Nuclear Magnetic Resonance Spectra of Adenosine Di- and Triphosphate

II. EFFECT OF COMPLEXING WITH DIVALENT METAL IONS*

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The requirement of a divalent metal ion for all enzymatic reactions involving adenosine di- and triphosphate is well established, but the role of the metal ion is not clearly understood. In some kinase reactions (1, 2) kinetic evidence has been presented that the metal complex of adenosine triphosphate is the true substrate in the enzymatic reaction. The present study was undertaken to obtain information concerning changes in the structure of the metal complex of adenosine triphosphate which might shed some light on the changes in the reactivity of specific bonds in the enzyme-substrate complex for these compounds.

In a previous paper (3), it was shown that chemical shifts of the nuclear magnetic resonance peaks of the phosphorus nuclei of adenosine di- and triphosphate could be measured on 0.1 M solutions of the nucleotides. The same techniques have been applied in the present investigation to observe the changes in the chemical shifts of the phosphorus magnetic resonance spectra in the presence of metal ions to give direct evidence of the nature of the complexes formed. With the use of high resolution technique, the chemical shifts of the proton magnetic resonance spectra in the nucleotides could be compared with the shifts in the metal complexes to determine the interaction of the metal ion with the adenine ring.

The measurements were extended to include the effect of paramagnetic ions such as Mn ++ and Cu ++ at low concentrations of the order of 10⁻⁴ to 10⁻⁵ M on the width of the absorption lines of both phosphorus and protons. Such spectra revealed preferential interactions with particular phosphorus nuclei and protons, thus specifying the nature of the metal complexes formed.

EXPERIMENTAL PROCEDURE

Preparation of Samples—The crystalline disodium salt of ATP and the sodium salt of ADP were obtained from the Sigma Chemical Company. The tetramethylammonium ion was substituted for the sodium ion by treatment of the nucleotides with Dowex 50 (California Corporation for Biochemical Research) in the tetramethylammonium form at 0°C. The metals were added as solutions of the metal chlorides which were standardized by chloride analysis. The pH was adjusted by the addition of (CH₃)₄NOH and was determined on a Beckman Zeromatic pH meter. The final concentration of nucleotide was determined by absorbance at 259 mµ with a Zehes spectrophotometer.

For measurement of the proton resonance spectra, the solution in D₂O was lyophilized, and the residue was dissolved in D₂O. The lyophilization and addition of D₂O was repeated to minimize the amount of H₂O in the final sample. Approximately 0.5 ml of 0.1 M ATP sufficed for each sample.

Recording of Spectra—The spectra were recorded with a Varian 4302 dual purpose NMR spectrometer. The broadline phosphorus spectra were obtained at 24.3 mc per second, as described previously (3). The proton spectra were obtained at 60.45 mc per second by standard high resolution technique with toluene as the external standard. The tetramethyl group of the tetramethylammonium ion present in all solutions could also serve as an internal standard for the proton spectra. The chemical shift data is expressed in terms of a dimensionless constant defined by:

\[ \delta = \frac{H_{\text{sample}} - H_{\text{reference}}}{H_{\text{reference}}} \times 10^6 \]

The reference peak for the P spectra is 85% H₃PO₄, used previously (3), and that for the H spectra is the aromatic peak of toluene as used by Jardetzky and Jardetzky (4) in their study of nucleotides. Four spectra were recorded for each sample, and the values of the chemical shifts were averaged. The chemical shifts were not corrected for bulk magnetic susceptibility differences which were considered negligible.

RESULTS

Effect of Mg ++ , Ca ++ , and Zn ++ on Chemical Shifts of Phosphorus in ATP and ADP—The chemical shifts of 0.1 M solutions of tetramethylammonium ATP with and without equimolar amounts of metal ion are compared at various pH values in Fig. 1. The effect of Mg ++ , Ca ++ , and Zn ++ are very similar, namely, a large shift in the \( \beta \)- and \( \gamma \)-P and practically no shift in the \( \alpha \)-P. From these chemical shifts, it may be inferred that these metal ions bind to the \( \beta \)- and \( \gamma \)-phosphate groups of ATP, but...
not to the \( \alpha \)-phosphate group. It should also be noted that addition of these metal ions shift the \( \gamma \)-P to lower fields as anticipated from lower shielding in contrast to addition of the last ionizable proton which shifts the \( \gamma \)-P to higher field. When the Mg concentration was doubled, no significant change was observed in the spectrum.

