# $^{31}P$ and $^{27}Al$ NMR Studies on the Coordination of Aluminum(III) with Tetraphosphate

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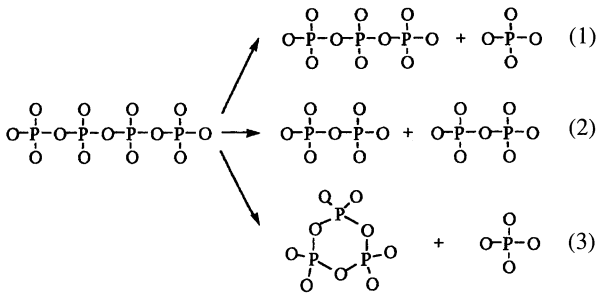
The binding isomerization of Al(III) with linear tetraphosphate has been investigated in the pH range of 1 to 9 using $^{31}P$ and $^{27}Al$ NMR spectroscopy. The complexes of 1 : 1 (=Al(III) : $P_4O_{13}$) stoichiometry with monodentate and didentate bindings, and the complex of 2 : 1 stoichiometry with two didentate bindings coexist in equilibrium. The concentration ratio of the 1 : 1 complexes with didentate to monodentate binding is 2.02±0.19 at pH 3. The relative concentration of the monodentate complex increases along with decreasing the pH. The possible binding positions of Al(III) to tetraphosphate in these complexes are discussed.

Recently, strong interest has been aroused about the biological roles of aluminum, because Al(III) has been implicated in a number of toxic processes.[1,2] Many toxic effects are manifested by inhibiting ATP-utilizing enzymes. Although ATP exists in the cell as a complex with $Mg^{2+}$, Al(III) binds ca. $10^7$-times more strongly to ATP than does $Mg^{2+}$.[1] Once Al(III) reacts with ATP, any consequent reactions requiring ATP participation are inhibited. In that context, it is important to specify the interaction of Al(III) with nucletiodes and other phospholigands.

There have been a large number of NMR studies on the interaction of Al(III) with condensed phosphates (e.g. mono-, di-, and triphosphate,[3,4] *cyclo*-polyphosphates,[5] and ATP[6—8]. Such bioinorganic studies were performed in order to clarify various aspects of the structures of these complexes, and, further, to fill the gap between the knowledge concerning biological and biochemical phenomena. It is now of interest to extend this study to the interaction of Al(III) with linear tetraphosphate.

Tetraphosphate is hydrolyzed in an aqueous solution to form monophosphate, diphosphate and *cyclo*-triphosphate in the following three different ways:[9—11]




The hydrolysis rates are significantly affected by the $H^+$ and $OH^-$ ion concentrations, and by the presence of various cations which form complexes or ion-pairs with the phosphate. Under the presence of Al(III) ions, linear pentaphosphate is also found in the degradation products of tetraphosphate.[12] In order to clarify the effects of Al(III) on hydrolysis, we examined the binding isomerization of Al(III) with tetraphosphate in the pH range of 1 to 9 using $^{31}P$ and $^{27}Al$ NMR spectroscopy.

This work has demonstrated the good resolution of the NMR peaks of complexed and uncomplexed species because of the slow exchange, as well as a complicated series of equilibria between various Al(III)–$P_4O_{13}$ complexes. The combination of the $^{31}P$ and $^{27}Al$ NMR results reveals that complexes of 1 : 1 (=Al(III) : $P_4O_{13}$) stoichiometry with monodentate and didentate bindings, and the complex of 2 : 1 stoichiometry with two didentate bindings, coexist. The possible binding structures of these complexes are discussed.

