Isotopic ^{18}O Shifts in ^{31}P NMR of Adenine Nucleotides Synthesized with ^{18}O in Various Positions

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Abstract: The analysis of phosphate ^{18}O labeled ADP and ATP by ^{31}P NMR spectroscopy at 145.7 and 235 MHz is described. The following isotopically labeled adenine nucleotide species were synthesized in various enzyme-catalyzed reactions: ADPβ-$P^{18}O_3$, ATPβ-$P^{18}O_3$, ADPβ-$P^{18}O_4$, ATPβ-$P^{18}O_3$(α-$P^{18}O_1$), ATPβ-$P^{18}O_3$($\gamma P^{18}O_1$), and ATP(β-P, γ-P)$^{18}O_6$. The magnitude of the isotopic (^{18}O) shift of the ^{31}P resonance varies from 0.0166 to 0.0285 ppm in the β-P bridge and nonbridge positions and these resonances which differ by 0.0118 ppm are completely resolved at 235 MHz. The γ-P(^{18}O) species are shifted by 0.0220 ppm per ^{18}O in both ATPγ-$P^{18}O_3$ (three nonbridge) and ATPγ-$^{18}O_4$ (one bridge, three nonbridge). For the latter compound, there was no indication of a difference in the chemical shift due to a bridge vs. a nonbridge ^{18}O even at 235 MHz.

It has been observed[1,2] that ^{18}O directly bonded to phosphorus causes a small shift of the order of 0.02 ppm in the chemical shift of ^{31}P. At high frequency, e.g., 145.7 MHz, the $^{18}O/^{16}O$ in inorganic phosphate can be determined with considerable accuracy at high values of ^{18}O.[1] Even at lower frequency, 40 MHz, in spite of the lack of resolution, good agreement of $^{18}O/^{16}O$ ratios between ^{31}P NMR and mass spectrometric analysis was achieved by using a curve analyzer.[3] In the current study, the ^{18}O analysis by ^{31}P NMR has been extended to ADP and ATP isotopically labeled in various positions of the molecule and the correlation of the magnitude of the isotopic shift to the double-bond character of the P–O bond was examined.

Experimental Section

KH_2PO_4 was exchanged twice with $H_2{}^{18}O$ (95.8% ^{18}O enrichment) to yield a product containing 93.6% ^{18}O.[4] Carbamate kinase, PEP,[5] AMP, and ATP were purchased from Sigma Chemical Co. and ADP was purchased from Boehringer Mannheim. KCNO was obtained from Fisher Scientific Co. $MgCl_2$ (99.999% pure) was a product of Accurate Chemical and Scientific Corp. ^{18}O-Enriched water was purchased from Miles. Pyruvate kinase was a gift from Dr. George Reed of the University of Pennsylvania and adenylate kinase was a gift from Dr. Noda Lafayette of Dartmouth Medical School. TEA from Eastman was redistilled before use. PEI plates were purchased from Brinkmann. All other reagents used were reagent grade without further purification. The composition of all synthesized ^{18}O nucleotides was checked on a PEI plate developed with 1.5 M LiCl.

NMR Measurements. The ^{31}P NMR spectra were recorded at 145.7 MHz on a Bruker WH360 NMR spectrometer; samples of 1.5 mL in 10-mm NMR tubes maintained at 25 °C were used. One sample was measured at 235 MHz on the Carnegie-Mellon 600-MHz spectrometer in a 5-mm sample tube at 19 °C.

Synthesis of ATP-$\gamma^{18}O_3$ (II). The ATP(γ-$P^{18}O_3$) (II) was synthesized in two steps by a modification of the procedure used to synthesize γ-^{32}P-ATP.[6] *l*-Carbamyl phosphate is synthesized chemically from $P_i(^{18}O)$ and KCNO and the ^{18}O phosphoryl group in the carbamyl phosphate produced is transferred to ADP to form ATP(γ-$P^{18}O_3$) (II) with three nonbridge ^{18}O atoms in a reaction catalyzed by carbamate kinase.[7]

