Effects of oxygen-17 and oxygen-18 on phosphorus-31 NMR: further investigation and applications

R. Douglas Sammons, Perry A. Frey, Karol Bruzik, and Ming Daw Tsai

_J. Am. Chem. Soc._, 1983, 105 (16), 5455-5461 • DOI: 10.1021/ja00354a045 • Publication Date (Web): 01 May 2002

Downloaded from http://pubs.acs.org on February 12, 2009

More About This Article

The permalink [http://dx.doi.org/10.1021/ja00354a045](http://dx.doi.org/10.1021/ja00354a045) provides access to:

- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article
Effects of $^{17}$O and $^{18}$O on $^{31}$P NMR: Further Investigation and Applications

R. Douglas Sammons, a,b,c Perry A. Frey, a,b Karol Bruzik, a,b and Ming-Daw Tsai a,b,c

Contribution from the Department of Chemistry, The Ohio State University, Columbus, Ohio 43210, and the Institute for Enzyme Research, University of Wisconsin at Madison, Madison, Wisconsin 53706. Received January 20, 1983

Abstract: An approximately linear relationship between the magnitude of the $^{18}$O isotope effect in $^{31}$P chemical shifts ($S$) and the spin–spin coupling constant between $^{17}$O and $^{31}$P ($J$) has been observed. Such a correlation is useful in systems where only one of the two parameters can be measured. In addition, we have discussed $^{31}$P–$^{17}$O interactions in $^{31}$P(1$^{17}$O) NMR using some model compounds and addressed the relationship $\Delta \delta = (35/3)J$, where $\Delta \delta$ and $J$ are line widths of the $^{31}$P(1$^{17}$O) NMR signal and the $^{17}$O NMR signal, respectively. By use of such correlations and chirally labeled [a-1$^{17}$O]adenosine 5'-diphosphate (ADP), the interactions of Mg$^{2+}$ and Co$^{2+}$ with ADP have been investigated in detail. The results unambiguously established that binding of Co$^{2+}$ with [a-1$^{17}$O]ADP results in an averaged signal due to new exchange of an oxygen or a quadrupolar effect in $^{31}$P NMR, and that binding of Mg$^{2+}$ with [a-1$^{17}$O]ADP results in an averaged signal due to rapid exchange of the two signals. Finally, we have shown that [1$^{17}$O] can be used as a “label” of oxygen and phosphate in macromolecular systems, which can be detected by $^{31}$P NMR due to quadrupolar or dipolar broadening.

Three NMR² techniques involving oxygen isotopes have recently been introduced in studies of various physical and biochemical problems involving biochemical phosphates.³ The $^{18}$O isotope effect in $^{31}$P chemical shifts,² which will be referred to as the $^{31}$P(1$^{18}$O) method in this paper, has been widely used to locate a labeled oxygen and to follow the exchange of an oxygen or a phosphor group.⁴,⁵ The $^{17}$O quadrupolar effect in $^{31}$P NMR,⁶ referred to as the $^{31}$P(1$^{17}$O) method,³ has become an indispensable tool in some stereochemical analyses⁷ and has been used to quantify $^{17}$O.⁸,⁹ Recently, $^{17}$O NMR has been useful for studying diamagnetic metal ion–nucleotide interactions,⁹,¹⁰ protonation of adenine nucleotides,¹¹,¹² and differentiation of diastereotropic oxgens.¹³

There are limitations in the applications of all three methods. The $^{31}$P(1$^{18}$O) method requires a high-resolution spectrometer and is limited to small molecules that give very sharp $^{31}$P(1$^{18}$O) NMR signals. There are limitations in the applications of all three methods. The $^{31}$P(1$^{18}$O) method has been widely used to locate a labeled oxygen and to follow the exchange of an oxygen or a phosphor group.⁴,⁵ The $^{17}$O quadrupolar effect in $^{31}$P NMR,⁶ referred to as the $^{31}$P(1$^{17}$O) method,³ has become an indispensable tool in some stereochemical analyses⁷ and has been used to quantify $^{17}$O.⁸,⁹ Recently, $^{17}$O NMR has been useful for studying diamagnetic metal ion–nucleotide interactions,⁹,¹⁰ protonation of adenine nucleotides,¹¹,¹² and differentiation of diastereotropic oxgens.¹³

Table I. Correlation between the $^{18}$O Isotope Shift ($S_{^{31}P-^{17}O}$) and the $^{31}$P–$^{17}$O Coupling Constant ($J_{^{31}P^{17}O}$).⁶