It was possible to study only the Mg complex of ADP, since the Ca and Zn complexes were not sufficiently soluble in the pH range of interest. A comparison of the change in chemical shifts caused by the addition of Mg\(^{++} \) to ADP and ATP, respectively, is shown in Table I. Unlike the \( \alpha \)-P of ATP, the \( \alpha \)-P of ADP is significantly shifted. The \( \beta \)-P of ADP is shifted as anticipated, thus indicating the formation of a complex involving the \( \alpha \)- and \( \beta \)-P. Again, the shift caused by addition of Mg\(^{++} \) is opposite in direction to the shift observed upon protonation of the second ionized group of the \( \gamma \)-phosphate.

Effect of Mg\(^{++} \), Ca\(^{++} \), and Zn\(^{++} \) on Proton Chemical Shifts in ATP.—The chemical shifts of the proton magnetic resonance peaks have been investigated by Jardetzky and Jardetzky (4). In confirmation of their work, it has been found that only the protons on \( C_1 \) and \( C_6 \) of the adenine ring and the carbon 1 of ribose are well resolved, and the data for these 3 protons\(^1 \) only are given in Table II. The values for the chemical shifts in 0.1 M ATP at pH 7.2 agree fairly well with those previously reported in 0.2 M ATP measured at 40 mc (4). Obviously, there is no change in the shift of the proton on carbon 1 of ribose, \( \Pi_1 \), caused by the addition of metal ions. The only significant shift is the shift to lower field, approximately \(-0.25 \) p.p.m., observed for the \( \Pi_1 \) peak upon the addition of Zn\(^{++} \). The shift found with zinc indicates that the zinc has formed a complex with the adenine ring with consequent diminution of the shielding of \( \Pi_1 \). For the \( \Pi_2 \) proton, the data is not so clear-cut. From the data in Table II, it can be seen that there is no significant shift of \( \Pi_2 \) caused by the addition of any metal ion. The addition of Zn\(^{++} \) affects only \( \Pi_3 \) and not \( \Pi_2 \). Jardetzky and Jardetzky (4) have reported a change in the chemical shift of \( \Pi_2 \) of \(-0.16 \) p.p.m. between pH 4.75 and 5.9 due to protonation of the ring. It must be concluded that Zn\(^{++} \) does not bind at the same site of the adenine ring as H\(^+ \). The equality of the chemical shift of \( \Pi_3 \) in the presence of metal ions and in the nonprotonated ring in the absence of metal ions, pH 7.1, and the constancy of the chemical shift over the pH range in the presence of added metal ions may be ascribed to a lowering of the pK of the ring due to the binding of the metal ion to the phosphate groups throughout the pH range measured as evidenced by the data in Fig. 1. If the constancy of the chemical shift of \( \Pi_3 \) were due to the binding of metal ion in place of H\(^+ \), the chemical shift would be expected to differ quantitatively from the shift observed when a proton is bound.

The line widths for the metal complexes are included in Table II since they decreased strikingly with pH, an effect not observed in the absence of metal ions. The Ca complex differed from the Mg and Zn complexes in that the line width was minimal at pH 6.0, and broadened at pH 8.5 as well as pH 4.9. It should be noted that \( \Pi_1 \), the doublet due to interaction with \( \Pi_s \), was resolved throughout the pH range in the Mg complex, but much better resolved at the higher pH values. For the Ca complex, the \( \Pi_1 \) peak appears as a singlet at pH 4.9, is well resolved at pH 6.6, and broadens again at pH 8.5, as do the \( \Pi_2 \) and \( \Pi_3 \) peaks. The Zn complex is unique in that \( \Pi_1 \) appears as a single peak at all pH values. The collapse of the doublet in the Zn complex may be due to the broadening characteristic of all the proton peaks in the complex, or alternatively, it may be due to a change in conformation of the ribose upon complex formation with a consequent change in the coupling constant between \( \Pi_1 \) and \( \Pi_2 \) (cf. Jardetzky (5)).