## Experimental

**Materials and NMR Samples.** Guanidinium tetraphosphate was prepared following a method described in the literature.[13] All other chemicals were of reagent grade and were used as received. In order to avoid the hydrolysis of tetraphosphate, Al(III)–$P_4O_{13}$ samples were prepared just prior to NMR experiments. The pH values of the solutions were adjusted with a small quantity of HCl or NaOH solution. All of the pH's were measured using a 3 mm electrode (Horiba 6069-10C) with a Horiba F-24C pH meter both before and after NMR measurements. Since the half-life of tetraphosphate at 25 °C and pH 3 in the presence of Al(III) is ca. 28 d,[11] hydrolysis was negligible during the NMR measurements.

**NMR Measurements.** The NMR spectra were recorded on a JEOL JNM-GSX 500 with a 10 mm multinuclear probe at room temperature. $^{31}P$ NMR spectra were recorded at 202.5 MHz. The standard NMR parameters were as follows: a flip angle of ca. 45° (10 μs), pulse repetition time of 3 s and a spectral width of 36 kHz. The chemical shifts were reported with respect to 85% $H_3PO_4$ as an external reference. $^{27}Al$ NMR spectra were recorded at 130.3 MHz.

The NMR parameters were typically: flip angle ca. 90° (34 μs), pulse repetition time 0.6 s and spectral width 31 kHz. The chemical shifts were reported with respect to a 0.1 mol dm$^{-3}$ $Al(NO_3)_3$ solution containing 0.1 mol dm$^{-3}$ $HNO_3$ as an external reference. A field/frequency lock was achieved with the $^2H$ resonance of $D_2O$ contained in a 2 mm tube. Overlapping signals were resolved into individual peaks using a Laurentzian curve-fitting method.

## Results and Discussion

A series of $^{31}P$ and $^{27}Al$ NMR spectra of aqueous solutions (pH 3) containing tetraphosphate and Al(III) at various concentration ratios ($R=[P_4O_{13}]_T/[Al(III)]_T=3$, 1, and 1/3) are shown in Fig. 1. As can be seen from the $^{31}P$ spectrum at $R=3$, the resonances of free tetraphosphate are observed at $-8$ ppm (two terminal phosphorus atoms) and $-21$ ppm (two middle phosphorus atoms). All other resonances are due to the Al(III)–$P_4O_{13}$ complexes. The $^{31}P$ spectra indicate that there is a slow exchange between the free and Al(III) bound tetraphosphates. The $^{27}Al$ spectra also illustrate a slow exchange between the hexaaqua Al(III) and phosphate bound forms. Al(III) binding to phosphates produces upfield shifts from the signal of $Al(H_2O)_6^{3+}$ in the $^{27}Al$ NMR spectrum. The chemical-shift values of Al(III)–phosphate complexes depend principally on the number of phosphate groups directly coordinating to the Al(III), and the effect, when a water molecule in the ligand field of Al(III) was substituted by a phosphate group, is almost additive.[4,5,14,15] The signals of Al(III) complexes coordinated with the single and two phosphate groups appear at $-3$ to $-4$ ppm and $-6$ to $-7$ ppm, respectively.

At $R=1/3$, the $^{31}P$ spectrum consists of two peaks with equal populations due to a complex at $-19.5$ and $-26.5$ ppm. The shift values were independent of the solution pH. For the $^{27}Al$ spectrum at $R=1/3$, a sharp line corresponding to $Al(H_2O)_6^{3+}$ and a broad one representing the complex of Al(III) coordinated with two phosphate groups were observed at 0 and $-6.6$ ppm, respectively. From integrating the $^{27}Al$ NMR spectrum, one can easily determine the normalized ratio of the complexed Al(III), $([Al]_T-[Al]_f)/[Al]_T$. The titration curve of Al(III) by tetraphosphate is shown in Fig. 2. A linear relationship is obtained for the solutions (pH 3) at $1/3<R<1/2$ and the slope of the plot gives an indication of the stoichiometry of the complex formed under this condition. It is equal to 2.1, strongly suggesting the formation of an $Al_2(P_4O_{13})$ complex. The possible binding structure of the complex is (**I**) or (**II**) (Chart 1), which gives two $^{31}P$ signals with equal populations (for terminal and middle phosphorus atoms), as can be seen for the $^{31}P$ spectra at $R=1/3$. However, the binding isomer (**I**) is more reasonable, because coordination forming 8-membered rings may not take place