<table>
<thead>
<tr>
<th>Compound</th>
<th>$S_{^{31}P-^{17}O}$, ppm</th>
<th>$J_{^{31}P^{17}O}$, Hz</th>
<th>Temp, °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>H$_3$PO$_4$·$^{18}$O$_4$</td>
<td>0.0188 ± 0.0007</td>
<td>83.0 ± 2.4</td>
<td>95</td>
</tr>
<tr>
<td>KH$_2$PO$_4$</td>
<td>pH 2.1</td>
<td>0.0201 ± 0.0007</td>
<td>87.9 ± 2.4</td>
</tr>
<tr>
<td>K$_2$HP$_2$O$_7$</td>
<td>pH 2.6</td>
<td>0.0200 ± 0.0011</td>
<td>88.7 ± 2.4</td>
</tr>
<tr>
<td>(CH$_3$)$_2$PO$_2$</td>
<td>$^{18}$O</td>
<td>0.0218 ± 0.0007</td>
<td>95.0 ± 2.4</td>
</tr>
<tr>
<td>PS$_2$PO$_3$</td>
<td>pH 8.6</td>
<td>0.0292 ± 0.0029</td>
<td>158.3 ± 2.4</td>
</tr>
<tr>
<td>PS$_2$PO$_3$</td>
<td>pH 6.4</td>
<td>0.0329 ± 0.0027</td>
<td>121.4 ± 2.4</td>
</tr>
<tr>
<td>[a-1$^{17}$O]ADP</td>
<td>pH 6.4</td>
<td>0.0286 ± 0.0015</td>
<td>123.4 ± 2.4</td>
</tr>
<tr>
<td>[a-1$^{17}$O]ADP</td>
<td>pH 6.4</td>
<td>0.0331 ± 0.0007</td>
<td>131.4 ± 2.4</td>
</tr>
<tr>
<td>CNEt-ADPaS</td>
<td>pH 6.4</td>
<td>0.0363 ± 0.0045</td>
<td>146.2 ± 2.4</td>
</tr>
</tbody>
</table>

The same sample was used for both $^{31}$P NMR (determining $J_{^{31}P^{17}O}$) and $^{18}$O NMR (determining $S_{^{31}P-^{17}O}$). Measured at 81 or 121 MHz, at ambient temperatures. Gaussian multiplication was applied to obtain a near-base-line separation of peaks. Although it is desirable to measure $S$ values at the same temperature as in $^{17}$O NMR experiments, it is hard to obtain a good resolution (to resolve $^{18}$O shifts) at near-boiling temperatures, particularly during a long accumulation. The correlation should be applied to only phosphates and derivatives of phosphates.

These limitations prompted us to investigate further the three NMR methods and their applicability. In this paper we present results of recent work on three aspects of these phenomena. Part A deals with a newly unmasked empirical correlation between the magnitude of $^{18}$O isotope shifts in $^{31}$P NMR (designated as $S$) and the magnitudes of the $^{31}$P–$^{17}$O spin–spin coupling constant (designated as $J$), as well as the interaction between $^{17}$O and $^{31}$P in small molecules. In Part B, we have used the above correlations and chirally labeled [a-1$^{17}$O]ADP to perform a detailed investigation of the interaction of Mg$^{2+}$ and Co$^{2+}$ with ADP. Part C further evaluates the use of $^{17}$O as a label of oxygen and phosphate in macromolecular systems.

Results and Discussion

(A) Further Investigation in the NMR Methods. (A) Correlation between $J$ and $S$. Determination of both $J$ and $S$ for a given phosphate is limited to certain conditions, so it would be useful


---


if the value of one could be obtained from the measured value of the other. Since both $J$ and $S$ were expected to be related to the P-O bond order, we have sought a correlation between the two parameters. The large amounts of data on both $J$ and $S$ available in the literature have been measured under various conditions, with variable resolution, and could be accurate to within only 20%. We therefore measured the $J$ and $S$ values given in Table I for a number of compounds, using the same sample to determine $J$ by $^{17}$O NMR and $S$ by $^{31}$P NMR; the shift is due to the $^{17}$O isotope always associated with $^{17}$O. In cases where peaks overlapped, the $J$ and $S$ values were determined by spectral simulation. When $J$ was plotted vs. $S$, as shown in Figure 1, an approximately linear relationship, $J$ (Hz) $\approx (3.65 \times 10^4) S$ (ppm) + 14, was obtained, confirming the existence of a relationship between these parameters for biochemical phosphates.

(2) $^{31}$P-$^{17}$O Interaction in Small Molecules. For small biochemical phosphates in solution, the line widths of $^{17}$O NMR signals ($\Delta$O) can be related to the quadrupolar relaxation time $T_q$ by eq 1:

$$\Delta O \approx \frac{1}{\pi T_q} \approx \frac{12\pi}{125} \left(1 + \frac{\eta^2}{3}\right) \left(\frac{e^2\eta Q}{h}\right)^2 \tau_r \tag{1}$$

where $e^2\eta Q/\hbar$ is the quadrupolar coupling constant, $\eta$ is the asymmetry parameter, and $\tau_r$ is the rotational correlation time. When $^{31}$P is bonded directly to $^{17}$O, the $^{31}$P nucleus will also be relaxed by virtue of its spin-spin coupling with $^{17}$O. This is termed "scalar relaxation of the second kind" by Abragam. Such a scalar relaxation is dependent upon the magnitudes of the longitudinal relaxation time $T_1$ of the quadrupolar nucleus (which is approximately equal to $T_q$ under present conditions) and the spin-spin coupling constant $J$. When the product $T_1J$ is sufficiently small, the scalar relaxation dominates the relaxation of $^{31}$P and results in the collapse of the multiplet. Suzuki and Kubo have calculated the line shape of a dipolar nucleus coupled to a quadrupolar nucleus with $I = 5/2$ at various values of $T_1J$.

