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Cohn M & Hughes T R. Nuclear magnetic resonance spectra of adenosine di- and triphosphate. II. Effect of complexing with divalent metal ions. *J. Biol. Chem.* 237:176-81, 1962. [Dept. Biol. Chem. and Dept. Phys., Washington Univ., St. Louis, MO and Johnson Res. Fdn., Univ. Pennsylvania, Philadelphia, PA]

^1H- and ^{31}P-NMR spectra of the diamagnetic divalent ion (Mg^{2+}, Ca^{2+}, Zn^{2+}) complexes of ADP and ATP showed the largest change in chemical shift for the β-P of ATP, ~3ppm from the uncomplexed nucleotides. The paramagnetic ions, Mn^{2+} and Co^{2+}, at low molar ratios to nucleotide caused a concentration-dependent broadening of the α-, β-, and γ-P resonances of ATP (Cu^{2+} broadened only β- and γ-P); all caused a broadening of the H_8 resonance of the adenine ring. [The *SCI*® indicates that this paper has been cited in over 400 publications.]

NMR of Metal Nucleotide Complexes

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In 1955 I was interested in understanding the specificity of obligatory divalent metal ions in enzymatic reactions of ATP. One possible source of these differences was a variation in the structures of the substrate, e.g., MgATP *vs.* CaATP, in the enzyme-substrate complexes. In seeking an experimental approach to differentiate these structures, it occurred to me that ^1H and ^{31}P-NMR had the potential to yield an answer.

When I returned to Washington University in 1956 after sabbatical leave in Oxford, I found that I could not do ^{31}P-NMR in St. Louis because the chemistry department's homemade instrument could only detect protons. I contacted Varian Associates, the only manufacturer of NMR spectrometers at that time. They were very interested in my project and invited me to come to California immediately, but it was not feasible until 1958 because of family obligations. In those two years, the organic chemists had discovered the usefulness of NMR, and Varian gave a rather cool reception to my project. Nevertheless, I was able to have one day on the spectrometer for proton NMR and, a month later, one day for ^{31}P-NMR. I could indeed distinguish the three ^{31}P peaks of ATP at 1 M concentration, and I could observe a chemical shift due to Mg^{2+} binding and a broadening of the resonances to invisibility by the paramagnetic Mn^{2+}.

I returned to St. Louis having established feasibility. The chemistry department of the University of Illinois generously allowed me to use their new acquisition, a 60 MHz Varian instrument, on occasional weekends. They requested me not to acknowledge them in my publications because they didn't want it known that they allowed outsiders to use their spectrometer. Sometime in 1959, Sam Weissman in our chemistry department obtained a Varian DP60, and I now had access to an instrument nearer to home.

Tom Hughes, a physics graduate student, both talented and a perfectionist, succeeded in obtaining high-resolution ^{31}P spectra of ADP and ATP at 0.5 M. To investigate the effect of pH and metal ions, we used a concentration of 0.09 M to record low-resolution spectra in 12 mm nonspinning plastic tubes (containers for Havana cigars that Tom smoked). I failed to observe an effect of the enzyme hexokinase (2 mg/ml) on the MgATP spectrum (90 mM); I anticipated a much larger effect than exists.[1]

The paramagnetic effect of Cu^{2+} on the ^{31}P spectrum, i.e., the broadening of the β- and γ-P resonances of ATP and the apparent narrowing of the α-P resonance, was discovered by serendipity. To avoid the effect of Na^+ binding, I had exchanged the Na^+ in the commercial ATP with the very weakly complexing $(CH_3)_4N^+$ ion with an ion-exchange column. Instead of improving the spectrum, I observed the unexpected broadening of the β- and γ-P resonances and the narrowing of the α-P resonance. When I showed the spectrum to my chemist colleagues, Dave Lipkin said, "That looks like a paramagnetic ion effect." My colleague Sid Velick, whose resin I had used, revealed that he had sized the resin by using brass sieves, a customary procedure at that time. I proved by chemical analysis that the ATP treated with this resin did indeed contain copper. Since Cu^{2+} can only form square planar complexes, only the β- and γ- were paramagnetically broadened; the apparent narrowing of the α-P resonance was due to saturation of the β-P, which caused the collapse of the unresolved α doublet. Mn^{2+} and Co^{2+}, which form octahedral complexes, broaden α-, β-, and γ-P resonances of ATP.

My colleague Sam Weissman suggested that I submit a communication to the *Journal of the American Chemical Society* on the high-resolution ^{31}P spectrum of ATP and the effect of metal ions on the spectrum. I did so and the manuscript was rejected. Subsequently, I published two papers in the *Journal of Biological Chemistry*, one on the effect of pH on the ^{31}P chemical shifts of ADP and ATP,[2] and the other, the subject of this commentary.

Since the publication of this work, the effect of paramagnetic ions on the NMR parameters (T_1 and T_2) of enzyme-bound ATP and of other substrates has been used to determine structure.[3,4] The chemical shift change in β-P of ATP due to Mg^{2+} has been used to determine the free Mg^{2+} concentration *in vivo* in cells[5] and in animal organs.[6]

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