Biochimica et Biophysica Acta, 589 (1980) 21—29

BBA 47784

COORDINATION OF MAGNESIUM WITH ADENOSINE 5'-DIPHOSPHATE AND TRIPHOSPHATE

FAUSTO RAMIREZ * and JAMES F. MARECEK

Department of Chemistry, State University of New York at Stony Brook, Stony Brook, NY 11794 (U.S.A.)

(Received April 13th, 1979)

Key words: ^{31}P-NMR; Mg-ATP complex; Mg-ADP complex

Summary

^{31}P NMR chemical shifts of salts of adenosine 5'-triphosphate and diphosphate: $ATPH_2^{2-}2(Me_4N^+) \cdot H_2O$, $ATPH_2^{2-}2\,Na^+ \cdot 3.5\,H_2O$, $ATPH_2^{2-}Mg^{2+} \cdot 4\,H_2O$, $ATPH_2^{2-}Ca^{2+} \cdot 2\,H_2O$, $ADPH^{2-}2(Me_4N^+) \cdot H_2O$ and $ADPH^{2-}Mg^{2+} \cdot 4\,H_2O$ have been measured in 0.02 M ^2H_2O solutions at 145.7 MHz (22°C) at constant p^{2}H values (8.20 and 6.20). The results are compared with those obtained from salts of adenosine 5'-monophosphate and other simpler phosphomonoesters, e.g. $AMP^{2-}2(Me_4N^+)$, $AMP^{2-}Mg^{2+}$, $AMPH^-Me_4N^+$ and $(AMPH^-)_2Mg^{2+}$. It is concluded that the effects exerted by Mg^{2+} and Ca^{2+} on the ^{31}P NMR shifts of dipoly- and tripolyphosphates relative to monovalent cations are due mainly to changes in conformation of the polyphosphate chain rather than to purely electronic factors associated with the binding of divalent cations to the phospho-oxyanions. The data are consistent with the existence of the following complexes at p^{2}H 8.20:

$(MgP_\alpha P_\beta)ADP^-$ and $(MgP_\alpha P_\gamma)ATP^{2-} \rightleftharpoons (MgP_\alpha P_\beta)ATP^{2-} \rightleftharpoons (MgP_\beta P_\gamma)ATP^{2-}$

with the latter equilibrium relatively fast in the NMR time scale. Monoprotonation of the terminal phosphate appears to weaken the Mg^{2+}-polyphosphate binding, particularly at P_β of MgADPH and at P_β and P_γ of $MgATPH^-$. The Mg^{2+}-polyphosphate binding weakens further at p^{2}H 3.70, i.e. in $MgATPH_2$. Possible implications of the results in the mechanism of actomyosin Mg^{2+}-ATPase in muscle contraction are discussed.

* To whom correspondence should be addressed.

Introduction

The role played by metal ions in biological systems has been the subject of much discussion [1—5]. Mg^{2+} is essential in a number of reactions in which water, alcohols, carboxylic acids, phosphomonoesters and other nucleophiles attack the P_α or P_γ electrophilic phosphate groups of nucleoside 5′-triphosphates under catalysis by enzymes of the ATPase and kinase types [6,7]. The observation of the ^{31}P NMR spectra of enzyme-bound substrates in 3-phosphoglycerate kinase [8] and pyruvate kinase [9] has been described recently. The interpretation of these observations relies on data concerning the effects on ^{31}P chemical shifts resulting from the binding of ATP, ADP and other phosphorus-containing substrates and products to the enzymes, both in the presence and in the absence of Mg^{2+}. The origin of these effects is not well understood at present, but it is apparent that the problem is related to the more general question of the sensitivity of the ^{31}P and ^{1}H NMR signals to effects exerted by metal ions in nucleoside triphosphates [10—20], and by the molecular conformation of phosphate esters [21].

Cohn and Hughes [22] first disclosed the significant effects of divalent metal ions on the ^{31}P NMR chemical shifts of ADP and ATP in aqueous solutions. These authors found that Mg^{2+} caused down-field shifts of the P_α and P_β signals of ADP, but only of the P_β and P_γ signals of ATP relative to Me_4N^+ cations in 0.1 M solutions of the nucleotides, and concluded from these results that the Mg^{2+} binds to these particular phosphate groups.