Figure 2 shows the $^{17}$O and $^{31}$P($^{17}$O) NMR spectra of $^{31}$P$^{17}$OCl$_3$ (Figure 2A), (CH$_3$O)$_3$P$^{17}$O (Figure 2B), (PhO)$_3$P$^{17}$O (Figure 2C), and Ph$_3$P$^{17}$O (Figure 2D). These compounds are all symmetrical small molecules with a $P=O$ bond that have relatively long $T_q$ and large $J$, thus showing fully or partially resolved $^{17}$O and $^{31}$P($^{17}$O) NMR spectra. It can be seen in Figure 2 that as the $^{17}$O NMR coupling pattern collapses (decreasing $T_2J$), the $^{31}$P NMR coupling pattern also collapses.

For biochemical phosphate molecules $T_1$ is generally shorter, due to a larger molecular size and a smaller degree of symmetry, and $J$ is generally smaller, due to a $P$-O bond with a smaller $\pi$-character, than for the molecules in Figure 2. Therefore, the

$$\frac{1}{T_1s} = \frac{8\pi^2 I(I + 1)}{3} \left[ \frac{T_q}{1 + (\omega_p - \omega_s)^2 T_q^2} \right] \tag{2}$$

$$\frac{1}{T_2s} = \frac{4\pi^2 I(I + 1)}{3} \left[ \frac{T_q}{1 + (\omega_p - \omega_s)^2 T_q^2} \right] \tag{3}$$

where $I = 5/2$, $J = J_{1p}, \omega_p, 1/T_1s$ and $1/T_2s$ are the contribution of scalar relaxation to the longitudinal and the transverse relaxations, respectively, of $^{31}$P. $T_1$ is the quadrupolar $T_1$ relaxation time of $^{17}$O, $\omega_p$ and $\omega_s$ are the angular precession frequencies of $^{31}$P and $^{17}$O, respectively.

For small biochemical phosphate molecules at the extreme narrowing limit ($\omega_p \tau_q^2 \ll 1$), $T_q$ is in the order of $10^{-10}$ s. Since $\omega_p - \omega_s \approx 10^7 - 10^8$ Hz, $\omega_p - \omega_s)^2 T_q^2 \gg 1$, and eq 4 and 5 can be reduced to

$$\frac{1}{T_1s} \approx 0 \tag{4}$$

$$\frac{1}{T_2s} \approx \frac{35}{3} \left(\frac{2J}{T_q}\right)^2 \tag{5}$$

Under this condition, $1/T_2s \approx 1/T_2s$ for $^{31}$P, and $T_1 \approx T_2 \approx T_q$.  

Effects of $^{17}$O and $^{18}$O on $^{31}$P NMR

![Figure 3](image)

Figure 3. $^{17}$O NMR spectra (at 27.1 MHz) and $^{31}$P($^{17}$O) NMR spectra (at 81.0 MHz) of $\text{H}_2\text{PO}_{4}^-\text{O}_3$ (50 atom % $^{17}$O) in $\text{D}_2\text{O}$ (A), $\text{H}_2\text{O}$/glycerol (1/1 volume ratio) (B), and glycerol (C). $^{31}$P NMR parameters: spectral width 10 kHz; acquisition time 0.05 s; $\text{H}$ decoupled; 1 K data points; $DE = 12$ $\mu$s; line broadening 20 Hz. Spectral parameters: spectral width 3012 Hz; acquisition time 2.7 s; acquisition delay 1 s; 75$\mu$s $\text{H}$ decoupling; line broadening 4 Hz. All spectra were obtained at 30 °C.

for $^{17}$O, which justifies the approximations of $\Delta \rho \approx 1/(\pi T_2)$ and $\Delta \rho \approx 1/(\pi T_2)$. The following approximate relationship can be obtained from eq 5

$$\Delta \rho \Delta \omega \approx (35/3) J^2 \tag{6}$$

where $\Delta \rho$ and $\Delta \omega$ represent the line widths of $^{31}$P($^{17}$O) and $^{17}$O NMR signals, respectively.

While the quantitative nature of eq 6 remains to be established by detailed experimental measurements, the relationship between $\Delta \rho$ and $\Delta \omega$ is approximately true in many systems. As one example, Figure 3 shows the $^{17}$O NMR and the $^{31}$P($^{17}$O) NMR signals of $\text{H}_2\text{PO}_{4}^-\text{O}_3$ in $\text{D}_2\text{O}$ (Figure 3A), $\text{H}_2\text{O}$/glycerol (Figure 3B), and glycerol (Figure 3C). In Figure 3A, the $\Delta \omega$ is 160 Hz (after correcting for a 20-Hz line broadening and $J_{\text{H}_2\text{O}} = 88$ Hz) while the $\Delta \rho$ is 390 Hz. The product $\Delta \rho \Delta \omega \approx 62400$ Hz$^2$, which is ca. 30% smaller than $(35J/3)^2 = 60350$ Hz$^2$. However, as $\Delta \rho$ increases due to an increased viscosity, which is not expected to change $J$, the $\Delta \rho$ decreases correspondingly, showing the inversely proportional relationship between $\Delta \rho$ and $\Delta \omega$. The significance of Figure 3C will be discussed further in part C.