Effect of Paramagnetic Ions on Phosphorus Resonance Spectra—Paramagnetic ions broaden the nuclear magnetic resonance lines of the nuclei of a compound with which they form complexes because the magnetic field of the ion changes the relaxation time of the magnetically active nuclei and because the complexed molecule is exchanging with the uncomplexed molecule in solution. The theory of such effects has been discussed by McConnell (6) and Pearson et al. (7). In the case of ATP, it has been possible to observe interactions with specific phosphate groups due to formation of complexes with Cu\(^{++} \), Mn\(^{++} \), and Co\(^{++} \) at concentrations of the paramagnetic ions between \( 10^{-5} \) and \( 10^{-4} \). The addition of Cu\(^{++} \) broadens only the \( \beta \) and \( \gamma \) peaks, as illustrated in Fig. 2, Curves C and D, indicating a complex with ATP which involves only the \( \beta \) and \( \gamma \) phosphate groups. On the other hand, Mn\(^{++} \) seems to form complexes with ATP involving all three phosphate groups as indicated by the broadening of all three P peaks in Fig. 2, Curve B. The

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\(^1\) H denotes hydrogen in the adenine moiety, \( H' \) denotes hydrogen in the ribose moiety, and the subscripts refer to the carbon atoms to which the hydrogens are bonded in adenine and ribose, respectively.
TABLE II
Chemical shifts and line widths of protons of ATP in presence of metal ions

Values are given for the 2 protons on the adenine, H₈ and H₉, and the proton on carbon 1 of ribose, H₁. The chemical shifts are referred to the ring protons of toluene. The concentration of ATP and metal ion is 0.1 M.

<table>
<thead>
<tr>
<th>Metal</th>
<th>pH</th>
<th>H₈</th>
<th>~ line width</th>
<th>H₂</th>
<th>~ line width</th>
<th>H¹</th>
<th>~ line width</th>
</tr>
</thead>
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<tr>
<td></td>
<td></td>
<td>δ</td>
<td>p.p.m.</td>
<td>δ</td>
<td>p.p.m.</td>
<td>δ</td>
<td>p.p.m.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>±</td>
<td></td>
<td>±</td>
<td></td>
<td>±</td>
</tr>
<tr>
<td>Mg</td>
<td>4.8</td>
<td>-2.02 ± 0.05</td>
<td>14.0</td>
<td>-1.73 ± 0.04</td>
<td>9.5</td>
<td>+0.37 ± 0.02</td>
<td>Doublet</td>
</tr>
<tr>
<td></td>
<td>6.3</td>
<td>-1.98 ± 0.01</td>
<td>5.6</td>
<td>-1.72 ± 0.06</td>
<td>3.0</td>
<td>+0.37 ± 0.03</td>
<td>Doublet</td>
</tr>
<tr>
<td></td>
<td>9.0</td>
<td>-2.04 ± 0.03</td>
<td>2.9</td>
<td>-1.70 ± 0.01</td>
<td>2.4</td>
<td>+0.35 ± 0.02</td>
<td>Doublet</td>
</tr>
<tr>
<td>Ca</td>
<td>4.0</td>
<td>-1.99 ± 0.03</td>
<td>15.1</td>
<td>-1.63 ± 0.02</td>
<td>13.8</td>
<td>+0.36 ± 0.03</td>
<td>Singlet, 13.8</td>
</tr>
<tr>
<td></td>
<td>6.6</td>
<td>-2.02 ± 0.01</td>
<td>9.2</td>
<td>-1.64 ± 0.01</td>
<td>3.1</td>
<td>+0.30 ± 0.003</td>
<td>Doublet</td>
</tr>
<tr>
<td></td>
<td>8.5</td>
<td>-2.01 ± 0.02</td>
<td>10.0</td>
<td>-1.64 ± 0.05</td>
<td>9.7</td>
<td>0.34 ± 0.05</td>
<td>Doublet</td>
</tr>
<tr>
<td>Zn</td>
<td>4.4</td>
<td>-2.21 ± 0.03</td>
<td>16.1</td>
<td>-1.70 ± 0.02</td>
<td>14.3</td>
<td>+0.41 ± 0.03</td>
<td>Singlet, 13.8</td>
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<tr>
<td></td>
<td>5.9</td>
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<td>13.5</td>
<td>-1.69 ± 0.03</td>
<td>7.0</td>
<td>0.30 ± 0.02</td>
<td>Singlet, 9.7</td>
</tr>
<tr>
<td></td>
<td>7.3</td>
<td>-2.24 ± 0.01</td>
<td>5.9</td>
<td>-1.67 ± 0.01</td>
<td>6.6</td>
<td>0.38 ± 0.02</td>
<td>Singlet, 9.4</td>
</tr>
<tr>
<td>None</td>
<td>7.1</td>
<td>-2.07 ± 0.03</td>
<td>-</td>
<td>-1.72 ± 0.03</td>
<td>-</td>
<td>+0.38 ± 0.02</td>
<td>-</td>
</tr>
</tbody>
</table>

*C.p.s. = cycles per second.

