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Interaction of Mg^{2+} ions with nucleoside triphosphates by phosphorus magnetic resonance spectroscopy

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SUMMARY The interaction of Mg^{2+} with nucleoside triphosphates: ATP, GTP, CTP and UTP has been studied by phosphorus Magnetic resonance spectroscopy in aqueous solution. The results show that these four nucleotides behave similarly. Purine and Purimidine bases have almost no effect on the phosphate groups even in the N_7 pK region of ATP and GTP. The Mg^{2+} ion binds not to the α and σ but only to the β phosphate group. The fixation is stronger at neutral pH than at acid pH.

INTRODUCTION

The important role of divalent ions in enzymatic reactions in which nucleotides participate as cofactors is well established. The interaction between metal ions and nucleoside triphosphates, in particular adenosine triphosphate, ATP, has been extensively studied[1-6]. Important contributions to the understanding of structure and mechanism of interaction in aqueous solution have been made by the use of nuclear magnetic resonance spectroscopy. Nevertheless many investigations have been devoted to paramagnetic ions[7-16] and few papers dealing with diamagnetic ions have been published[7,17-18]. The main difficulty when using these ions is the purity of the metallic salts. They very often contain paramagnetic impurities which even in very small amounts can considerably add to the broadening and shift of the resonance signals.

The results obtained from studies with paramagnetic ions[7,10-15] have shown that these ions interact with the phosphate groups and the adenine ring : Mn^{2+}, Ni^{2+}, Co^{2+} bind to all three phosphates, while Cu^{2+} interacts with β and σ phosphates only. Concerning diamagnetic ions, Cohn and Hughes[7] observed, for a given pH between 5 and 7, a large shift for Pβ and Pσ and practically no shift for Pα. They concluded that the metal ions :

Mg^{2+}, Ca^{2+}, Zn^{2+} bind to the β and ϖ phosphate groups of ATP but not to the α phosphate group. No attention was paid to an eventual pK change of the solution when adding ions.

In this paper we should like to report a quantitative phosphorus magnetic resonance study of Mg^{2+} complex with four nucleoside triphosphates : ATP ; guanosine-, cytidine- and uridine-, 5'-triphosphate (GTP, CTP, UTP). Mg^{2+} was used throughout this work because it is the natural activator of most of the enzymes which act on phosphorylated substrates, particularly the kinases and synthetases. Activation with Mg^{2+} is usually a fast process while with other ions, activation may take an appreciable time to reach equilibrium. Secondly the salts of Mg^{2+} are more soluble than those of Ca^{2+} or Zn^{2+} at neutral pH, the most suitable pH for enzymatic reactions. Particular attention was paid to the effect of Mg^{2+} concentration, pH and the nature of the base. The results yield information about the nature of the complex, the Mg^{2+} binding site and the conformation of the phosphate chain in presence of Mg^{2+} ions.

MATERIALS AND METHODS

The nucleoside triphosphates : ATP, GTP, CTP and UTP (disodium salts) of highest grade were purchased from CALBIOCHEM AG (Loewengraben, Lucerne, SWITZERLAND) and magnesium chloride (reagent grade $MgCl_2$ $6H_2O$ crystals) from MERCK.

Bivalent metallic ions were systematically removed by shaking D_2O solutions of nucleotide with Chelex 100. The pH was adjusted with concentrated solutions of DCl or NaOD and measured with a Tacussel pH meter. The pD was taken to be equal to pH + 0.4[19].

The ^{31}PMR spectra were recorded at 40.48 MHz on a Varian XL100 - 12WG spectrometer at about 30°C. The apparatus operated in the pulsed Fourier transform mode associated to a 16K 620f Computer. Proton noise decoupling was obtained with a Varian gyrocode spin decoupler. An acquisition time of 3 sec was used throughout this work (resolution = 0.3 Hz). Neither sensitivity nor resolution enhancement was used in any of the spectra. For all the measurements D_2O used as solvent provided the deuterium lock signal. The ^{31}P chemical shifts of nucleotides (in ppm) are

measured from a 85 % H_3PO_4 external reference.