(B) Interactions of $\text{Mg}^{2+}$ and $\text{Co}^{2+}$ with ADP: Complete Study by Three Techniques and Chiral $\text{[a-}^{17}$O$\text{]}$ADP. Recently we have introduced the use of $^{17}$O NMR to study the binding of $\text{Mg}^{2+}$ with adenine nucleotides,$^{11}$ which is based on the observation that binding of $\text{Co}^{2+}$ with $\text{[a-}^{17}$O$\text{]}$ADP (and other $^{17}$O-labeled nucleotides) resulted in two signals: one slightly shifted downfield (1.9 ppm) and significantly broadened; the other greatly shifted upfield (180-200 ppm) and significantly broadened. In $\text{Mg}^{2+}$ complexes only a single signal with a small upfield shift (<6 ppm) has been observed. Although it has been concluded, on an empirical basis, that $\text{Mg}^{2+}$ interacts with both the $\alpha$-phosphate and $\beta$-phosphate of ADP, and with all of the $\alpha$, $\beta$, and $\gamma$-phosphates of ATP (with a smaller extent of interaction with the $\alpha$-phosphate of ATP), several important problems on the methodology remain to be established.

In the following sections we described detailed study of $\text{Mg}^{2+}$ and $\text{Co}^{2+}$ binding with ADP by use of all three NMR techniques and chirally labeled ADP.

1. Effects of Metal Ions on $S$ and $J$ in Metal-Nucleotide Complexes. The effect of $\text{Co}^{2+}$ binding on the $S$ values of nucleotides has been reported$^{19,18}$ but not the effect of $\text{Mg}^{2+}$ binding.

A possible reason is that the $^{31}$P NMR signals of $\text{Mg}^{2+}$ complexes are slightly broadened at high magnetic fields.$^{20}$ At a medium magnetic field, we have observed the $^{17}$O isotope effect on the $P$, $\omega$ value of $\text{Mg}^{2+}$ complex, which is ca. 30% smaller than $(35J/3)^2 = 69350$ Hz$^2$. However, as $\Delta \rho$ increases due to an increased viscosity, which is not expected to change $J$, the $\Delta \rho$ decreases correspondingly, showing the inversely proportional relationship between $\Delta \rho$ and $\Delta \omega$. The significance of Figure 3C will be discussed further in part C.

Figure 4. $^{31}$P NMR spectra (81.0 MHz) showing the effect of metal ion binding on the $^{17}$O isotope shift (at the $P$, signal) of $\text{[a-}^{17}$O$\text{]}$ADP. (A) $\text{Free} [\text{a-}^{17}$O$\text{]}$ADP, randomly labeled, 25 $\text{mM}$ in $\text{D}_2\text{O}$, pD 7.8; (B) $\text{Mg} [\text{a-}^{17}$O$\text{]}$ADP, randomly labeled, 25 $\text{mM}$ in $\text{D}_2\text{O}$, pD 7.8; (C) $\text{Co} [\text{NH}_\text{3})_\text{4}-\text{[a-}^{17}$O$\text{]}$ADP, A plus $\Delta$ isomers, in $\text{50% D}_2\text{O}$, pH 5.5. Spectral parameters for (A) and (B): spectral width 2500 Hz; acquisition time 3.3 s; 75$\mu$s pulse; 16K data points; resolution 0.305 Hz/pixel; temperature 30 °C; $\text{H}$ decoupled; Gaussian multiplication (LB = 0.8, GB 0.04). Spectrum C was obtained as previously described.$^{18}$

The $J$ values of $\text{CoADP}$ and $\text{MgADP}$ are not readily measurable due to the relatively broad $^{17}$O NMR signals. However, on the basis of the correlation in Figure 1 between $S$ and $J$, the $J$ values of $\text{MgADP}$ ($J_\text{a}$) and $\text{CoADP}$ (as an average of $\text{O=P-}^{17}$O$-\text{Co}^{2+} + ^{17}$O$-\text{Co}^{2+}$) should be within 10% of that of $\text{free ADP}$ ($J_\text{b}$).

(2) Unequivocal Assignments of $^{17}$O NMR Signals. As indicated in an earlier paper,$^{17}$ the unequivocal assignment of the two $^{17}$O NMR signals of $\text{Co(NH}_3)_4[\text{a-}^{17}$O$\text{]}$ADP awaited the preparation of sterosepecifically labeled compounds. Following the procedure previously developed for the synthesis of chiral $[\text{a-}^{17}$O$\text{]}$ADP,$^{18}$ we have synthesized $\text{R}_2$- and $\text{S}_2$-[a-17O]ADP. Interaction of $\text{S}_2$-[a-17O]ADP with $\text{Co(NH}_3)_4\text{CO}_3$ gave a mixture of the $\Delta$ isomer (I) and the $\Delta$ isomer (II) of $\text{Co(NH}_3)_4-\text{[a-}^{17}$O$\text{]}$ADP.$^{21}$


upfield signal being too broad to be detected. This question has
3279-3286.

In the Mg2+ complexes of I70-labeled ADP and ATP only one
of ADP is randomly labeled with
spectrum subsequent to the
also shown are the
specifically located at the uncoordinated position, and the
(98 ppm) is due to '70=P-O--C03+. We attribute the presence
of ca. 20% downfield signal in Figure 2C to epimerization between
the isomer and the
isomer (and vice versa) is due to epimerization between the two isomers
during accumulation.