$KH_2P^{18}O_4$ (300 μL of 1 M) (93.6% ^{18}O), 300 μL of 1 M sodium acetate, pH 4.8, and 375 μL of 6 M KCNO (prepared immediately before use) were incubated in a water bath at 30 °C. The pH was monitored at 5-min intervals; acetic acid was added to maintain the pH at about 6.5. An additional 100 μL of KCNO was added to the mixture after 30 min and incubated at pH 6.5 for an additional 15 min. The reaction mixture was then chilled in ice. To the cold mixture were added 550 μL of 550 mM ADP, pH 7.6, 1.5 mL of 1 M Tris-HCl, pH 7.3, 300 μL of 1 M $MgSO_4$, and 30 units of carbamate kinase in 50 μL. The mixture was incubated at 38 °C for 30 min. The yield of ATP-$\gamma^{18}O_3$ from ADP was 95%; the unreacted 5% was mainly due to the contamination of AMP in the ADP used. On the basis of [^{18}O]orthophosphate, the yield was 76%. The reaction was stopped by the addition of 450 μL of 50% trichloroacetic acid; the mixture was centrifuged, and the supernatant was extracted three times with five volumes of ether.

The components of the reaction mixture were separated on a diethylaminoethylcellulose (DE-52) column (3 × 25 cm). The adenine nucleotides were eluted with a 4-L gradient, 0–0.5 M, of TEA-CO_2 buffer, pH 7.6 (buffer was prepared by bubbling CO_2 into a TEA solution). P_i was eluted between 0.165 and 0.205 M salt, AMP at 0.265 M, ADP at 0.285 M, and ATP at 0.35 M salt. All three peaks were well resolved from one another. After the ATP fractions were concentrated in a rotary evaporator to near dryness, the ATP was dissolved in a few milliliters of H_2O and titrated to pH 8–9. The metal ions were removed from ATP by extraction with 1% 8-hydroxyquinoline.[8] The P_i-^{18}O could also be recovered.

Synthesis of ADP-$\beta^{18}O_3$ (I) and ATP-$\beta^{18}O_3$,$\gamma^{18}O$ (VI). The isotopically labeled ATP and ADP were synthesized from isotopically labeled carbamyl phosphate, AMP, a trace of ATP, and the enzymes adenylate kinase and carbamate kinase as indicated schematically in eq 1–4.

$KH_2P^{18}O_4$ (20 mg), 1 mL of 1 M sodium acetate, pH 4.8, and 1.25 mL of 6 M KCNO were incubated as described above. After 25 min, 0.4 mL of 6 M KCNO was added and incubation was continued for 15 min. The mixture was then chilled in ice. To the cold mixture were added the following: 500 μmol of AMP in 0.875 mL, 8 μmol of ATP in 0.6 mL, 0.6 mL of 1 M $MgCl_2$, 5 mL of 1 M Tris-HCl, pH 7.3, and

$$\text{AMP} + \text{ATP (trace amount)} \underset{}{\overset{\text{adenylate kinase}}{\rightleftharpoons}} 2\text{ADP} \quad (1)$$

$$\text{ADP} + \text{carbamyl-P}(^{18}O_4) \xrightarrow{\text{carbamate kinase}} \text{ATP}(\gamma P^{18}O_3) + CO_2 + NH_3 \quad (2)$$

$$\text{AMP} + \text{ATP}(\gamma P^{18}O_3) \overset{\text{adenylate kinase}}{\rightleftharpoons} \text{ADP} + \text{ADP}(\beta P^{18}O_3) \text{ three nonbridge O atoms} \quad (3)$$

$$\text{ADP}(\beta P^{18}O_3) + \text{carbamyl-P}(^{18}O_4) \xrightarrow[\text{kinase}]{\text{carbamate}} \text{ATP}(\beta\text{-}\gamma\text{-}P^{18}O_6) \quad (4)$$