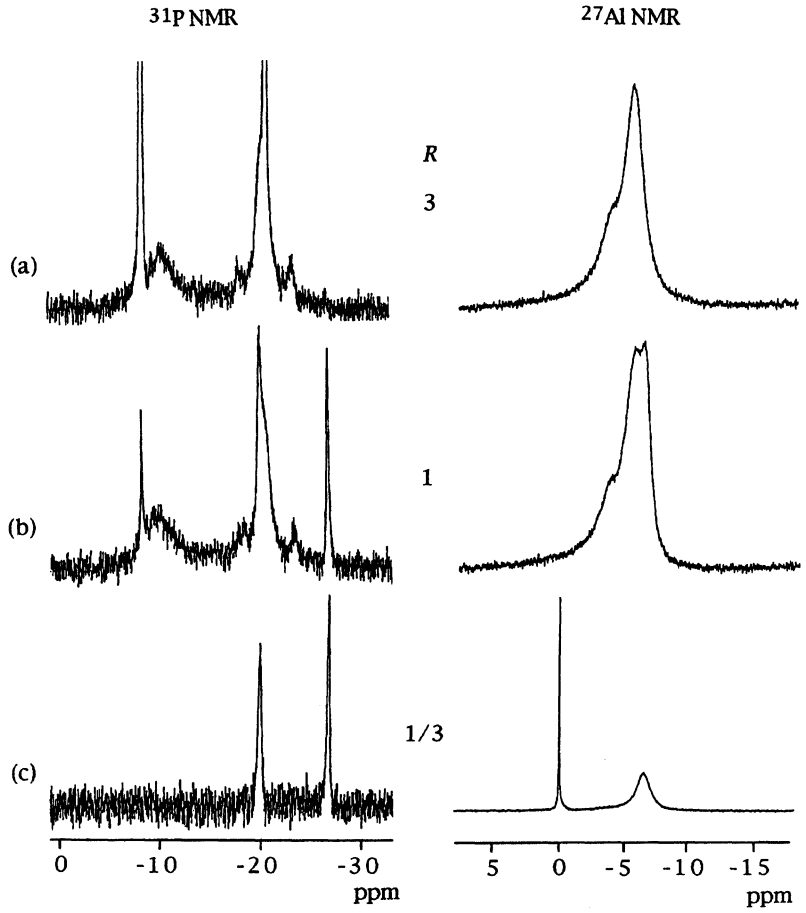



Fig. 1. $^{31}P$ and $^{27}Al$ NMR spectra of aqueous solutions (pH 3) containing tetraphosphate and Al(III) at various concentration ratios: $[Al(III)]_T=2.7$ mM; $R=[P_4O_{13}]_T/[Al(III)]_T=3$ (a), 1 (b), and 1/3 (c).

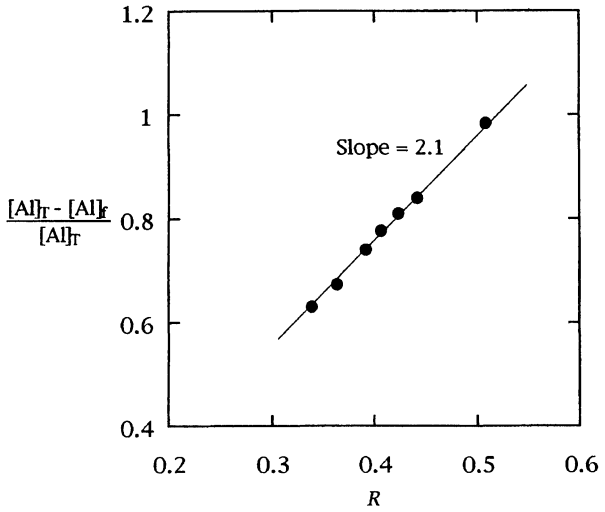



Fig. 2. Titration curve of Al(III) by tetraphosphate obtained from $^{27}$Al NMR. The fraction of complexed Al(III), $([Al]_T-[Al]_f)/[Al]_T$, is expressed as a function of $R=[P_4O_{13}]_T/[Al(III)]_T$: $[P_4O_{13}]_T=2.7$ mM; pH 3.

