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^{18}O Isotope Shift Effect in the ^{31}P-NMR Spectra of the Terminal Phosphate Groups of ADP and ATP: A Reinvestigation

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The ^{18}O isotope-induced chemical shift on the ^{31}P resonance of the terminal phosphate groups of adenosine 5′-triphosphate and adenosine 5′-diphosphate was found to be 0.0206 ppm per ^{18}O in a bridge position, and 0.0226 ppm per ^{18}O in a nonbridge position.

It is well established that the ^{18}O-induced chemical shift per ^{18}O bonded to phosphorus (S value) is larger for a P=O double bond (0.038–0.044 ppm) than for a P–O single bond (0.015–0.025 ppm) (*1*). In the case of multiple substitution, the magnitude of the shift is generally additive. Therefore, ^{18}O in the $\beta\gamma$-bridge position of ATP produces a smaller S value (0.0167 ppm) at the β-phosphorus atom than ^{18}O in the β-nonbridge position (0.0285 ppm) (*1b*). In contrast, no difference in the S values has been found for $\beta\gamma$-bridge and γ-nonbridge ^{18}O substituted phosphate groups of ATP and for the $\alpha\beta$-bridge and β-nonbridge ^{18}O substituted phosphate group of ADP. This has been related to the axial symmetry of the chemical shift tensor of the dianionic form of terminal phosphate groups as compared to the asymmetry of the β-phosphate group of ATP or the α-phosphate group of ADP (*1b*), a fact which has been established for a number of polyphosphates (*2a*).

During our investigations on enzymatic phosphoryl transfer reactions we found that an ^{18}O isotope in the bridge position to the terminal phosphate group of ADP and ATP causes a smaller chemical shift than ^{18}O isotopes in the nonbridge position. Therefore, terminal phosphate groups show no exception to other phosphate groups with respect to heavy isotope-induced chemical shifts. This observation has previously been made by Frey and co-workers (*2b,c*).

The various compounds listed in Table 1 were prepared by enzymatic phosphoryl transfer reactions using myokinase, carbamate kinase, or pyruvate phosphate dikinase. Incubation of [$\alpha\beta$-^{18}O, β-$^{18}O_2$]ATP (**1**) with myokinase in the presence of ADP lead to [$\alpha\beta$-^{18}O, β-$^{18}O_2$]ADP (**2**) and [$\alpha\beta$-^{18}O, β-^{18}O, $\beta\gamma$-^{18}O]ATP (**3**) in the first step. Further reaction produces [$\alpha\beta$-^{18}O, β-$^{18}O_2$, γ-$^{18}O_2$]ATP (**4**), [$\alpha\beta$-^{18}O, β-^{18}O, $\beta\gamma$-^{18}O, γ-$^{18}O_2$]ATP (**5**), and [$\alpha\beta$-^{18}O]ADP (**6**) (Scheme 1). Incubation of ATP

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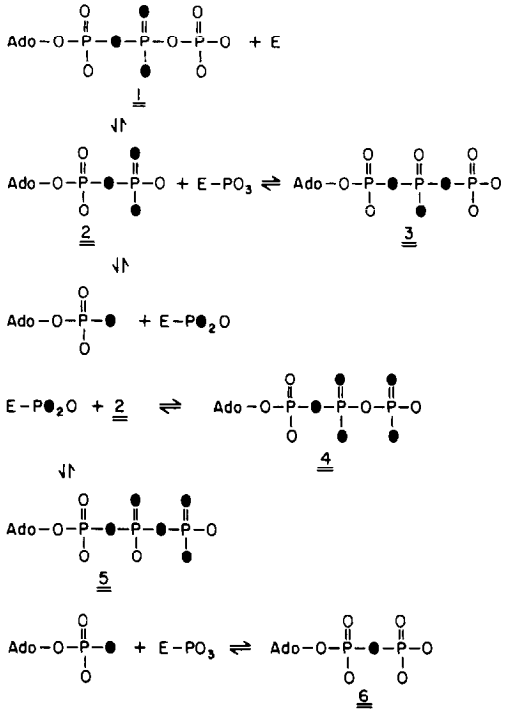
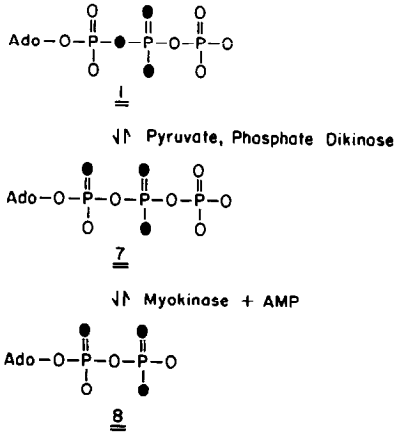


SCHEME 1

1 in the presence of pyruvate phosphate dikinase produces [α-^{18}O, β-$^{18}O_2$]ATP (**7**). Further incubation of that mixture with myokinase produces [α-^{18}O, β-$^{18}O_2$]ADP (**8**). [Pyruvate, phosphate dikinase also causes positional isotope exchange of ^{18}O from the β-nonbridge position into the $\beta\gamma$-bridge position of ATP **1**. However, the ADP produced are the same with or without that positional isotope exchange (von der Saal and Villafranca, unpublished data) (Scheme 2).]