RESULTS AND DISCUSSION

The chemical shifts of the phosphorus and the ^{31}P-^{31}P coupling constants $J_{\alpha-\beta}$, $J_{\beta-\gamma}$ were followed as a function of the Mg^{2+} concentration at constant nucleoside triphosphate concentration (0.05 M) and constant pD (7.5-8.7). In general when the ratio R = $[Mg^{2+}]/[Nucleotide]$ varies from 0 to 2, the chemical shift variation observed is large for P_β and small for P_α and P_γ . On the whole, the nucleoside triphosphates studied : ATP, GTP, CTP, UTP, behave similarly.

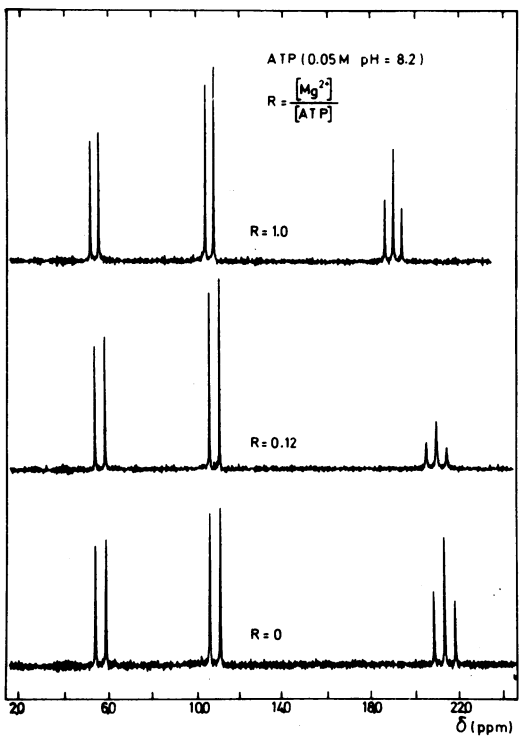

Figure 1 : ^{31}NMR spectra of ATP (0.05 M, pD = 8.2 ; 30°C) at different concentrations of Mg^{2+}.

Figure 1 shows the phosphorus spectra (with proton noise decoupling) of :

- ATP (0.05 M)
- ATP plus a small quantity of Mg^{2+} ion ($\simeq$ 0.006 M)

- ATP plus an equivalent amount of Mg^{2+} (0.05 M).

For R values between 0 and 1, the β line is considerably broadened, whereas those of P_α and P_γ hardly change. When $R \geqslant 1$, the phosphorus spectra of the nucleoside triphosphates return to normal, i.e. the P_β broadening disappears, which shows that the magnesium salt used contains practically no paramagnetic ions.

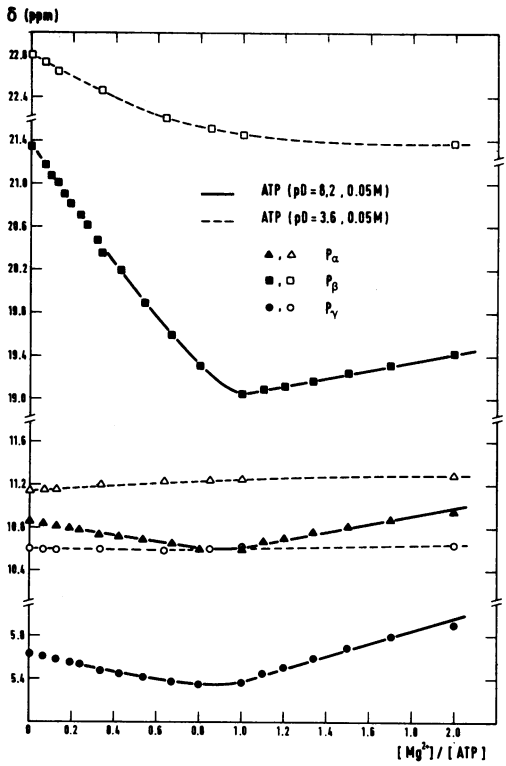

Figure 2 : Chemical shifts of the three phosphorus signals of ATP (in ppm upfield from 85 % H_3PO_4) as a function of $R = [Mg^{2+}]/[ATP]$; —— at pD = 8.2 ; --- at pD = 3.6.