We separated the Δ and Δ isomers of Co(NH3)4ADP by high-pressure liquid chromatography (HPLC) as described under Experimental Section and identified them as Δ and Δ isomers based on the 31P NMR spectra. Shown in Figure 5 are the P signals of the resolved Δ and Δ isomers of Co(NH3)4(Sp)-[a-170]ADP, which exhibit 17O isotope shifted lines due to the 17O species present in the starting 17O-enriched water. Both the stereochemical purity of (S)-[a-170]ADP and the diastereomeric purity of I and II must be >95% on the basis of Figure 5.

Figure 6 shows the 17O NMR spectra (at 40.65 MHz) of Co(NH3)4[a-17O]ADP (Figure 6A), in which the α-phosphate of ADP is randomly labeled with 17O at nonbridging positions. Also shown are the Δ isomer, I (Figure 6B), in which 17O is specifically located at the uncoordinated position, and the Δ isomer, II (Figure 6C), in which 17O is directly coordinated to Co3+.

These results unambiguously establish that the upfield signal (82 ppm) is due to O=P--17O--Co3+, whereas the downfield signal (98 ppm) is due to O=P--17O--Co3+. We attribute the presence of ca. 20% downfield signal in Figure 2C to epimerization between the Δ isomer and the Δ isomer during 2 h of data accumulation at 50 °C. We confirmed this by re-determining the 31P NMR spectrum subsequent to the 17O experiments and verifying the presence of 31P NMR signals corresponding to the two isomers. No appreciable dissociation to free ADP or monodentate CoADP was detected by 31P NMR.

(3) 31P(17O) NMR Studies of Mg2+ and Co3+ Binding to ADP.

In the Mg2+ complexes of 17O-labeled ADP and ATP only one signal at the low field (broadened by 2-4 times) was observed. It was not clear whether this signal was due to the average of 17O=O=P--O--Mg2+ and 17O=O=P--O--Mg2+, or whether it represented essentially only the signal of 17O=O=P--O--Mg2+, the upfield signal being too broad to be detected. This question has
now been resolved by the 31P(17O) NMR method, as described below.

Figure 7 shows the 31P NMR spectra of free ADP (Figure 7A) and free [α-17O]ADP (Figure 7B), the difference spectrum B - A (Figure 7C), the 31P NMR spectra of MgADP (Figure 7D) and Mg[α-17O]ADP (Figure 7E), and the difference spectrum E - D (Figure 7F). By comparing the broad P signals in parts C and F of Figure 7, it is obvious that the apparent ΔP of MgADP has decreased by ca. 50%. Such a “line sharpening effect” in 31P(17O) NMR is predictable based on eq 6. The line widths of the broad P signals, measured at the half-height and corrected for the spin–spin coupling constant between P and P, are 470 Hz for free ADP (ΔPf) and 250 Hz for MgADP (ΔP).

Figure 8 shows the 31P NMR spectra of Co(NH3)4ADP, the Δ isomer (Figure 8A), and the corresponding 17O-labeled compound I (Figure 8B) and II (Figure 8C), the difference spectrum B - A (Figure 8C), the 31P NMR spectra of CoADP (Figure 8D) and the corresponding 17O-labeled compound II (Figure 8E), and the difference spectrum E - D (Figure 8F). The ΔP of the broad P signals of I and II, as measured from parts C and F of Figure 8, respectively, and corrected for J, are 290 and 170 Hz, respectively. If the Δ and Δ isomers were in rapid exchange, as in MgADP, the average ΔP would be 230 Hz, which is the same as the ΔP of MgADP within experimental error. The ratio of ΔPf/ΔP is ca. 1.9 for MgADP and 2.0 for CoADP.

Therefore, Mg2+ and Co3+ have approximately the same effect on both J (as described in section 1) and ΔP upon binding with [α-17O]ADP. On the basis of eq 6, they should also have the same effect on ΔO. According to the previous report for Co(NH3)4[α-17O]ADP, ΔO/ΔO ≈ 3.0-5.2 for the upfield signal and ≈ 1 for the downfield signal, which give an average value of 2.0-3.1. For Mg[α-17O]ADP, ΔO/ΔO ≈ 2.2-2.8 for the single

---

Effects of $^{17}$O and $^{18}$O on $^{31}$P NMR

The conclusion that Mg-[α-17O]ADP is in the "fast exchange limit" on the time scale of 17O NMR may not seem reasonable considering the fact that the two signals of Co-[α-17O]ADP are separated by ca. 200 ppm (8 × 10^6 Hz) at 40 MHz. However, it can easily be explained by the "epimerization" process mentioned in section 2 of part B. The epimerization is intramolecular and should be much faster than the chemical exchange (MgADP = Mg[2+ + ADP]. In the case of Co-[α-17O]ADP, no dissociation to free ADP or the monodentate complex was detectable when ca. 30% of epimerization had occurred.

It is not impossible that the "quadrupolar relaxation" is partially or fully responsible for the observed broadening, if the bound ADP has a large internal rotational freedom and therefore a very small $\tau$.

(23) It is not impossible that the "quadrupolar relaxation" is partially or fully responsible for the observed broadening, if the bound ADP has a large internal rotational freedom and therefore a very small $\tau$.

Effects of $^{17}$O and $^{18}$O on $^{31}$P NMR

The epimerization had occurred. "Epimerization" process mentioned in section 2 of part B. The epimerization is intramolecular and should be much faster than the chemical exchange (MgADP = Mg[2+ + ADP]. In the case of Co-[α-17O]ADP, no dissociation to free ADP or the monodentate complex was detectable when ca. 30% of epimerization had occurred.

