, doublet) appeared transiently at the initial stage of incubation, probably assignable to unstable peroxophosphonate or peroxodiphosphonate. The oxidation of diphosphonate with hydrogen peroxide is slow, as well as in electrochemical oxidation of phosphonate,$^{7,8)}$ and is likely to be based on more complicated reactions than the simplified pathways in Eqs. 2 and 3.

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