Figures 2 and 3 give respectively the chemical shifts of the P_α , P_β , P_γ and the ^{31}P-^{31}P coupling constants $J_{\alpha-\beta}$, $J_{\beta-\gamma}$ of ATP versus the ratio $R = [Mg^{2+}]/[Nucleotide]$. Table I sums up the main chemical shift values and those of $J_{\alpha-\beta}$, $J_{\beta-\gamma}$ for the four nucleoside triphosphates investigated. It is found that $\delta = f(R)$ curves of the P_α, P_β, P_γ at neutral pD all show a minimum when R = 1.

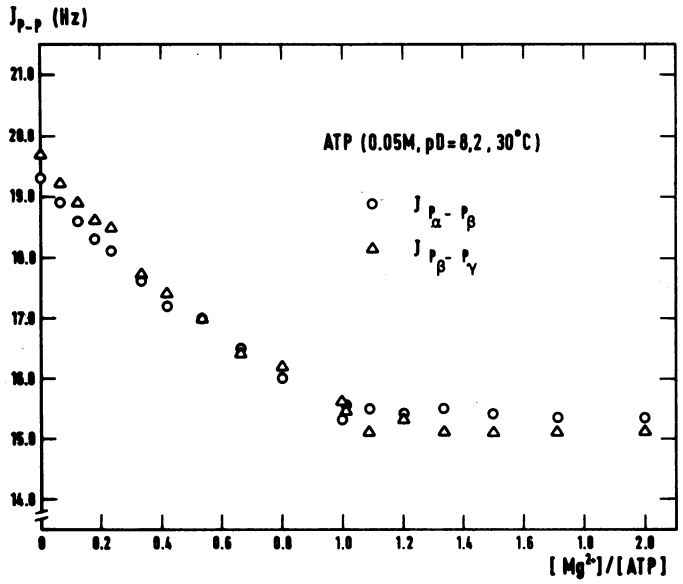

Figure 3 : Change in $^{31}P-^{31}P$ coupling constants of ATP as a function of R = $[Mg^{2+}]/[ATP]$.

When R=0→1, P_α, P_β, P_γ shift towards low field, $\Delta\delta_\beta$ is about 2.3 ppm, whereas $\Delta\delta_\alpha$ and $\Delta\delta_\gamma$ are comparable and much smaller. The δ_β variation is roughly proportional to the Mg^{2+} concentration, while $J_{\alpha-\beta}$ and $J_{\beta-\gamma}$ decrease in the same way (Fig.3) from 19.5 ± 0.3 Hz to 15.5 ± 0.3 Hz.

When R=1→2, P_α, P_β, P_γ shift in the opposite direction (to high field) but now $\Delta\delta_\alpha$, $\Delta\delta_\beta$ and $\Delta\delta_\gamma$ are comparable. On the other hand $J_{\alpha-\beta}$ and $J_{\beta-\gamma}$ hardly change.

If the $J_{\alpha-\beta}$ and $J_{\beta-\gamma}$ values observed are plotted as abscissae and the chemical shift of P_β as ordinates, a straight line is obtained for the R values between 0 and 1 (fig.4).This means that under such conditions (0 R 1), the δ_β variation is only due to fixation of the Mg^{2+} ion on the phosphate chain. When the Mg^{2+} concentration is higher than that of the nucleotides, $J_{\alpha-\beta}$ and $J_{\beta-\gamma}$ remain practically constant which means that the ratio [complexed Mg^{2+}]/[nucleotide] is still nearly equal to one. The shifts observed for the three phosphorus must then be due to another cause. The simultanous occurence of the following observations :

Tableau I : ^{31}P Chemical shifts (in ppm upfield from 85 % H_3PO_4 as external standard), and ^{31}P-^{31}P coupling constants $J_{\alpha-\beta}$, $J_{\beta-\gamma}$ (in Hz) of nucleoside triphosphates at different concentrations of Mg^{2+} (R = $[Mg^{2+}]/[Nucleotide]$).