"Epimerization" process mentioned in section 2 of part B. The epimerization is intramolecular and should be much faster than the chemical exchange (MgADP = Mg[2+ + ADP]. In the case of Co-[α-17O]ADP, no dissociation to free ADP or the monodentate complex was detectable when ca. 30% of epimerization had occurred.

The epimerization had occurred. "Epimerization" process mentioned in section 2 of part B. The epimerization is intramolecular and should be much faster than the chemical exchange (MgADP = Mg[2+ + ADP]. In the case of Co-[α-17O]ADP, no dissociation to free ADP or the monodentate complex was detectable when ca. 30% of epimerization had occurred.

"Epimerization" process mentioned in section 2 of part B. The epimerization is intramolecular and should be much faster than the chemical exchange (MgADP = Mg[2+ + ADP]. In the case of Co-[α-17O]ADP, no dissociation to free ADP or the monodentate complex was detectable when ca. 30% of epimerization had occurred.

The epimerization had occurred. "Epimerization" process mentioned in section 2 of part B. The epimerization is intramolecular and should be much faster than the chemical exchange (MgADP = Mg[2+ + ADP]. In the case of Co-[α-17O]ADP, no dissociation to free ADP or the monodentate complex was detectable when ca. 30% of epimerization had occurred.

"Epimerization" process mentioned in section 2 of part B. The epimerization is intramolecular and should be much faster than the chemical exchange (MgADP = Mg[2+ + ADP]. In the case of Co-[α-17O]ADP, no dissociation to free ADP or the monodentate complex was detectable when ca. 30% of epimerization had occurred.

The epimerization had occurred. "Epimerization" process mentioned in section 2 of part B. The epimerization is intramolecular and should be much faster than the chemical exchange (MgADP = Mg[2+ + ADP]. In the case of Co-[α-17O]ADP, no dissociation to free ADP or the monodentate complex was detectable when ca. 30% of epimerization had occurred.

"Epimerization" process mentioned in section 2 of part B. The epimerization is intramolecular and should be much faster than the chemical exchange (MgADP = Mg[2+ + ADP]. In the case of Co-[α-17O]ADP, no dissociation to free ADP or the monodentate complex was detectable when ca. 30% of epimerization had occurred.

The epimerization had occurred. "Epimerization" process mentioned in section 2 of part B. The epimerization is intramolecular and should be much faster than the chemical exchange (MgADP = Mg[2+ + ADP]. In the case of Co-[α-17O]ADP, no dissociation to free ADP or the monodentate complex was detectable when ca. 30% of epimerization had occurred.

"Epimerization" process mentioned in section 2 of part B. The epimerization is intramolecular and should be much faster than the chemical exchange (MgADP = Mg[2+ + ADP]. In the case of Co-[α-17O]ADP, no dissociation to free ADP or the monodentate complex was detectable when ca. 30% of epimerization had occurred.
ments ($^{16}O/^{17}O/^{18}O \approx 0.52/0.29/0.19$). Due to this pattern of enrichment, the major labeled species are the singly labeled ones, as is evident from Figure 4. The $^{17}O/^{18}O$ (52.4%, $^{17}O$, 35.1%, $^{18}O$) obtained was from Monsanto. The puratronic-grade (99.999%) Mg(NO$_3$)$_2$ was purchased from Ventron Co. Arginine kinase was purified and assayed as previously described.$^{25}$ Other biochemicals were obtained from Sigma. Other chemicals used were of reagent grade or highest purity available commercially.

Synthesis of $^{17}O$DPPC. Scheme I outlines the synthesis of $^{17}O$-DPPC. To a solution of 5.25 mol of $^{17}O$Cl$_2$ (52 atom % $^{17}O$) in dry THF was added ca. 6 mmol of triethylamine, followed with 2.0 g of (S)-(-)-1,2-di-palmitin (I) in THF. After being stirred for 3 h at room temperature, the solvent and excess PCl$_5$ and triethylamine were removed under vacuum, and then the resulting phosphorochloridate 2 was dissolved in THF at 0 °C and then added to a mixture of 2-(methylamino)-ethanol (0.32 g) and triethylamine (2.2 mL) in THF. The reaction was allowed to proceed for 1 h at room temperature. After filtration and evaporation, 1.6 g of the product 3 was isolated by column chromatography on silica gel. The structure of 3 was characterized by $^1H$ and $^{13}C$ NMR. $^3P$ NMR analysis in CDCl$_3$ showed two peaks due to $^{13}P$-$^{17}O$ and $^{13}P$-$^{18}O$ (0.039 ppm upfield), which is characteristic of a P=O double bond. Calculation on the basis of the known $^{17}O/^{18}O$ ratio and the observed $^{16}O/^{17}O$ ratio indicated that the atom percent $^{17}O$ enrichment is 50%. $^{17}O$ NMR analysis (60 °C, in CDCl$_3$) showed $\delta = 67$ and $J_{pH} = 150$ Hz. Hydrolysis of 3 in H$_2$O gave $[^{17}O]$-N-methyl-palmitoylphosphatidylethanolamine (4). Methylation of 4 in CH$_2$I$_2$ using a heterogeneous catalyst (2 M aqueous K$_2$CO$_3$ containing benzyltrimethylammonium chloride), gave $[^{17}O]$DPPC (5), which is characterized by $^1H$ and $^{13}C$ NMR.