The addition of Co²⁺ affects the spectrum in a qualitative manner similar to Mn²⁺.

The error in the quantitative estimation of line widths is approximately ±15% under the conditions of the measurements. Nevertheless, the values in Table III indicate that the change in line width varies in an approximately linear manner with Mn²⁺ concentration. The broadening with Mn²⁺ was observed over the pH range 3.3 to 7.6, yielding evidence that Mn²⁺ interacts with α-, β-, and γ-phosphates of HATP and ATP.

The effect of Mn²⁺ on the phosphorus peaks of ADP was similar to that on ATP, causing broadening of the α and β peaks. With the addition of Cu²⁺ to ADP, both the α and β peaks are broadened. Again, as deduced from the chemical shift data with Mg²⁺, it appears that when three phosphate groups are available in ATP, no complex formation occurs with the α-phosphate group but if only two phosphate groups are available in ATP, the metal will form a complex with them. The addition of EDTA in concentrations slightly in excess of the Cu²⁺ completely obliterates the broadening effect of either ADP or ATP.

Effect of Paramagnetic Ions on Proton Spectra—The broadening of the proton peaks due to the addition of Mn²⁺ or Cu²⁺ is illustrated in Fig. 3. Although all the proton peaks are somewhat broadened, the most dramatic effect is observed on H₈. In fact, at Mn²⁺ concentration of 8 X 10⁻⁵ M, the H₈ peak is so broad that it is no longer observable. The forming of complexes with Cu²⁺ and Mn²⁺ affects the same proton, H₈, as does formation of complexes with Zn²⁺, and it may be tentatively concluded that the three metal ions form a complex with the adenine ring at the same site. The same kind of proton spectra

FIG. 2. Phosphorus magnetic resonance spectra of ATP (0.1 M), pH 7.2, in the presence of paramagnetic metal ions. A, no additions; B, 8 X 10⁻⁵ M MnCl₂; C, 1.65 X 10⁻⁶ M CuCl₂, D, 3.15 X 10⁻⁶ M CuCl₂.

TABLE III
Effect of Mn²⁺ concentration on line widths of phosphorus spectra of 0.1 M ATP, pH 7.2

<table>
<thead>
<tr>
<th>Concentration of Mn²⁺</th>
<th>Line width</th>
<th>Δ</th>
<th>Line width</th>
<th>Δ</th>
<th>Line width</th>
<th>Δ</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>37.2</td>
<td>42.5</td>
<td>40.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>41.6</td>
<td>4.4</td>
<td>48.0</td>
<td>5.5</td>
<td>49.1</td>
<td>8.6</td>
</tr>
<tr>
<td>4</td>
<td>56.7</td>
<td>19.5</td>
<td>58.5</td>
<td>16.0</td>
<td>68.0</td>
<td>27.5</td>
</tr>
<tr>
<td>8</td>
<td>79.5</td>
<td>42.3</td>
<td>83.2</td>
<td>40.7</td>
<td>94.5</td>
<td>54.0</td>
</tr>
</tbody>
</table>
were obtained for ADP upon addition of Cu$^{++}$ or Mn$^{++}$, as shown in Fig. 3 for ATP.

**DISCUSSION**

A variety of possible structures of the strong complexes (8-14) of ATP and ADP with divalent metal ions have been proposed on the basis of the chemical and enzymatic reactivity of these complexes. Indirect evidence has been adduced to support particular structures from models (13, 14), infrared spectra data (15). The present study is the first to give direct experimental evidence distinguishing the specific phosphate groups which are involved in the complexes of 0.1 M solutions of metal nucleotides. From the chemical shifts of the phosphorus peaks, it is clear that Mg$^{++}$, Ca$^{++}$, and Zn$^{++}$ in equimolar concentrations form a complex predominantly with the $\beta$- and $\gamma$-phosphate groups of ATP. If a small fraction of the ATP was in a form which involved metal binding to the $\alpha$-phosphate, it would not have been detected. A striking difference is observed between the Mg$^{++}$ complex of ATP and ADP; Mg$^{++}$, the only metal ion measured with ADP, forms a complex with the $\alpha$- and $\beta$-phosphate groups of ADP. The difference in the stability constants of the ADP and ATP complexes (12) may be ascribed to the fact that the ADP complex involves the $\alpha$- and $\beta$-phosphate groups and the ATP complex involves the $\beta$- and $\gamma$-phosphate groups.