Tran-Dinh and Neumann [23], working in 0.05 M ^2H_2O solutions of ADP reached the conclusion that the Mg^{2+} binds exclusively to the P_α atom of the diphosphate. Tran-Dinh et al. [24] had previously concluded that, under comparable conditions, Mg^{2+} binds exclusively to the P_β atom of ATP. These authors ascribed the differences between their findings and those of Cohn and Hughes concerning the binding of the metal ion to the terminal phosphate group of the nucleotides to p*K* variations of this group in the presence of the divalent metal cation. More recently, Jaffe and Cohn [25], in the course of ^{31}P NMR studies on thioanalogs of adenine nucleotides, repeated their early work on the effect of added magnesium salts to ATP. They concluded that this method cannot be employed to specify the metal binding site in a polynucleotide chain.

In view of the importance of the correct structural definition of MgATP complexes, we have reexamined the ^{31}P NMR spectra of pure salts of ADP and ATP at 145.7 MHz in ^2H_2O solutions maintaining constant p^2H values of 8.20, 6.20 and 3.70, and at concentrations low enough to avoid significant self-association of the nucleotides [26]. We have also compared the effects of Ca^{2+} with those of Mg^{2+} under comparable conditions, and have extended the measurements to monovalent cation salts of the nucleotides. The main thrust of our conclusions is that the effects of divalent metal ions on ^{31}P NMR shifts of nucleoside 5′-polyphosphates are the result of changes in the conformation of the polyphosphate chain, and therefore bear no simple correlation to the particular phosphate group with which the metal cation is bound.

Materials and Methods

Preparation of ADP and ATP salts. The salts were prepared as follows. Sigma Chemical Co. grade I $ATPH_2^{2-}2\,Na^+ \cdot 3.5\,H_2O$ and grade VI $ADPH^{2-}2\text{-}(c\text{-}C_6H_{11}NH_3)^+ \cdot 2\,H_2O$ were dissolved in water and rapidly converted into aqueous solutions of $ATPH_4$ and $ADPH_3$ using a column of BioRad AG50W-X8 resin (H^+ form) at 5° C. The column eluate was added to an aqueous solution containing 1 mol equiv. of $Ba(OH)_2$ at 5° C. The mixture was brought to 25° C and was treated with a solution containing 1 mol equiv. of $MgSO_4$. The $BaSO_4$ was removed by centrifugation. The solution was evaporated to 5—10 ml at 15° C (1 mm), frozen, and the remaining water removed by lyophilization. The salt was dried to constant weight over P_2O_5 (18 h at 20° C, 0.5 mm). The aqueous solution of $ATPH_4$ or $ADPH_3$ was added to 2 mol equiv. of $(CH_3)_4N^+OH^-$ in water at 5° C. The salt was isolated as described above. The aqueous solution of $ATPH_4$ was added to 1 mol equiv. of $(CH_3COO)_2Ca$ in water at 20° C. The salt was obtained as described above. The purity of the salts (greater than 99%) was established by TLC (cellulose, isobutyric acid/2.0 N NH_4OH (66 : 34)) and values of the neutralization equivalents obtained by titration of 0.01 N solutions of the salts with 0.100 N $(CH_3)_4N^+OH^-$. The elemental analysis of two of the salts are as follows. $ATPH_2^{2-}Mg^{2+} \cdot 4\,H_2O$, Calcd. for $C_{10}H_{22}N_5O_{17}P_3Mg$: C, 19.9; H, 3.7; Mg, 4.0; N, 11.6; P, 15.4; N.E., 300.8. Found: C, 19.9; H, 3.5; Mg, 4.1; N, 11.6; P, 15.3; N.E., 301.0. $ATPH_2^{2-}2(Me_4N^+) \cdot H_2O$, Calcd. for $C_{18}H_{40}N_7O_{14}P_3$: C, 32.2; H, 6.0; N, 14.6; N.E., 335.7. Found: C, 32.2; H, 6.4; N, 14.5; N.E., 338.5.