Synthesis of the A and $\Delta$ Isomers of CO(NH)$_2$-[~[~]~$^{17}O$]ADP. Isomers of CO(NH)$_2$-[~[~]$^{17}O$]ADP were synthesized according to the procedure used for the synthesis of (R)$_2$- and (S)$_2$-[~[~]$^{17}O$]ADP, except that $^{17}O$-$^{17}O$ was introduced in the first step (synthesis of $^{17}O$-$^{17}O$-AMP) and desulfurization was carried out in unlabeled H$_2$O. The procedure of Cornelius et al.$^{24}$ was followed to prepare CO(NH)$_2$-[~[~]$^{17}O$]ADP from (S)$_2$-[~[~]$^{17}O$]ADP, which was then purified as previously described.$^{28}$ The $^{17}O$ enrichment was calculated as 52% on the basis of the $^{17}O$ enrichment (measured from $^{31}$P NMR and the known $^{17}O/^{18}O$ ratio in the starting H$_2$O.$^{17}O$).

The A and $\Delta$ isomers of CO(NH)$_2$-$^{17}O$ADP had been separated previously on a cyclohexylamyllose column, but we have separated the two isomers on a Waters uBondapak C$_8$ reverse-phase HPLC column using 50 mM acetate at pH 6.3 as the eluting buffer. The A and $\Delta$ isomers were eluted at 33 and 39 min, respectively. The assignment of peaks was based on the known $^{31}$P chemical shifts of the two diastereomers.$^{24}$ The first band gave the more upfield $P_S$ resonance (corresponding to the $\Delta$ isomer) and the second band gave the more downfield resonance (corresponding to the A isomer).Remixing of half of the two isomers in a 2/1 ratio gave the expected pattern of the $P_S$ signal.

Synthesis of Model Compounds. $^{31}$PCl$_5$ was prepared by hydrolyzing 10.4 g of PCl$_5$ with 1 mL of H$_2$O at ~78 °C followed by distillation under vacuum (88% yield). Treatment of PCl$_5$ with a severalfold excess of a MeOH/triethylamine mixture at room temperature gave (CH$_3$H)$_2$P$_2$O$_5$. The atom percent $^{17}O$ enrichment in (CH$_3$H)$_2$P$_2$O$_5$ was 52% on the basis of the percent $^{17}O$ enrichment (determined by $^{31}$P NMR) and the known ratio of $^{17}O/^{18}O$. (Ph$_3$P)$_2$O$_5$ was prepared

Figure 10. $^3P$ NMR spectra (at 81.0 MHz) of unsonicated lipid bilayers. (A) Dipalmitoylphosphatidylcholine (DPPC), unlabeled; (B) $[^{17}O]$DPPC, 50 atom % $^{17}O$ at phosphorus; (C) subtraction of A from B. Sample conditions: 100 mg of DPPC mixed with 1.5 mL of D$_2$O by vortexing at 50 °C. Spectral parameters: spectral width 25,000 Hz; $^1H$ decoupling (decoupler power 2.5 W); acquisition time 0.082 s; 40,000 scans; line broadening 100 Hz; 45 °C

Scheme I

\[ \text{RC} = \text{CH}_2(\text{CH}_3)_2 \rightarrow \text{RC} = \text{CH}_2(\text{CH}_3)_2 \]

(b) Blethen, S. L.; Kaplan, N. O. Biochemistry 1967, 6, 1413-1421.
analogously to (CH$_3$)$_2$P$_{17}$O except that phenol was used instead of methanol. Ph$_3$P$_{17}$O (49 atom % $^{17}$O) was prepared by oxidizing triphenylphosphine with the mixture Et$_2$N/CCl$_4$/H$_2$O (5 equiv) in dry dimethoxyethane$^{28}$ followed by silica gel chromatography. (Ph$_3$)$_2$P$_{17}$O$_3$ was a byproduct of the coupling reaction of [a-$^{17}$O]AMPS to cyanoethyl dimethoxyethane$^{26}$ followed by silica gel chromatography. (Ph$_3$)$_2$PO$_{17}$O$_4$ was obtained by dissolving H$_3$PO$_4$ (1 mmol) in 5 mL of D$_2$O followed by addition of 63% 1 M HCIO$_4$. The final solution contained 1.4 M HClO$_4$ and 0.2 M H$_3$PO$_4$.

Spectral Methods. $^{17}$O NMR spectra were obtained from a Bruker WM-300 spectrometer and $^{31}$P NMR spectra from both WP-200 and WM-300 spectrometers. A deuterium lock was used in all cases. The $^{17}$O chemical shifts reported are relative to external HClO$_4$ (at 25 °C), and the $^{31}$P chemical shifts are referenced to external 1 M H$_3$PO$_4$. The positive sign represents a downfield shift in both $^{17}$O and $^{31}$P NMR. The estimated error in the measurements of "broad $^{31}$P($^{17}$O) NMR signals" is ±10%.

