This Week’s Citation Classic


1H and 31P-NMR spectra of the diamagnetic divalent ion (Mg2+, Ca2+, Zn2+) complexes of ADP and ATP showed the largest change in chemical shift for the 3P of ATP. ∆3Ppm from the uncomplexed nucleotides. The paramagnetic ions, Mn2+ and Cu2+, at low molar ratios to nucleotide caused a concentration-dependent broadening of the ν-α, β, and γ-P resonances of ATP (∆ν = broadened only β- and γ-P; α-P is unaffected). This caused a broadening of the Hα resonance of the adenine ring. (The SCP 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 obligate 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, I contacted my colleague Sam Weissman, who had sized the metal ions on the enzyme-bound substrate doublet, revealed that he had sized the resin by using brass sieves, a customary procedure at that time, and consequently, I was able to have one day on the spectrometer for proton NMR and, a month later, one day for 31P-NMR. I could indeed distinguish the three 31P peaks of ATP at 1 M concentration, and I could observe a chemical shift due to Mg2+ binding and a broadening of the resonances to invisibility by the paramagnetic effect. When 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 occasion 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, 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 31P 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. The paramagnetic effect of Cu2+ on the 31P spectrum, i.e., the broadening of the β- and γ-P resonances of ATP and the apparent narrowing of the γ-P resonance, was discovered by serendipity. To avoid the effect of Na+ binding, I had exchanged the Na+ for the commercial ATP with the very weakly complexing (CH3)2N+ 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 tissue-injury after brain trauma. This Week’s Citation Classic

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