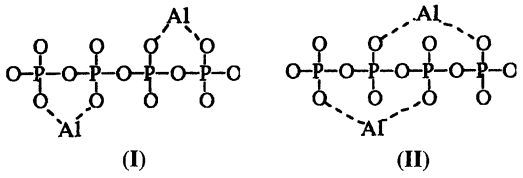



Chart 1.

as a predominant reaction.

At $R=1$, the $^{31}$P spectrum in Fig. 1 contains some broad resonances, which indicate that some complexes coexist under this condition. As the $R$ increases from 1 to 3, the $^{31}$P signal intensity of the free tetraphosphate increases with increasing $R$, while the signals of $Al_2(P_4O_{13})$ at $-19.5$ and $-26.5$ ppm become hardly visible and the broad resonances remain substantially unchanged. The $^{27}$Al spectrum of the equimolar solution (pH 3) illustrates the coexistence of complexes coordinated with a single phosphate group ($-3.6$ ppm) and complexes coordinated with two phosphate groups ($-5.8$ and $-6.6$ ppm). As the $R$ increases from 1 to 3, the $^{27}$Al signal at $-6.6$ ppm corresponding to the $Al_2(P_4O_{13})$ complex becomes hardly visible, while the resonances at $-3.6$ and $-5.8$ ppm remain unchanged in appearance. The intensity ratio of the signal at $-5.8$ ppm to $-3.6$ ppm ($K_{iso}$) was determined by integrating individual peaks which had been resolved by Laurentzian curve-fitting. The $K_{iso}$ value was constant ($K_{iso}=2.02\pm0.19$) at pH 3 (Fig. 3). It seems reasonable to conclude that complexes of 1 : 2 (=Al(III) : $P_4O_{13}$) could not be stoichiometry formed, even in a solution containing excess tetraphosphate. The broad $^{31}$P resonances are attributed to the 1 : 1 complexes of Al(III) coordinated with single and two phosphate groups. The $^{27}$Al peaks at $-3.6$ and $-5.8$ ppm are also ascribed to 1 : 1 complexes with monodentate and didentate bindings, respectively. Therefore, $K_{iso}$ refers to an intramolecular binding isomerization constant for the equilibria between the 1 : 1 complexes with

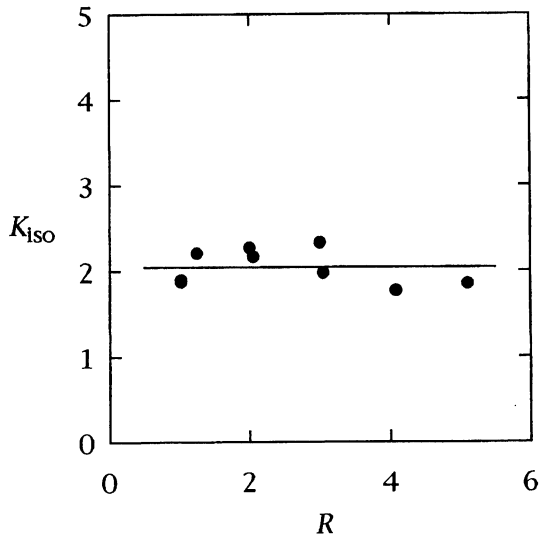



Fig. 3. Binding isomerization of the 1 : 1 Al(III)–tetraphosphate complexes between monodentate and didentate bindings at pH 3. $K_{iso}=$[1 : 1 didentate complex]/[1 : 1 monodentate complex]; $R=[P_4O_{13}]_T/[Al(III)]_T$.