High resolution ^{31}P NMR measurements. Each measurement was carried out on a freshly prepared solution containing 0.10 mmol of the salts: $ATPH_2^{2-}2\text{-}[(CH_3)_4N]^+ \cdot H_2O$, $ATPH_2^{2-}2\,Na^+ \cdot 3.5\,H_2O$, $ATPH_2^{2-}Ca^{2+} \cdot 2\,H_2O$, $ATPH_2^{2-}\text{-}Mg^{2+} \cdot 4\,H_2O$, $ADPH^{2-}2[(CH_3)_4N]^+ \cdot H_2O$, and $ADPH^{2-}Mg^{2+} \cdot 4\,H_2O$, in 5.0 ml of ^2H_2O. For measurements on ATP at $p^2H = 3.70$, the solution of the corresponding salt was adjusted to the p^2H values with several μl of 1 N $(CH_3)_4\overset{+}{N}\overset{-}{O}H$ in ^2H_2O. For measurements on ATP at p^2H values 6.20 and 8.20, the corresponding salt was treated with 1 or 2 mol equiv. of $(CH_3)_4\overset{+}{N}\overset{-}{O}H$ in ^2H_2O, respectively. For measurements on ADP at $p^2H = 6.20$ and 8.20, the same procedure was utilized without added $(CH_3)_4\overset{+}{N}\overset{-}{O}H$ solution, or after addition of 1 mol equiv. of the base.

All ^{31}P NMR chemical shifts are given in ppm from 85% H_3PO_4; positive values are down-field from the reference.

pK determinations. The pK_a measurements were carried out by the procedure of Albert and Serjeant [27]. Values for all salts, except Ca^{2+}, are accurate to ±0.1 unit; that for Ca^{2+} to ±0.2 unit. The pH measurements were carried out with an Orion Model 801A Digital Ionanalyzer. The p^2H values are pH + 0.4 [28].

Salts of phosphomonoesters. Phenyl phosphate, $(ArO)P(O)(OH)_2$ or adenosine 5′-phosphate, $AMPH_2 \cdot 2.5\,H_2O$ (Sigma Chemical Co.) were converted into the bis-tetramethylammonium salts by titration with 2 mol equiv. of $(CH_3)_4\overset{+}{N}\overset{-}{O}H$ solution in 0.20 M water. Solutions of the salts were converted into Mg^{2+} salts (1 : 1 stoichiometry) by an ion-exchange resin in the Mg^{2+} form. The mono-tetramethylammonium salts were made by titration of the

acids with 1 mol equiv. of $(CH_3)_4\dot{N}\bar{O}H$ solution. The 2 : 1 phosphate-magnesium salts were made from 2 mol equiv. of the acids and 1 mol equiv. of $Ba(OH)_2$, followed by 1 mol equiv. of $MgSO_4$ as described in the previous section.

Results and Discussion

Acid-base equilibria in ADP and ATP solutions

The values given in the literature for pK_{a3} and pK_{a4} of $ADPH^{2-}$ and $ATPH^{3-}$ are 7.20 and 7.68, respectively, at 25° C, extrapolated to zero ionic strength [29]. The corresponding pK_a values obtained in this study from titrations of 0.01 M H_2O solutions of the salts with 0.100 N $(CH_3)_4\dot{N}\bar{O}H$ at 25° C are as follows: $ADPH^{2-}$ 2 Me_4N^+, 6.9; $ADPH^{2-}Mg^{2+}$, 5.3; $ATPH^{3-}$ 3 Me_4N^+, 7.2; $ATPH^{3-}$ 2 Na^+-Me_4N^+, 7.0; $ATPH^{3-}Ca^{2+}Me_4N^+$, 5.6; and $ATPH^{3-}Mg^{2+}Me_4N^+$, 5.4. It is apparent that the presence of Mg^{2+} increases significantly the acidity of the last ionizable proton of ADP and ATP, (approx. 1.6 and 1.8 pK_a units, respectively), relative to the Me_4N^+ salts. This effect was noted earlier by Smith and Alberty [30,31], who used different techniques to prepare their nucleotide-metal complexes.