From the chemical shifts of the proton peaks, it may be concluded that Zn$^{++}$, but not Mg$^{++}$ or Ca$^{++}$, forms a complex with the adenine ring of ATP involving the nitrogen in position 7, since it is the proton on C8 which is shifted. The uniqueness of the Zn complex, relative to the Mg and Ca complexes, is consistent with the results of McCormick and Levedahl (16) on rotatory dispersion. Although the data for phosphorus and proton shifts for Zn$^{++}$ are consistent with an intramolecular chelate structure, such as postulated by Szent-Györgyi (14), they do not eliminate the possibility of an intermolecular complex. Further studies on the proton shift as a function of Zn$^{++}$ and ATP concentration should elucidate this point.

The spectrophotometric studies of Hotta, Brahms, and Morales (17) led them to conclude that Mg forms a complex to the adenine as well as to phosphate. However, their data show no absolute shift in the ultraviolet spectrum of ATP due to addition of Mg$^{++}$, which would imply that the complex has the same spectrum as free ATP. The shift observed by these investigators in the region of pH 4 could be explained by a lowering in pK of protonation of the ring caused by Mg$^{++}$ complexing with...
the \( \beta \)- and \( \gamma \)-phosphate groups. This interpretation, namely a lowering of pK of the ring, would be consistent with our finding that \( H_2 \) proton in the metal complexes has the same chemical shift at pH \( \sim 4.5 \) as the nonprotonated ring in free ATP, i.e. pH 6. Furthermore, in the metal complexes there is no change in the chemical shift with pH in region of 4.5 to 6.0, as there is in free ATP. In contrast to the \( Mn^{++} \) effect on the ultraviolet absorption spectrum of ATP, \( Zn^{+} \), which exhibits complexing by the proton resonance data, also causes an absolute spectroscopic shift in the spectrum of ATP at pH 7.7. It may be concluded that if \( Mg^{++} \) and \( Ca^{++} \) do bind to the adenine ring as well as to the \( \beta \)- and \( \gamma \)-phosphates of ATP, the complex must be of a different type from the \( Zn \) complex, and only a small fraction of the total ATP molecules, too small to be detected by NMR spectrometry, exists in such a form in 0.1 M solutions.

The lack of reactivity of the \( Mg \)- and \( Ca \) ATP complexes and the reactivity of the \( Zn \) ATP have been demonstrated in two enzymatic reactions, namely, the hydrolysis catalyzed by inorganic pyrophosphatase of yeast (18) and the \( CO_2 \)-dependent hydroxylamine kinase activity of pyruvate kinase (19). A special role of \( Zn^{+} \), in contrast to \( Mg^{++} \) and \( Ca^{++} \), has been described in muscle relaxation (20) and may also be related to the difference in structure of the ATP complexes. It is tempting to suggest that the \( N_\gamma \) position of ATP must be chelated to the metal ion for the enzyme to interact with the substrate for these reactions. For example, inorganic pyrophosphatase from yeast catalyzes the hydrolysis of either \( Mg \) or \( Zn \) pyrophosphate, but only the hydrolysis of \( Zn \) ATP. It may be that \( Mg \) ATP is bound to the enzyme through \( N_\gamma \), which is available, thereby forming an inactive enzyme-substrate complex. In the case of \( Zn^{++} \), the \( N_\gamma \) position of ATP is chelated to \( Zn \) so that the \( Zn \) ATP resembles \( Zn \) pyrophosphate much more than \( Mg \) ATP resembles magnesium pyrophosphate.

