^{18}O-Isotope Shifts on the ^{31}P Nuclear Magnetic Resonance of Adenosine-5′-phosphate and Inorganic Phosphate

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Summary An isotope shift of 0·025 ± 0·003 p.p.m. to higher field is observed in the ^{31}P n.m.r. spectrum of adenosine-5′-[^{18}O]phosphate; for inorganic phosphate a shift to higher field of 0·020 ± 0·001 p.p.m. is observed for each ^{18}O atom incorporated.

THE effect of isotopic substitution on the magnetic shielding of nuclei was predicted by Ramsey,[1] and has subsequently been widely observed in high resolution n.m.r. spectroscopy. Almost invariably, substitution by a heavier isotope shifts the n.m.r. signal of a neighbouring nucleus to higher magnetic field, the magnitude of the shift being related to the fractional change in mass and the number of bonds separating the nuclei involved. The isotope shift in general is approximately proportional to the number of atoms in the molecule that have been substituted by isotope.[2] However the effect of ^{18}O substitution on ^{31}P chemical shifts has not been reported hitherto. If an isotope shift could be detected in the ^{31}P n.m.r. spectrum of phosphate esters and anhydrides, ^{31}P n.m.r. spectroscopy would provide a simple analytical tool for mechanistic studies of enzyme-catalysed phosphoryl transfer reactions.

Adenosine-3′,5′-phosphate was hydrolysed by bovine heart cyclic AMP phosphodiesterase in [^{18}O]water (38 atom % ^{18}O; containing 43 mM glycylglycine buffer adjusted to pH 7·5, and 9 mM magnesium acetate) to adenosine-5′-[^{18}O]phosphate and AMP. The lyophilised sample in 50 mM triethanolamine buffer (in 50% D_2O, pH 7·6 containing 5 mM ethylenediaminetetra-acetic acid) was agitated with

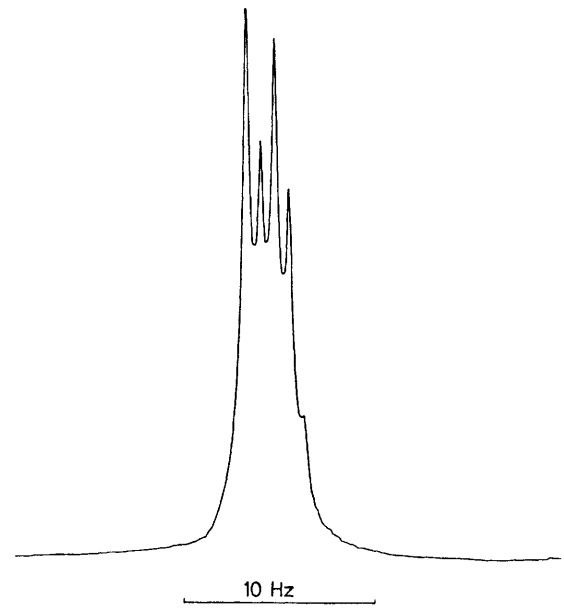

FIGURE. The broad band proton noise decoupled ^{31}P n.m.r. spectrum of [^{18}O]inorganic phosphate (0·64 M; obtained by hydrolysing PCl_5 with 50 atom % [^{18}O]water) with added normal inorganic phosphate (0·24 M). The spectrum was recorded at 36·43 MHz on a Bruker WH90 FT spectrometer in 2-amino-2-methylpropane-1,3-diol hydrochloride buffer (2 ml, 100 mM, 50% D_2O, pH 9·0) containing ethylenediaminetetra-acetic acid (5 mM). 145 transients were collected in 4K and 4K zeros added, with offset 2200 Hz, bandwidth 100 Hz, repetition rate 20·48 s, pulse width 15 μs, at 311K.

chloroform to denature the protein. The filtered aqueous solution was brought to pH 8·0. The ^{31}P n.m.r. signal of the [^{18}O]AMP (identified by its relative intensity) was 0·9 ± 0·1 Hz to higher field than that of AMP at 36·43 MHz.

The hydrolysis of phosphorus pentachloride with [^{18}O]-water (50 atom % ^{18}O) gave [^{18}O]inorganic phosphate with the expected statistical distribution of isotope (*viz.*, 1 : 4 : 6 : 4 : 1) into the five species (HPO_4^{2-}, $HPO_3{}^{18}O^{2-}$, $HPO_2{}^{18}O_2{}^{2-}$, $HPO{}^{18}O_3{}^{2-}$, and $HP{}^{18}O_4{}^{2-}$). The ^{31}P n.m.r. spectrum of this sample to which normal inorganic phosphate has been added is shown in the Figure. It can be seen that the ^{18}O isotope shift is additive, each atom of isotope causing an upfield shift of 0·74 ± 0·05 Hz at 36·43 MHz (*i.e.*, 0·020 ± 0·001 p.p.m.) and pH 9·0. It should be possible therefore to determine directly the distribution of ^{18}O in the polyphosphate group of nucleotides by ^{31}P n.m.r. spectroscopy; this is indeed the case.[3]

We gratefully acknowledge the award of a S.R.C. research studentship (to B.S.S.). This is a contribution from the Oxford Enzyme Group supported by the S.R.C.

(*Received*, 20*th April* 1978; *Com.* 415.)

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