The ionic strengths prevailing in the solutions employed for our NMR measurements are comparable to those which exist in the solutions used for pK measurements. Therefore, it can be concluded that the NMR spectra observed at p^2H 8.20 reflect the behavior of species ADP^{3-} and ATP^{4-}. The solutions at p^2H 6.20 should contain significant amounts of ADP^{3-} or ATP^{4-}, in addition to species $ADPH^{2-}$ or $ATPH^{3-}$ when Mg^{2+} and Ca^{2+} are present. The predominant species in solutions at p^2H 3.70 should be $ATPH_2^{2-}$.

Effect of Mg^{2+} on the ^{31}P NMR shifts of phosphomonoesters

An examination of the ^{31}P NMR spectra of 0.20 M H_2O solutions of salts derived from phenyl phosphate at 24.3 MHz (Varian T60A NMR at 35°C) disclosed two effects: (a) salts of the phosphate and Mg^{2+} having 1 : 1 and 2 : 1 stoichiometry have very similar ^{31}P chemical shifts relative to the corresponding Me_4N^+ salts. (b) The first protonation of the dianion is accompanied by a significant up-field shift of the signal ($\Delta \approx 3$—4 ppm), but further protonation does not have much effect on the signal (negative values are up-field from the reference): $ArOP(O)O_2^{2-}$ 2 Me_4N^+, —0.8; $ArOP(O)O_2^{2-}Mg^{2+}$, —0.4; $ArOP(O)(OH)O^-Me_4N^+$, —3.5; $[ArOP(O)(OH)O^-]_2Mg^{2+}$, —3.5; $ArOP(O)(OH)_2$, —3.9 ppm.

An analogous situation is observed among salts of AMP (0.25 M H_2O solutions: AMP^{2-} 2 Me_4N^+, +3.6; $AMP^{2-}Mg^{2+}$, +3.2; $AMPH^-Me_4N^+$, +1.1; $(AMPH^-)_2Mg^{2+}$, +1.0 ppm. The insensitivity of the ^{31}P signal to salt formation with Mg^{2+} is also shown by phosphodiesters, e.g. diphenyl phosphate: $(ArO)_2P(O)O^-Me_4N^+$, —8.7; $[(ArO)_2P(O)O^-]_2Mg^{2+}$, —8.8 ppm;

High resolution ^{31}P NMR studies on ADP and ATP salts

Fig. 1 shows that, at p^2H 8.20, Mg^{2+} exerts the same effect, namely a down-field shift of 0.9 ppm, on the P_α and P_β signals of ADP^{3-} relative to Me_4N^+.

