P 15
PHOSPHORUS

ANASAZI **APPLICATION SERIES**

BROADBAND PROBE / MULTINUCLEAR PROBE

^{31}P NMR OF ADENOSINE PHOSPHATES

Anasazi Instruments

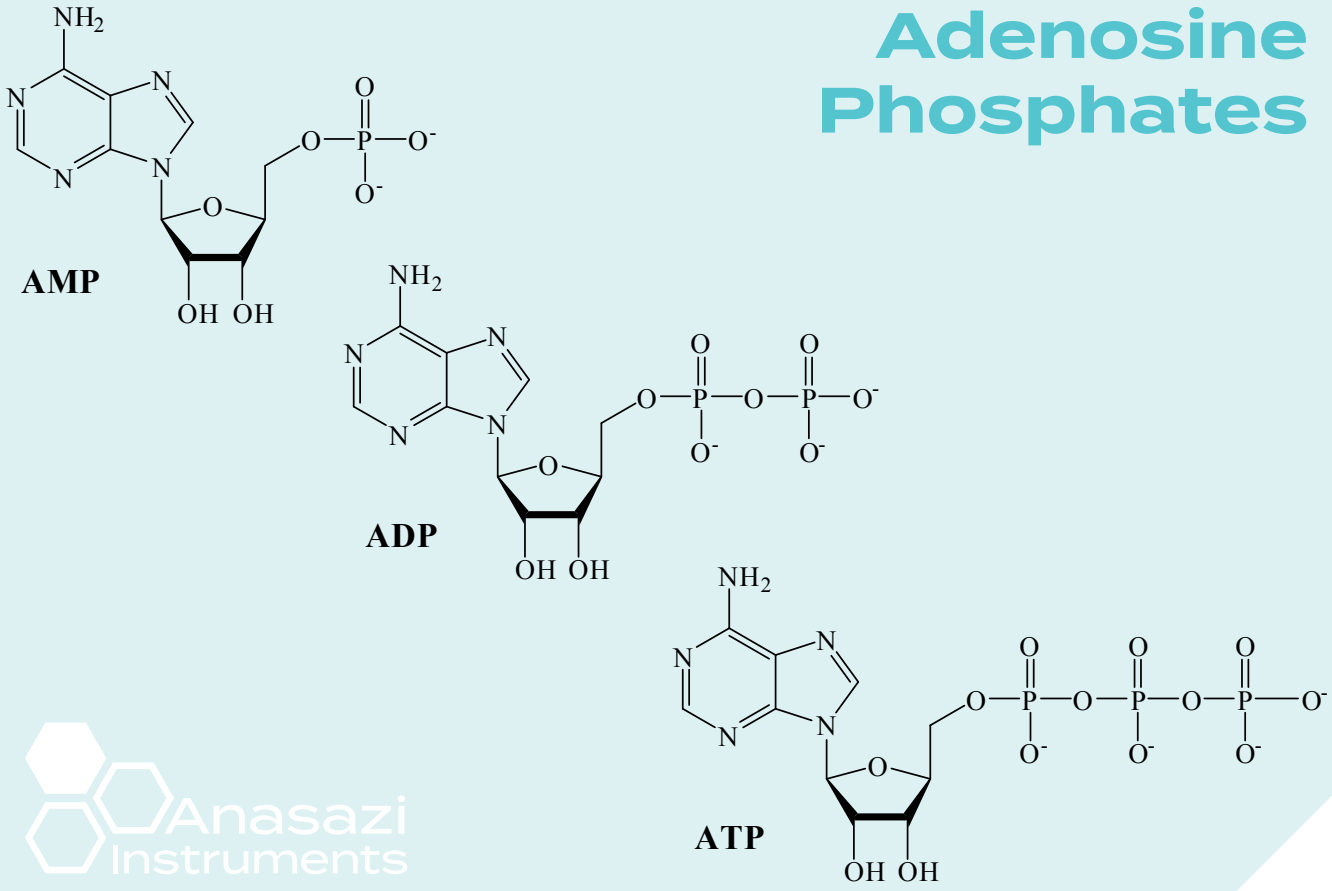
DID YOU KNOW?