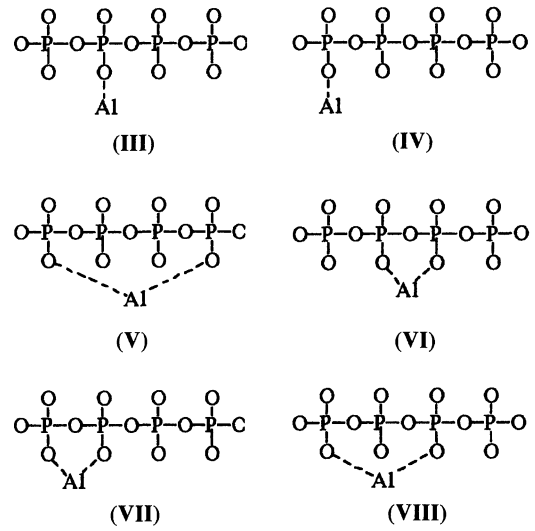



Chart 2.

monodentate and didentate bindings under the experimental condition, viz., $K_{iso}=$[1 : 1 didentate complex]/[1 : 1 monodentate complex]. Possible binding isomers for the 1 : 1 complexes are as follows (Chart 2).

As mentioned above, various binding isomers for the 1 : 1 complexes would coexist. The equilibria between these species may be greatly affected by the pH. The p$K_a$ values of tetraphosphate are p$K_3$=1.36, p$K_4$=2.23, and p$K_5$=7.38,[16] so that the protonation state of the ligand should change depending on the p$K_a$'s of the phosphate at pH<3. Fewer binding sites might become available for complexation with Al(III) as the pH decreases. Figure 4 illustrates the $^{31}$P and $^{27}$Al NMR spectra of equimolar mixtures of tetraphosphate and Al(III) at pH<3. As the pH decreases, the $^{27}$Al signal intensity of the monodentate complex increases, and the signal of free Al(III) appears at pH 1.1. Such a spectral change was

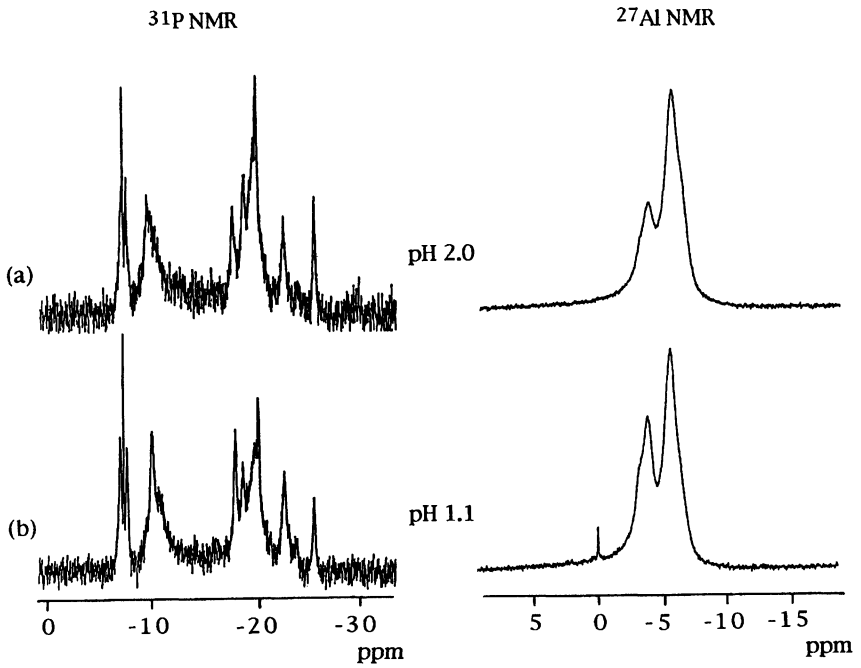



Fig. 4. $^{31}P$ and $^{27}Al$ NMR spectra of equimolar solutions of 5 mM tetraphosphate and Al(III) at pH<3: pH 2.0 (a) and pH 1.1 (b).