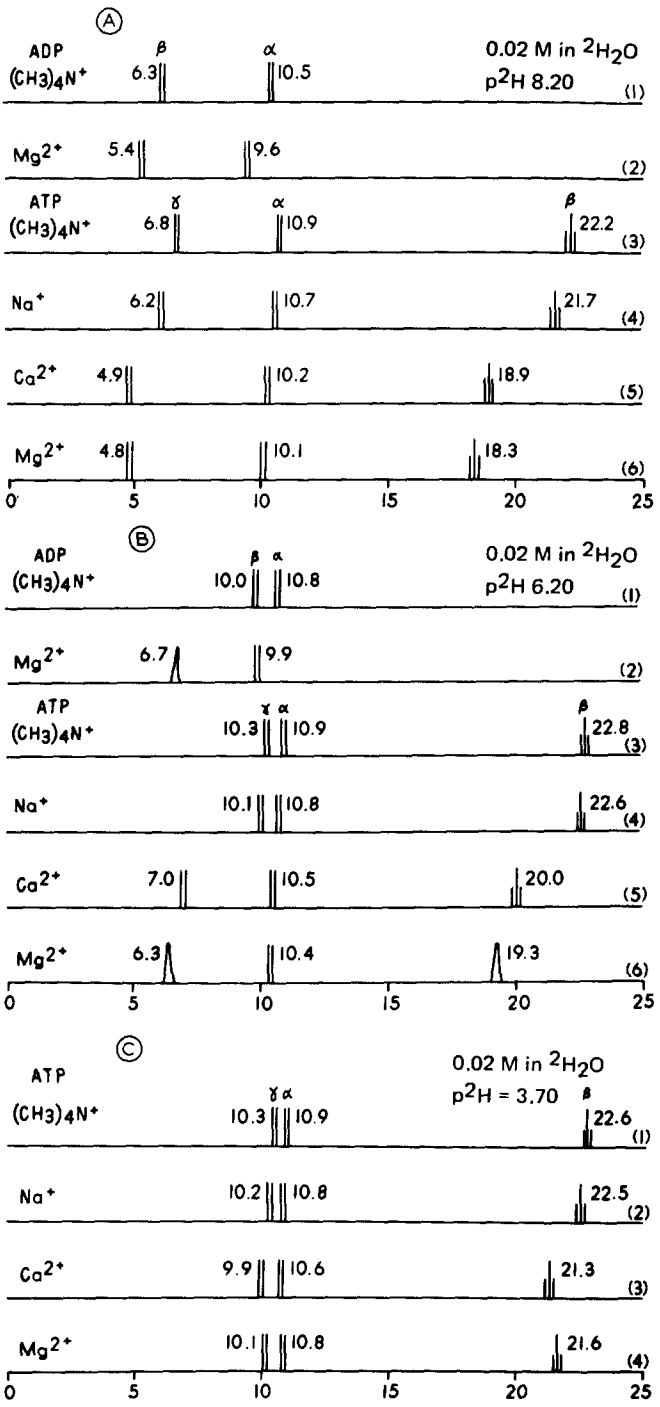
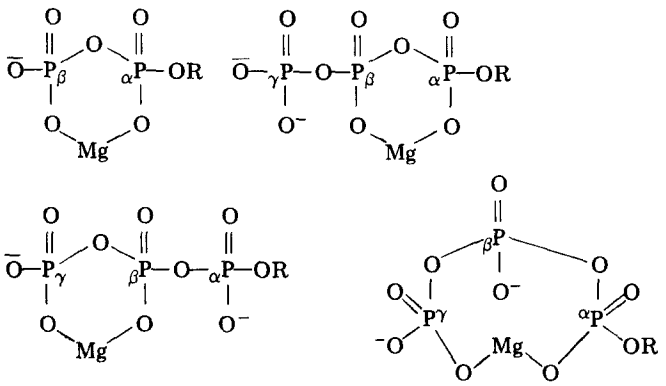


Fig. 1. ^{31}P NMR chemical shifts of adenosine 5'-diphosphate and triphosphate in 0.02 M ^2H_2O in the presence of different metal ions: (A) p^2H 8.20; (B) p^2H 6.20; (C) p^2H 3.70 (p^2H = pH (measured) + 0.4; Ref. 28). Shifts are in ppm to high field of external 85% H_3PO_4, measured in a Brucker WH-360 instrument at 145.7 MHz (22°C), and are accurate to ±0.01 ppm. In the current convention all values should be preceded by a negative sign.

The effect of Mg^{2+} vs. Me_4N^+ on dianions from phosphomonoesters is about one-half as large (0.4 ppm) and varies in direction depending on the structure of the ester, e.g. the shift is up-field for AMP^{2-} and down-field for phenyl phosphate. There is no obvious reason why the effect on ^{31}P NMR shift should be significantly different when Mg^{2+} binds to oxyanions of phospho- and pyrophosphomonoesters, if the effect is due exclusively to electronic factors. Therefore we conclude that the effects on ^{31}P NMR shifts observed when Mg^{2+} binds to ADP are the result mainly of changes in the conformation of the polyphosphate chain.

The conclusion reached above is strengthened by the data obtained with ATP^{4-}. Mg^{2+} causes down-field shifts of 0.8, 3.9 and 1.9 ppm on the P_α, P_β and P_γ signals of ATP^{4-}, respectively, relative to Me_4N^+. As shown in Scheme I, the

Scheme I



formation of six-membered cyclic complexes $(MgP_\alpha P_\beta)ADP^-$, $(MgP_\alpha P_\beta)ATP^{2-}$ and $(MgP_\beta P_\gamma)ATP^{2-}$, and of the eight-membered cyclic complex, $(MgP_\alpha P_\gamma)$-ATP^{2-}, should alter the polyphosphate chain conformation with respect to the conformation of the same chain in salts of monovalent ions. These conformational changes may well account for most of the effects noted in Fig. 1A.