Nucleotide	R	δ_α	δ_β	δ_γ	$J_{\alpha-\beta}$	$J_{\beta-\gamma}$
ATP	0	10.85	21.34	5.63	19.2	19.5
0.05 M	1.0	10.63	19.08	5.37	15.3	15.6
pD = 8.0	2.0	10.95	19.42	5.89	19.3	15.1
$\Delta\delta$, ΔJ	0→1.0	0.22	2.26	0.26	3.9	3.9
	1→2.0	-0.32	-0.34	-0.52	0	0.5
GTP	0	10.82	21.30	5.51	19.2	19.4
0.05 M	1.0	10.56	19.02	5.31	15.5	15.8
pD = 8.1						
$\Delta\delta$, ΔJ	0→1.0	0.26	2.28	0.20	3.7	3.6
CTP	0	10.89	21.39	5.53	19.1	19.5
0.05 M	1.0	10.50	19.09	5.38	15.4	15.6
pD = 8.4	2.0	10.73	19.33	5.80	15.2	15.0
$\Delta\delta$, ΔJ	0→1.0	0.39	2.30	0.15	3.7	3.9
	1.0→2.0	-0.23	-0.24	-0.42	0.2	0.6
UTP	0	10.92	21.28	5.48	19.5	19.6
0.05 M	1.0	10.60	19.08	5.37	15.5	15.6
pD = 8.7						
$\Delta\delta$, ΔJ	0→1.0	0.32	2.20	0.11	4.0	4.0
UTP	0	10.99	21.60	6.20	19.5	19.5
0.05 M	1.0	10.60	19.26	5.69	15.7	15.6
pD = 7.6	2.0	10.84	19.50	6.12	15.4	15.0
$\Delta\delta$,ΔJ	0→1.0	0.39	2.34	0.51	3.8	3.9
	1.0→2.0	-0.24	-0.24	-0.43	0.3	0.6

- The exchange broadening of the P_β line whereas P_α and P_γ are not broadened.

- $J_{\alpha-\beta}$ and $J_{\beta-\gamma}$ varying linearly with R only for $0 \leq R \leq 1$ and constant for $R > 1$

- The plot of δ_β vs J_{P-P} which reveals a break of the slope for R=1.

show clearly that :

- The Mg^{2+} ion binds not to the α and ϭ but only to the β phosphate group.

- The interaction arises from a 1:1 metal-nucleotide complex.

- The Mg^{2+}-(nucleotide)$_2$ species, does it exists, is only a minor component.

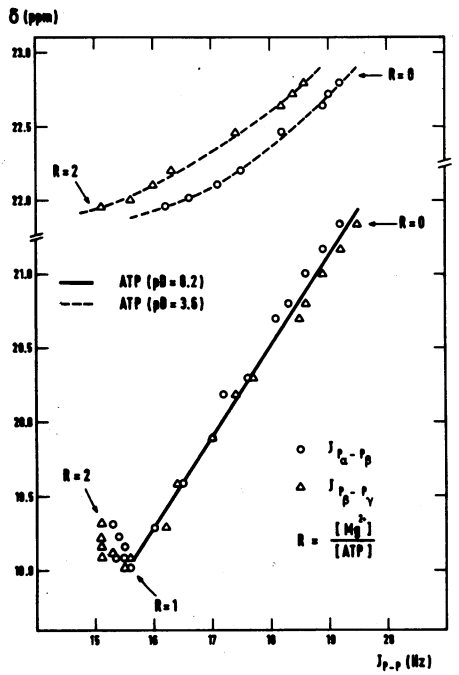

Figure 4 : Chemical shift of P_β versus $J_{\alpha-\beta}$ and $J_{\beta-\gamma}$.

The first conclusion contradicts that reached by Cohn and Hughes[7], who thought that Mg^{2+} is fixed on the β and ϭ phosphate groups of ATP. These authors studied the chemical shift variation of ATP phosphorus in the presence and absence of Mg^{2+} as a function of pH, then compared the difference in chemical shift of the respective ^{31}P's at a given pH between 5 and 7. However the first pK of the P_γ phosphate group lies in this pH region and the presence of metellic ions could therefore modify it.