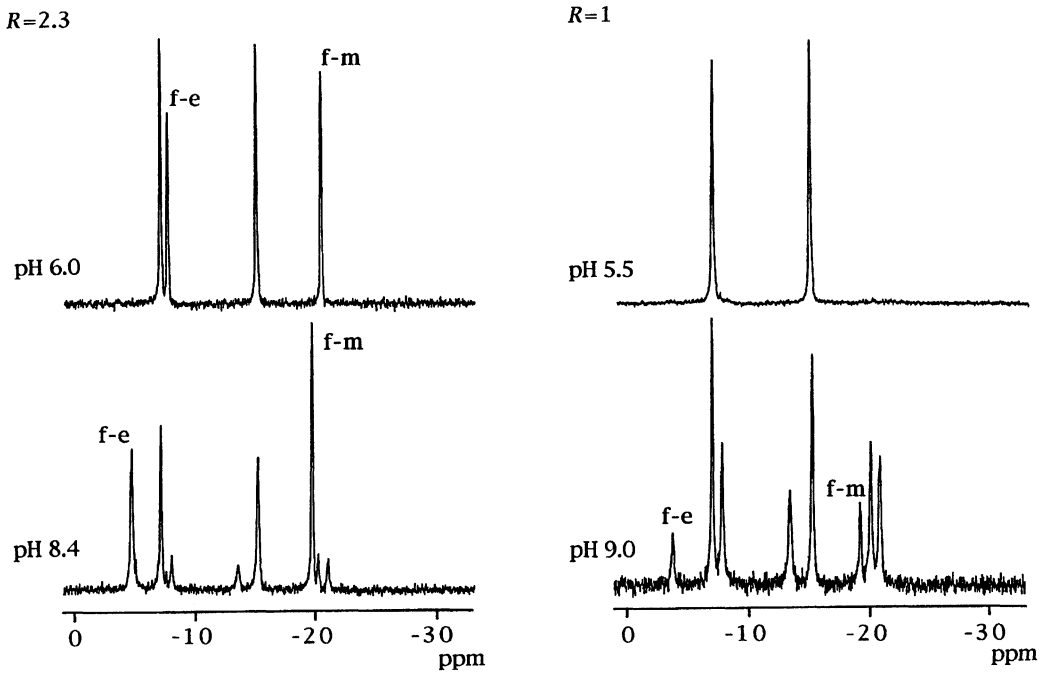



Fig. 5. $^{31}P$ NMR spectra of aqueous solutions containing tetraphosphate and Al(III) in the pH region of 5.5 to 9.0: $[P_4O_{13}]_T$=5.0 mM. The signals of terminal and middle phosphorus atoms of free tetraphosphate are labeled "f-e" and "f-m", respectively.

caused by a rearrangement of the coordinating Al(III) due to the protonation of tetraphosphate in the complexes. There are two kinds of binding isomers for the 1 : 1 complex with the monodentate binding, i.e., (III) and (IV) (Chart 2).

We could not obtain reliable information from the $^{27}Al$ NMR spectra at pH>4 because of the line broadening and downfield shift. This phenomenon, which has also been observed for complexes of Al(III) with carboxyl ligands,[17,18] may be attributed to hydroxide-ion participation in the Al-(III) coordination sphere. Because the aluminum nucleus is quadrupolar, asymmetry in the ligand field produces an increase in the $^{27}Al$ line width. Thus, the ligand substitution by a variable number of hydroxide ions can yield a variety of nonsymmetric aluminum environments to increase the line width.