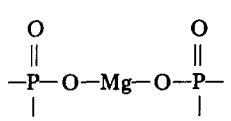
Na^+ resembles Me_4N^+, while Ca^{2+} resembles Mg^{2+}, in their effects on the ^{31}P shifts of ATP^{4-} at p^2H 8.20.

Fig. 1B shows that an increase in acidity of the solutions from p^2H 8.20 to 6.20 has little effect on the P_α signal of ADP and ATP, in the absence and in the presence of Mg^{2+}. However, interesting effects are noted on the terminal phosphate signals, ADP-P_β and ATP-P_γ, as a result of this increase in acidity. In the absence of Mg^{2+}, one observes only the up-field shift to be expected as a result of the protonation of the relatively strong terminal oxyanion base (corresponding to the pK_a values of 6.9 and 7.2 for ADP and ATP, respectively). In the presence of Mg^{2+}, the effect of changing p^2H from 8.20 to 6.20 on the terminal phosphate signal is virtually the same for ADP-P_β and ATP-P_γ, namely, a relatively small (approx. 1.3—1.5 ppm) up-field shift accompanied by a significant broadening of the signal (this loss of resolution is not seen in the respective P_α signals). The broadening effect disappears at p^2H 3.70 (Fig. 1C), where the ATP-P_γ signal becomes again a sharp doublet, with very similar chemical shifts, in the presence and in the absence of Mg^{2+}.

The response of the ATP-P_β signal to the increase in acidity from p^2H 8.20 to 6.20 is also of interest. In the absence of Mg^{2+}, the signal undergoes only a slight (approx. 0.6 ppm) up-field shift, as expected of a relatively weak oxyanion base (i.e. a relatively strong conjugate acid). However, in the presence of Mg^{2+}, the increase in acidity causes a 1.0 ppm up-field shift and a significant broadening of the ATP-P_β signal, as was the case for the ATP-P_γ signal. Again, the broadening effect of the ATP-P_β signal disappears at p^2H 3.70, and the shift approaches the value it has at this acidity in the absence of Mg^{2+}.

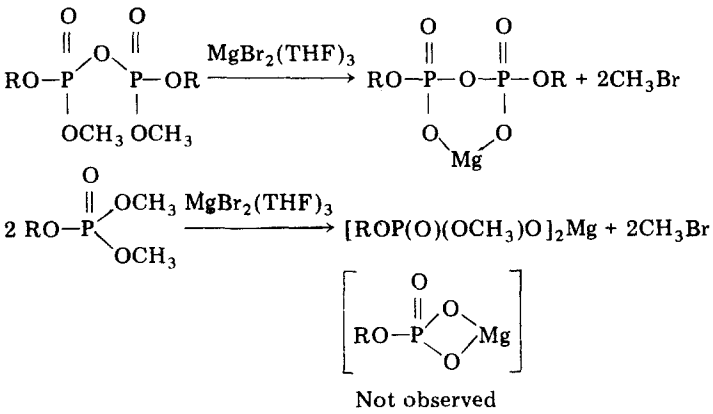
It seems to us that the data in Fig. 1 do not allow a definitive choice between the three possible complexes of $MgATP^{2-}$ in aqueous solution. Indeed, it may very well be that the three complexes exist in a dynamic equilibrium which is established rapidly in the time scale of the NMR phenomenon. Even the relative amounts of the three complexes present at equilibrium cannot be deduced with assurance from these data; however, the close correspondence of the Mg^{2+} effect on the P_α atoms of ADP^{3-} and ATP^{4-}, and the relatively large differences in the Mg^{2+} effects on the P_β atoms of the two nucleotides may indicate that the predominant complex is $(MgP_\alpha P_\gamma)ATP^{2-}$, since the conformation of this complex should differ the most from that of the only complex possible for $MgADP^-$, i.e. the six-membered cyclic $(MgP_\alpha P_\beta)ADP^-$ (cf. Scheme I).