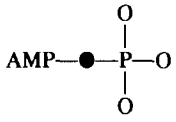
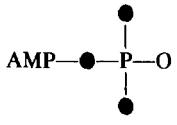
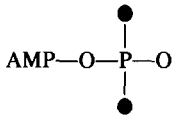
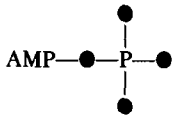
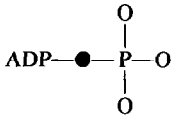
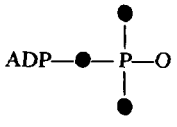
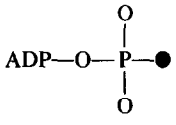
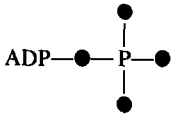
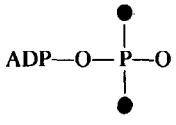
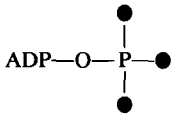
SCHEME 2

[γ-$^{18}O_3$]ATP (**9**) was produced by incubating ADP and *P*-[$^{18}O_4$]carbamylphosphate in the presence of carbamate kinase (*1b*). Because the ^{18}O : ^{16}O ratio was only 80 : 20 in the carbamylphosphate, [γ-$^{18}O_2$]ATP (**10**) and [γ-^{18}O]ATP (**11**) were also produced. [β-$^{18}O_4$]ATP (**12**) was prepared from *P*-(adenosine-5′)-*P*-(4-morpholino)-phosphate. Compound **12** was then converted to β-$^{18}O_3$-labeled P^1-(adenosine-5′)-P^2-(4-morpholino)-diphosphate and subsequently to [$\alpha\beta$-^{18}O, β-$^{18}O_2$]ATP (**1**) (*3*). [γ-$^{18}O_4$]ATP (**13**) was synthesized in the same way (*3*).

The results (Table 1) clearly show that a bridge ^{18}O atom causes a smaller shift

TABLE 1

^{18}O ISOTOPE EFFECT IN THE 145.81-MHz ^{31}P-NMR SPECTRA ($S_{^{31}P-^{18}O}$) OF THE TERMINAL PHOSPHATE GROUPS OF VARIOUS ^{18}O SUBSTITUTED ADPs AND ATPs AT 18°C AND pH 8.5

Compound	No.	$S_{^{31}P-^{18}O}$ (ppm)	Compound	No.	$S_{^{31}P-^{18}O}$ (ppm)
Part I: compounds containing ^{18}O either in the bridge or in nonbridge positions			Part II: compounds containing ^{18}O both in the bridge and in the nonbridge positions		
	**6**	0.0205		**2**	0.0219
	**8**	0.0227		**12**	0.0221
	**3**	0.0206 0.0208[a]		**5**	0.0219
	**11**	0.0225		**13**	0.0219
	**4, 10**	0.0225			
	**9**	0.0227			

Note. All values are ±0.0003 ppm. For each spectrum, 2000 scans were accumulated using a tip angle of 30°, an acquisition time of 3.9 s, a sweep width of 4200 Hz, and 32 K data points. Zero filling to 64 K was applied prior to Fourier transformation.

[a] From Ref. (*4*).

(0.0206 ppm, bond order = 1) in the ^{31}P-NMR spectrum of the terminal phosphate group of ATP or ADP than a nonbridge ^{18}O atom (0.0226 ppm, bond order = 1.3). These shift differences are additive. However, the shift differences are too small to obtain separate signals if the solutions contain mixtures of compounds with equal numbers of ^{18}O isotopes bonded to the terminal phosphate group. These differences are only apparent with one compound having ^{18}O in the bridge and the other compound only in the nonbridge position. For example, a mixture of the ATP molecules **5** and **9** gives rise to only one γ-phosphate doublet on all currently available NMR spectrometers (up to 243 MHz ^{31}P resonance). This doublet has a slightly larger linewidth compared to the pure compounds. It is therefore not possible to use peak heights instead of peak areas to obtain quantitative results in these cases. Furthermore, one should be cautious in manipulating the free induction decay to obtain better resolved spectra (e.g., Lorentzian-to-Gaussian line shape transformation), because this results in different effects on lines with different linewidths (*1a*). In the experiments with myokinase (Scheme 1), the ^{18}O content of **1** was 95%. Therefore, the ^{31}P signals attributed to compound **3** resulted from a mixture of **3** and a small amount of **4**, in which one ^{18}O in the γ position was replaced by ^{16}O. The same is true for several other compounds. However, because of the high ^{18}O : ^{16}O ratio, the chemical shifts obtained by our method are still within the limits of the digital resolution (0.0003 ppm).

The magnitude of the S value of nobridging ^{18}O atoms on terminal phosphate groups may be explained with the bond order (*1b*), but the reason why ^{18}O in the bridge position to terminal phosphate groups causes a larger S value on the terminal phosphorus (0.0206) than on the central phosphorus (0.0167) (*1b*) is not known. The one-bond coupling constant $^1J_{(^{31}P-^{17}O)}$ also depends on the bond order (*5*). Unfortunately, the ^{17}O-NMR signals of bridging oxygen atoms in polyphosphates are too broad to allow the detection of a difference in the coupling $\beta^{31}P$–$\beta\gamma^{17}O$ and $\beta\gamma^{17}O$–$\gamma^{31}P$ (*5*). The ^{17}O-NMR spectra of nucleoside 5′-(β,γ-peroxytriphosphates) (*6*) might help to solve this problem.

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