Adenosine phosphates are vital for all living organisms. The interconversion of adenosine diphosphate (ADP) and adenosine triphosphate (ATP) not only generates energy for your cells, but also stores energy for when you need it later. When a phosphate group is cleaved from ATP, energy is released and used by your cells. On the other hand your cells capture energy by synthesizing ATP. ATP is now free to move about the cell to other locations that need energy. As a result, the amount of ADP and ATP in a cell changes constantly.

ATP and ADP's little cousin, adenosine monophosphate (AMP) plays a role in this energy cycle. Interestingly enough, AMP is also used commercially as a 'bitter blocker', a food additive to alter human perception of taste. AMP is also in your RNA... weird huh?

^{31}P NMR can be used to study the change in concentration of various phosphorus species in human muscles during exercise and rest. Using the ^{31}P spectra of AMP, ADP, and ATP students learn how to interpret ^{31}P NMR spectra of adenosine phosphates and use ^{31}P NMR to determine the quantities of ADP and ATP in an unknown sample.

Adenosine Phosphates



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[1] Friebolin, H. Basic One-and Two-Dimensional NMR Spectroscopy, pp. 377-9.

SPECTRA & INTERPRETATION

The figures below show typical ^{31}P NMR spectra of AMP, ADP, and ATP with and without proton decoupling on an Eft-60 spectrometer. The α, β, γ notation often confuses students. So herein, the phosphate closest to the adenosine in all three molecules is referred to as α, and the middle phosphate of ATP as β. The last phosphate in ADP and ATP is referred to as terminal, regardless of whether it is β or γ.

The proton-decoupled AMP spectrum (Fig. 1a) shows a singlet at δ 3.5. With no decoupling (Fig. 1b), the signal becomes smaller due to the lack of NOE and begins to resolve into a triplet (J = 4.3 Hz) from coupling to the CH_2.

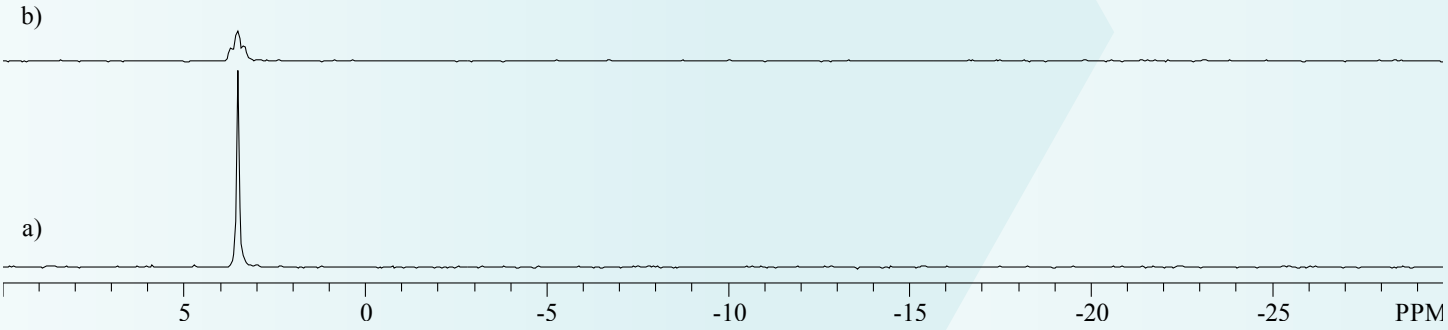

Figure 1. ^{31}P NMR spectra of AMP (a) with and (b) without proton decoupling.