The broadening of the ADP-P_β and the ATP-P_β and P_γ signals at p^2H 6.20 in the presence of Mg^{2+} is puzzling. This apparent loss of resolution is not observed with Mg^{2+} at 0.1 M concentration, other parameters being constant. The effect is not observed with Ca^{2+} under the same experimental conditions that produce the effect with Mg^{2+}. The only explanation we can offer is that protonation of the terminal phosphate weakens the Mg^{2+}-polyphosphate binding, and that this allows the establishment of intermolecular complexes of the type:



For example, terminal protonation of ADP and weakening of the $P_\beta O$-Mg binding would produce intermolecular complexes of type $[(\dot{M}g\bar{P}_\alpha\bar{P}_\beta H)ADP]_n$, $n > 1$, in equilibrium with the intramolecular complex, $(MgP_\alpha P_\beta H)ADP$. At the lowest concentration studied, the rate of formation of the intermolecular complexes could be sufficiently slow in the NMR time scale to produce the line-broadening effect noted. The picture would be analogous, although more complicated, in the ATP solutions. Terminal protonation generates the intramolecular complexes: $(MgP_\alpha P_\gamma H)ATP^-$, $(MgP_\alpha P_\beta \cdot P_\gamma H)ATP^-$ and $(MgP_\beta P_\gamma H)$-ATP^-, which would be in equilibrium with the corresponding intermolecular complexes resulting from weakening of the $P_\gamma O$-Mg, $P_\beta O$-Mg and $P_\gamma O$-Mg binding, respectively. In this picture, the complex-weakening effect of terminal protonation is assumed to be weakest at the more remote P_α group, i.e. the $P_\alpha O$-Mg binding is preserved. It would be reasonable also to expect a further weakening of the Mg^{2+}-polyphosphate binding at p^2H 3.70.

Scheme II



Not observed

Previous studies in this laboratory may be pertinent to the present work. X-ray diffraction analysis discloses the ability of Mg^{2+} to bind two phosphate groups quite tightly, $\diagup$P(O)OMgOP(O)$\diagdown$, at least in the crystalline state [32]. The reaction of a magnesium bromide-tetrahydrofuran complex with methyl pyrophosphate esters shows that six and eight-membered cyclic magnesium pyrophosphates can be prepared, as illustrated in Scheme II [33]. However, two methyl groups on the same phosphate atom cannot be displaced by one Mg^{2+}; i.e., four-membered cyclic complexes with Mg, O and P atoms in the ring, which have been suggested in the literature, cannot be made by this procedure. Quite probably, salts with a 1 : 1 dianion : magnesium stoichiometry are either cyclic dimers or linear polymers: $[ROP(O)O_2^{2-}Mg^{2+}]_n$ rather than monomers with a four-membered ring.

In summary, the results summarized in Fig. 1 differ to some extent from those previously described [22—24], although one of our conclusions, namely, that the effects of Mg^{2+} on the ^{31}P NMR shifts of ATP do not provide a straightforward indication of the sites of cation attachment, are in line with a reevaluation of earlier work [25]. Differences between our data and previous work are due to the following factors: (a) lower concentration of the nucleotides, which minimizes self-association [26], and (b) use of pure salts of the nucleotides free from extraneous ions.

The significance of our results lies in a better understanding of the ^{31}P NMR data in relation to the binding of Mg^{2+} to ATP and ADP. Moreover, the data support the recent hypothesis [34,35] that an important element in actomyosin Mg^{2+}-ATPase in muscle contraction involves the tight binding of an eight-membered cyclic $(MgP_\alpha P_\gamma)ATP^{2-}$ complex to the myosin active-site pocket, and the hydrolysis of this complex to another tightly bound myosin-ADP-Mg-P_i complex, which is transformed into actomyosin-MgADP-P_i, with a six-membered cyclic $(MgP_\alpha P_\beta)ADP^-$ complex and loosely bound P_i, by interaction with the protein actin.

Acknowledgments

This work was supported by Grant GM-20672 from the National Institutes of General Medical Sciences, and was partially carried out at Brookhaven National Laboratories under auspices of the U.S.A. Department of Energy. We are grateful to Dr. Alan McLaughlin and Mr. Donald Lawler for their assistance in obtaining NMR spectra.