The data from the broadening due to paramagnetic ions shows that \( Cu^{++} \) may form the same type of complex with ATP as \( Zn^{++} \), namely, it interacts with the \( \beta \)- and \( \gamma \)-phosphate groups and \( H_\alpha \) of the adenine ring. It is difficult to assess the quantitative aspects of these interactions which are observed at a ratio of \( Cu:ATP \) of approximately 1:10,000. Without a considerably extended investigation, one cannot estimate if the \( Cu^{++} \) is interacting with the phosphates and adenine at the same rate, i.e. does a single molecule of ATP necessarily have a conformation involving simultaneous binding to both the phosphates and the ring, or are they independent? For ADP, \( Cu^{++} \) again shows interaction with \( H_\alpha \) of the adenine, but the \( \alpha \)-phosphate as well as the \( \beta \)-phosphate is now bound. The difficulty of interpretation of this type of data mentioned above is further emphasized by the fact that the P peak and \( H_\alpha \) peak of AMP, which forms a relatively weak complex with \( Cu^{++} \), are broadened by \( Cu^{++} \) in a manner analogous to ADP.

The detailed interpretation of the data for \( Mn^{++} \) is beset with even greater uncertainties. Although there is no question that \( Mn^{++} \) interacts with the \( \alpha \)-, \( \beta \)-, and \( \gamma \)-phosphate groups of ATP and the \( H_\alpha \) of the adenine ring, only further study can determine whether there exists a single complex or a mixture of complexes. The fact that \( Mn^{++} \) can serve in place of \( Zn^{++} \) in those enzymatic reactions of ATP in which \( Mg^{++} \) is inactive (18, 19), as well as replace \( Mg^{++} \) in those enzymatic reactions of ATP that require \( Mg^{++} \), suggests that \( Mn \) ATP may exist in several forms. The effect of \( Mn^{++} \) on the broadening of the resonance peaks of ADP indicates an interaction of \( Mn^{++} \) with the \( \alpha \)- and \( \beta \)-phosphate groups and the \( H_\alpha \) of the adenine ring. Evidence that the structure of the predominant species of \( Mn \) ATP and ATP differ in \( 10^{-4} \) M solutions containing equimolar ratios of metal and nucleotide has been obtained from electron spin resonance spectra.

In conclusion, it should be pointed out that the two methods used in the present study for the investigation of metal complexes of the nucleotides, the chemical shift data obtained with non-paramagnetic metal ions lends itself more readily to straightforward interpretation in terms of molecular structure. Nevertheless, the change in line width observed with paramagnetic metal ions is potentially of greater value and may lead to the elucidation of the kinetics of interaction as well as structural information.

SUMMARY

The structure of the complexes of adenosine di- and triphosphate with divalent metal ions has been investigated by studying the nuclear magnetic resonance spectra of the hydrogen and phosphorus nuclei of the nucleotides. The chemical shifts of the phosphorus nuclei in the presence of equimolar concentrations of \( Mg^{++} \), \( Ca^{++} \), and \( Zn^{++} \) indicate that these metals form complexes with the \( \beta \)- and \( \gamma \)-phosphate groups of adenosine triphosphate and with the \( \alpha \)- and \( \beta \)-phosphate groups of adenosine diphosphate. The chemical shifts of the proton resonance peaks in the adenosine triphosphate complexes showed that only \( Zn^{++} \) causes a change, namely, the \( H_\alpha \) resonance peak is shifted to lower field due to binding to the adenine ring. All the proton peaks were greatly broadened due to the addition of metal ions at pH of approximately 4.5.

The effect of low concentrations of the order of \( 5 \times 10^{-5} M \) of paramagnetic ions on line broadening of the phosphorus resonance demonstrated that \( Cu^{++} \) interacts solely with the \( \alpha \)- or \( \beta \)-phosphate groups of adenosine triphosphate, but \( Mn^{++} \) and \( Ca^{++} \) interact with the \( \alpha \)-, \( \beta \)-, and \( \gamma \)-phosphate groups. With adenosine diphosphate, \( Cu^{++} \) as well as \( Mn^{++} \) interacts with the \( \alpha \)- and \( \beta \)-phosphate groups. The paramagnetic ions also show specific broadening of the \( H_\alpha \) peak. The data is discussed in terms of the molecular configuration of the metal complexes and the implications for specificity as substrates in enzymatic reactions.

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Addendum—Since this work was completed, a note by Hammes et al. (21) appeared, in which he and his co-workers measured the chemical shift of the proton resonance peaks of ATP and of the Mg and Cs complexes of ATP and observed, as in the present investigation, that there was no significant difference.

REFERENCES

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