Figure 5 shows the $^{31}P$ NMR spectra of solutions containing tetraphosphate and Al(III) at pH 5.5—9.0. The signals of free tetraphosphate shift to downfield with increasing pH. In the spectrum for an equimolar solution at pH 9.0, the appearance of resonances of free tetraphosphate re-

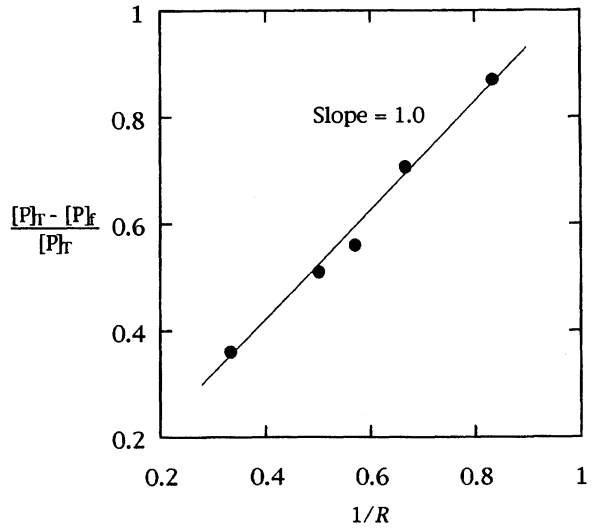



Fig. 6. Titration curve of tetraphosphate by Al(III) obtained from $^{31}$P NMR at pH 6. The fraction of complexed tetraphosphate, $([P_4]_T-[P_4]_f)/[P_4]_T$, is expressed as a function of $1/R=[Al(III)]_T/[P_4O_{13}]_T$: $[Al(III)]_T=2.5$ mM.

veal the dissociation of Al(III)–$P_4O_{13}$ complexes, owing to the hydrolysis of Al(III). In addition to the resonances, two signals with an equal population due to a complex appear at −7.0 and −15 ppm. The shift values were independent of the solution pH. In the pH region of 5.5 to 7, no $^{31}$P resonances other than those of the complexed and free tetraphosphates were recorded. Since the p$K_a$ values of tetraphosphate are p$K_4$=2.23 and p$K_5$=7.38,[16] the predominant ionic species of free tetraphosphate is $H_4P_4O_{13}^{2-}$ at pH 3.5—6. The normalized ratio of the complexed tetraphosphate, $([P_4]_T-[P_4]_f)/[P_4]_T$, was determined by integrating the $^{31}$P NMR spectrum at $0.3<1/R<0.8$ and pH 6. Figure 6 illustrates a linear relationship between $([P_4]_T-[P_4]_f)/[P_4]_T$ and $1/R$. The slope of the plot is equal to 1.0, suggesting the formation of a 1 : 1 complex under this condition. The possible binding structure of the complex is (V) or (VI), which gives two $^{31}$P signals with equal population. However, the binding isomer (VI) is more reasonable, because the coordination forming a 10-membered ring cannot take place as a predominant reaction. At pH>7, four characteristic $^{31}$P signals with equal population due to a complex appear at −8.0, −13.5, −20, and −21 ppm independent of the pH. In the case of the 1 : 1 complex, binding structures (III), (IV), (VII) or (VIII) could exhibit four $^{31}$P signals with equal populations. However, an unambiguous assignment of the binding structure is difficult at the present stage of the NMR results.

More binding sites in tetraphosphate should become available for complexation as the pH increases. One may not rule out the formation of 1 : 1 complexes with tridentate or tetradentate binding at a high pH. However, Al(III) seems impossible to bind with more than two phosphate groups because of a partial replacement of water molecules by hydroxide ions in the coordination sphere. Furthermore, the existence of a complex with tridentate or tetradentate binding should have been expected, even at pH<4, like the formation of tridentate complexes of Al(III) with *cyclo*-hexaphosphate[5] and polyphosphate.[15]

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