References

1 Dhar, S.K. (1973) Metal Ions in Biological Systems, Plenum Press, New York
2 Eichhorn, G.L. (1973) Inorganic Biochemistry, Chapter 33, Elsevier, Amsterdam
3 Sissoeff, I., Grisvard, J. and Guille, E. (1976) Prog. Biophys. Mol. Biol. 31, 165—180
4 Sigel, H. (ed.) (1974—1976) Metal Ions in Biological Systems, Vols. 1—6, Marcel Dekker, New York
5 Kluger, R. (1977) Bioinorganic Chemistry (Van Tamelen, E., ed.), Vol. 4, Chapter 9, Academic Press, New York
6 Koshland, D.E., Jr. (1955) Discuss. Faraday Soc. 20, 142—151
7 Boyer, P.D. (ed.) (1970; 1973—1974) The Enzymes 3rd edn., Vol. III, Chapter 9; Vol. III, Chapter 7—16, Vol. IX, Chapter 1—3; Vol. X, Chapter 11—13, Academic Press, New York
8 Nageswara Rao, B.D., Cohn, M. and Scopes, R.K. (1978) J. Biol. Chem. 253, 8056—8060
9 Nageswara Rao, B.D., Kayne, F.J. and Cohn, M. (1979) J. Biol. Chem. 254, 2689—2696
10 Sternlicht, H., Shulman, R.G. and Anderson, E.W. (1965) J. Chem. Phys. 43, 3133—3143
11 Sternlicht, H., Shulman, R.G. and Anderson, E.W. (1965) J. Chem. Phys. 43, 3123—3132
12 Labotka, R.J., Glonek, T. and Myers, T.C. (1976) J. Am. Chem. Soc. 98, 3699—3704
13 Burt, C.T., Glonek, T. and Barany, M. (1976) J. Biol. Chem. 251, 2584—2591
14 Cozzone, P.J. and Jardetzky, O. (1976) Biochemistry 15, 4853—4865
15 Cornelius, R.D., Hart, P.A. and Cleland, W.W. (1977) Inorg. Chem. 16, 2799—2805
16 Hammes, G.G., Maciel, G.E. and Waugh, J.S. (1961) J. Am. Chem. Soc. 83, 2394—2395
17 Hoppe, J.A. and Morales, M. (1966) J. Am. Chem. Soc. 88, 2077—2078
18 Glassman, T.A., Cooper, C., Harrison, L.W. and Swift, T.J. (1971) Biochemistry 10, 843—859
19 Granot, J. and Fiat, D. (1977) J. Am. Chem. Soc. 99, 70—79
20 Sternlicht, H., Jones, D.E. and Kustin, K. (1968) J. Am. Chem. Soc. 90, 7110—7118
21 Gorenstein, D.G. (1975) J. Am. Chem. Soc. 97, 898—900
22 Cohn, M. and Hughes, T.R., Jr. (1962) J. Biol. Chem. 237, 176—181
23 Tran-Dinh, S. and Neumann, J.M. (1977) Nucleic Acids Res. 4, 397—403
24 Tran-Dinh, S., Roux, M. and Ellenberger, M. (1975) Nucleic Acids Res. 2, 1101—1110
25 Jaffe, E.K. and Cohn, M. (1978) Biochemistry 17, 652—657
26 Lam, Y.F. and Kotowycz, G. (1977) Can. J. Chem. 55, 3620—3630
27 Albert, A. and Serjeant, E.P. (1971) The Determination of Ionization Constants, Chapter 2 and 3, Chapman and Hall, London
28 Glasoe, P.K. and Long, F.A. (1960) J. Phys. Chem. 64, 188—192
29 Phillips, R., Eisenberg, P., George, P. and Rutnam, R.J. (1965) J. Biol. Chem. 240, 4393—4397
30 Smith, R.M. and Alberty, R.A. (1956) J. Am. Chem. Soc. 78, 2376—2380
31 Alberty, R.A. (1969) J. Biol. Chem. 244, 3290—3302
32 Narayanan, P., Ramirez, F., McCaffrey, T., Chaw, Y.F. and Marecek, J.F. (1978) J. Org. Chem. 43, 24—31
33 Ramirez, F., Marecek, J.F., Chaw, Y.F. and McCaffrey, T. (1978) Synthesis 519—522
34 Ramirez, F., Shukla, K.K. and Levy, H.M. (1979) J. Theor. Biol. 76, 351—357
35 Shukla, K.K., Ramirez, F., Marecek, J.F. and Levy, H.M. (1979) J. Theor. Biol. 76, 359—367