The proton-decoupled ADP spectrum (Fig. 2a) consists of a pair of doublets δ -6.4 and -10.4 arising from the two mutually-coupled phosphate groups, with J = 21 Hz. The integral of the δ -10.4 signal is 5-10% larger than that of the δ -6.4 signal. With no decoupling (Fig. 2b), the integrals are approximately equal and the δ -10.4 signal begins to split into a doublet of triplets from the addition of coupling to the CH_2 (J = 5.2 Hz). These changes identify the δ -10.4 signal as arising from the α position and the δ -6.4 signal as the terminal phosphate

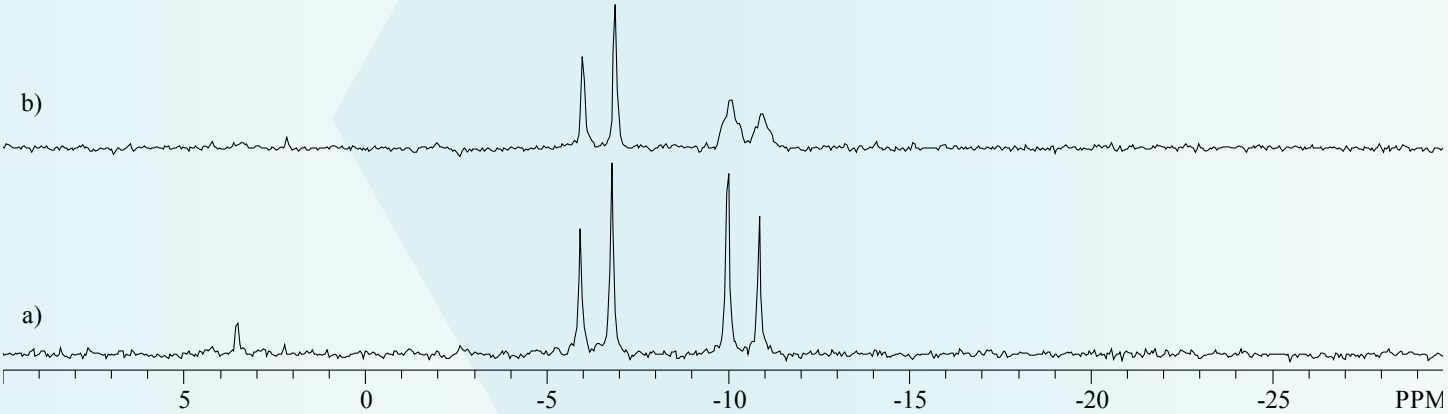

Figure 2. ^{31}P NMR spectra of ADP (a) with and (b) without proton decoupling.

SPECTRA & INTERPRETATION

The proton-decoupled ATP spectrum (Fig. 3a) consists of two doublets and an apparent triplet δ -6.2 (d, J = 19.5 Hz), -10.9 (d, J = 18.5 Hz), -21.4 (dd). The integral of the δ -10.9 doublet is about 20% larger than the other two, which are approximately equal. The apparent triplet arises from the β-phosphate, which is coupled to each of the other two in a doublet of doublets with almost identical coupling constants. With no decoupling (Fig. 3b), the δ -10.9 signal begins to split into a doublet of triplets from the additional coupling to the CH_2 (J = 6.0 Hz). Without decoupling, the integrals of all three signals are approximately equal. These changes confirm that the δ -10.9 signal is the α-phosphate, and the δ -6.2 signal is the terminal phosphate.