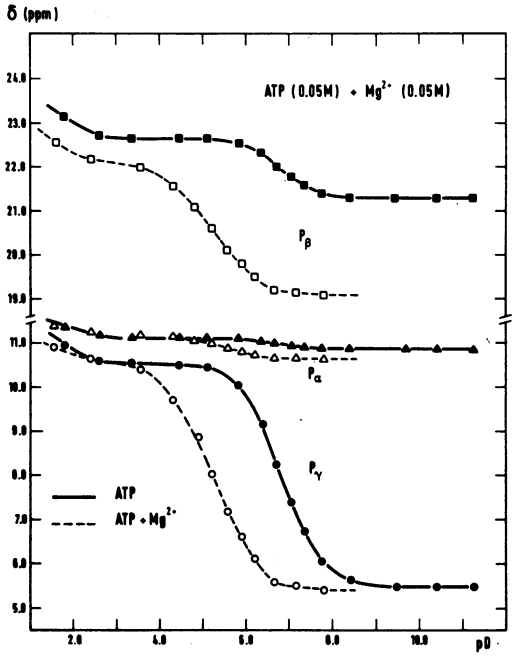

Figure 5 : ^{31}P titration curves of ATP with and without Mg^{2+}.

Figure 5 shows the ATP titration curves in the absence and presence of Mg^{2+}. In both cases the δ_{σ} variation is found to be comparable before (pH=8) and after (pH=3) protonation of this group and only the pK values differ by 1.5 units. This explains a difference of 2.3 and 3.6 ppm for δ_{σ} observed by Cohn and Hughes[7] at pH 4.8 and 6.3 (pD=5.2 and 6.7). We also performed the titration of the three other nucleotides GTP, CTP and UTP, in the absence and presence of Mg^{2+} ions. In the former case (absence of Mg^{2+}) at a given pH the respective chemical shifts of P_{α}, P_{β}, P_{σ} are the same for all the nucleotides. The titration curves between pH 1.5 and 11 are practically superposable. Very similar results are obtained in the latter case (presence of Mg^{2+}). Purine and pyrimidine bases are found to have almost no effect on the phosphate groups, even in the N_7 pK region of ATP and GTP. These results suggest that the α, β and σ phosphorus are relatively far from the base. Kennard et al.[20] have studied the conformation of ATP by X-ray diffraction. The calculated distances between N_7 and the P_{α}, P_{β}, P_{σ} are 5.0, 6.2 and 4.5 Å respectively. Glasman et al.[13] found by proton magnetic resonance that Mn ATP, Co ATP complexes

possess no metal ion-nitrogen coordinate bond and are outer sphere complexes. For the diamagnetic ions Mg^{2+}, Ca^{2+} similar conclusions have been reported [7,17].

It must be noted that although the Mg^{2+} nucleotide complex is always in the ratio 1:1 at both neutral and acid pH (fig.2), the δ_β variation is different in both cases : $\Delta\delta_\beta$=2.3 ppm at neutral and $\Delta\delta_\beta$=0.7 ppm at acid pH. Polarisation of the β phosphate group due to the presence of Mg^{2+} thus seems more important at neutral pH. In addition the large variation of δ_β as compared with those of δ_α and δ_σ when Mg^{2+} complexes with the nucleotides suggests that this ion binds not to the two oxygen atoms of the phosphate chain (P_α-O-P_β-O-P_σ) but to the other two of the β phosphate group (fig.6) otherwise the P_α and P_σ chemical shifts variations should be equal to half $\Delta\delta_\beta$.

Nucleotidyl Transferases ATPases — Phosphotransferases ATPases

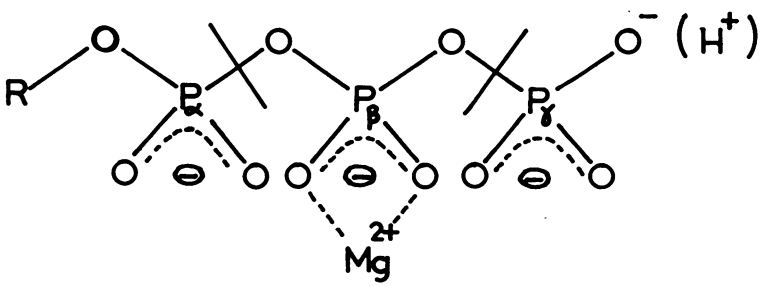

Figure 6 : Possible Mg^{2+} binding sites on the phosphate chain of nucleoside triphosphates and bond cleavage in the kinase and pyrophosphorylase reactions.

The Mg^{2+} fixation on the β phosphate group could easily explain why in phosphotransferase reactions[21], the σ phosphate group of the nucleoside triphosphates alone is eliminated. Steric reasons can also account for the fact that the P_α-O bond are always cleaved by nucleotidyltransferases[22-23] and the P_σ-O bond in phosphotransferases.

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