Acknowledgment. This work was supported by National Institutes of Health Grants GM 29041 (M.-D.T.) and GM 30480 (P.A.F.). The NMR spectrometers used (at The Ohio State University) were supported by National Institutes of Health Grant 27431 and National Science Foundation Grant CHE 7910019. We thank Drs. A. G. Marshall and C. Cottrell at The Ohio State University for providing computer programs for curve fitting, Ru-Tai Jiang and Yeun-Jung Shyy (also at The Ohio State University) for assistance in the synthesis of stereoisomers of Co(NH$_3$)$_4$([S]$_2$-$^{17}$O)ADP, and Judy Hart for isolating arginine kinase.

Registry No. Δ-Co(NH$_3$)$_4$([S]$_2$)-[a-$^{17}$O]ADP, 86119-73-5; Δ-Co(NH$_3$)$_4$([S]$_2$)-[a-$^{17}$O]ADP, 86119-74-6; Co(NH$_3$)$_4$ADP, 63937-09-7; Co(NH$_3$)$_4$([a-$^{17}$O]ADP, 80539-98-6; Mg[a-$^{17}$O]ADP, 86119-85-9; MgADP, 7384-99-8; [a-$^{17}$O]DPPC, 86119-75-7; DPPC, 2644-64-4; [a-$^{17}$O]ADP, 81246-59-5; (S$_2$)-[a-$^{17}$O]ADP, 83541-22-4; (S$_2$)-[a-$^{17}$O]ADP, 85550-14-7; [a-$^{17}$O]ADP, 80547-17-7; [a-$^{17}$O]AMPS, 80547-08-6; [a-$^{17}$O-€-CN)-Pt(O)(CN)$_2$, 86119-83-7; H$_3$PO$_4$O$_4$, 86119-77-9; KH$_2$PO$_4$, 86119-78-0; K$_3$HPO$_4$, 86119-79-1; CH$_3$PO$_3$, 8077-98-6; Ph$_2$PO$_4$, 86119-80-4; (Ph)$_2$PO$_4$, 86119-81-5; (Ph)$_2$PO$_4$, 86119-82-6; PCl$_3$, 69643-75-7; H$_3$PO$_4$, 86119-84-8; P, 7772-14-0; $^{17}$O, 13968-48-4; $^{11}$O, 14797-71-8. We thank Drs. R. J. Ackerman, J. A. Borst, D. W. B. Cole, J. L. D. Rees, R. S. Smith, and C. W. Webster for their helpful discussions and criticism of this work.

Abstract. The stereochemistry of lysine 2,3-aminomutase in Clostridium subterminale strain SB4 has been elucidated. Deuterium NMR has been used to show that the transformation of (2S)-a-lysine to (3S)-β-lysine proceeds with transfer of the 3-pro-R hydrogen of α-lysine to the 2-pro-R position of β-lysine. The 3-pro-R hydrogen of α-lysine is retained at C-3 of β-lysine. Also the C-2 hydrogen of α-lysine is retained at the 2-pro-S position of β-lysine. Thus, the reaction proceeds with inversion of configuration at C-2 and C-3. Experiments with [2-15N,3-13C]-α-lysine have shown that the amino group transfer takes place essentially completely intramolecularly. However, conversion of α-lysine-3,3-d$_2$ led to the formation of mainly β-lysine-d$_4$ indicating the reaction is not absolutely completely intramolecular.

Streptomyces, in which the metabolic product, β-l-lysine, occurs as a constituent of several antibiotics, including myomycin$^2$ and related compounds,$^4$ viomycin,$^5$ roseothricin,$^6$ geomycin,$^7$ tuberactinomycin (containing γ-hydroxy-β-lysine),$^8$ and the strepto-

---

1 Worcester Foundation for Experimental Biology.
2 University of Connecticut.

---

Sterechemistry of Lysine 2,3-Aminomutase Isolated from Clostridium subterminale Strain SB4

D. John Aberhart,* Steven J. Gould,³ Horng-Jau Lin,¹ T. K. Thiruvengadam,¹ and Bruce H. Weiller¹

Contribution from the Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts 01545, and School of Pharmacy, Section of Medicinal Chemistry and Pharmacognosy, The University of Connecticut, Storrs, Connecticut 06268. Received December 2, 1982

Abstract: The stereochemistry of lysine 2,3-aminomutase in Clostridium subterminale strain SB4 has been elucidated. Deuterium NMR has been used to show that the transformation of (2S)-α-lysine to (3S)-β-lysine proceeds with transfer of the 3-pro-R hydrogen of α-lysine to the 2-pro-R position of β-lysine. The 3-pro-R hydrogen of α-lysine is retained at C-3 of β-lysine. Also, the C-2 hydrogen of α-lysine is retained at the 2-pro-S position of β-lysine. Thus, the reaction proceeds with inversion of configuration at C-2 and C-3. Experiments with [2-15N,3-13C]-α-lysine have shown that the amino group transfer takes place essentially completely intramolecularly. However, conversion of α-lysine-3,3-d$_2$ led to the formation of mainly β-lysine-d$_4$ indicating the reaction is not absolutely completely intramolecular.

The transformation of α-1-lysine, 1a, into β-1-lysine, 2a, by the enzyme lysine 2,3-aminomutase constitutes the first step of a major metabolic pathway of lysine in Clostridia and other bacteria.$^9$ The transformation also takes place in several species of Nocardia or

---