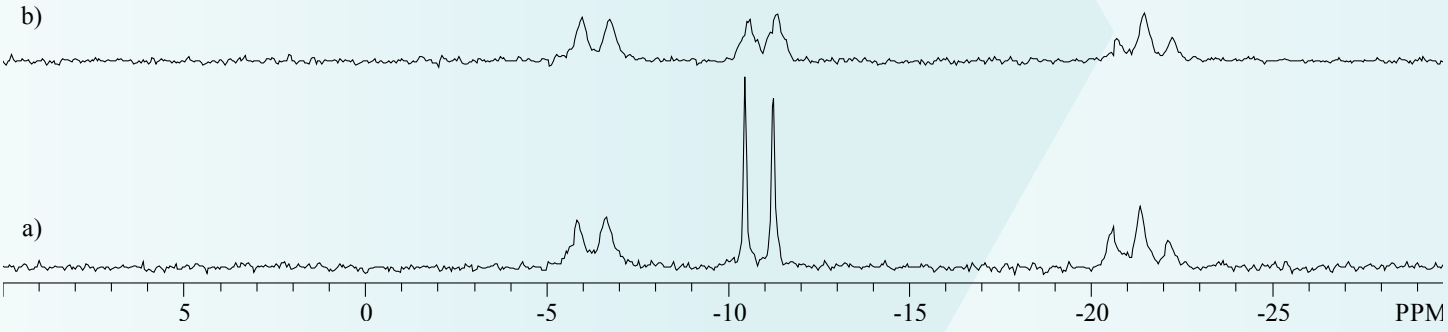

Figure 3. ^{31}P NMR spectra of ATP (a) with and (b) without proton decoupling.

Figure 4 shows the 31P NMR spectrum of a mixture containing 61% ATP and 39% ADP. The multiplets at δ -6 and δ -11 contain the overlapping signals of the terminal and α signals respectively of ATP and ADP. The terminal resonance at δ -6 is chosen for the quantitative analysis because the integral of the α resonance is affected by the NOE. Using the terminal signal allows the analysis to be done on a decoupled spectrum with better S/N. The signal at δ -6 arises from both the ATP and ADP in the solution. The β resonance at δ -21 arises only from ATP. So, setting the integral of the δ -6 signal to 100 allows the percentage of ATP in the mixture to be read directly off of the spectrum. This spectrum is particularly good. A ±3% variation in the measured percentage is typical under these conditions.

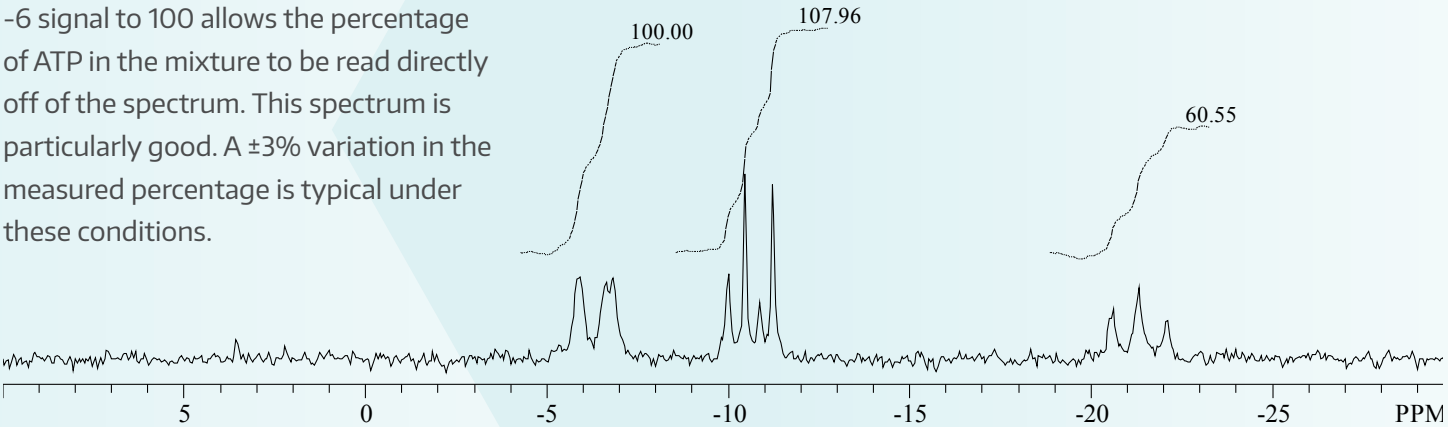

Figure 4. Proton-decoupled ^{31}P NMR spectrum of an ATP/ADP mixture containing 61% ATP.