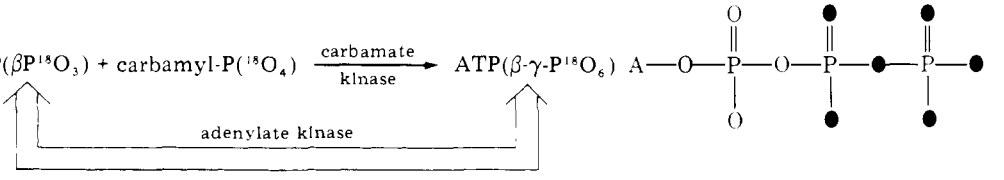

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Table I. ^{31}P Chemical Shift (ppm) (Upfield) per ^{18}O Bonded to P Atoms of ADP and ATP (B = Bridge, NB = Nonbridge)

nucleotide species	α	β	γ
I. ADP-$\beta^{18}O$		0.0220	
II. ATP-$\gamma^{18}O_3$			0.0220
III. ADP-$\beta^{18}O_4$	0.0166 (α-β B)	0.0215	
IV. ATP-$\gamma^{18}O_4$		0.0165 (β-γ B)	0.0220
V. ATP$\beta^{18}O_3$, $\gamma^{18}O_1$		0.0281 (NB) 0.0165 (β-γ B)	0.0220 (β-γ B)
VI. ATP$\beta^{18}O_2$, $\gamma^{18}O_2$		0.0285 (NB)[a] 0.0163 (B)[a]	0.0217
VII. ATP$\beta^{18}O_1$, $\alpha^{18}O_1$	0.0172 (α-β B)	0.0281 (NB) 0.0165 (α-β B)	

[a] Determined from resolved peaks at 235 MHz; all other bridge and nonbridge values were estimated from spectra at 145.7 MHz.

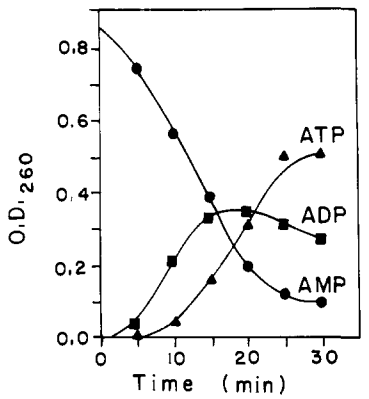
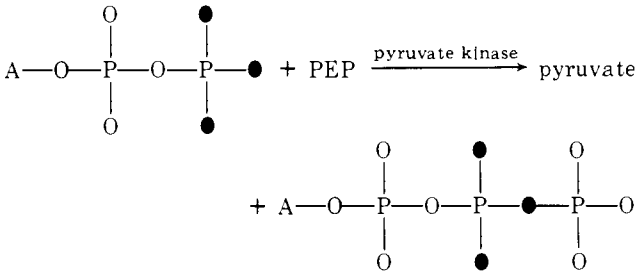


Figure 1. Distribution of nucleotides as a function of time in the preparation of ADP$\beta^{18}O_3$ (I) and ATPβ-, γ-$^{18}O_6$ (VI).

2.5 mL of 1 M KCl. The reactions were initiated with 1500 units of adenylate kinase (0.15 mL) and 50 units of carbamate kinase (0.1 mL) and the reaction mixture was incubated at 38 °C for 30 min. The course of the nucleotide concentrations over the 30-min period is shown in Figure 1. The mixture was chilled in ice immediately and cold $HClO_4$ was added to a final concentration of 6%. After removal of the precipitated protein by centrifugation, the supernatant was titrated to pH 7.6 with KOH. After $KClO_4$ was allowed to precipitate at 0 °C for at least 30 min, the precipitate was centrifuged and the supernatant was applied to a DE-52 column. The column was eluted with 4 L of TEA-CO_2 buffer, pH 7.6, gradient 0–0.5 M.

The fractions containing ADP-$\beta^{18}O_3$ (I) and ATP(β,γ)-$^{18}O_6$ (VI), respectively, were collected, and, after the solutions were concentrated with a rotary evaporator to near dryness, metals were removed from ATP (VI) as described above. An ADP-$\beta^{18}O_3$ solution at pH 8.0 was applied to a 1.9 × 5 cm column of acid-washed Chelex 100 to remove metals.

Synthesis of ATP-βP$^{18}O_3$ (V) (β–γ Bridge ^{18}O). To 6 mL of solution containing 100 mM Hepes, pH 7.6, 25 mM $MgSO_4$, 100 mM KCl, 15 mM ADP-$\beta^{18}O_3$, and 25 mM *P*-enolpyruvate, 0.4 mg of pyruvate kinase was added. The reaction mixture was incubated at 25 °C for 30 min. The reaction was stopped by diluting the mixture with 0.2 M TEA-CO_2 buffer, pH 7.6. The mixture was applied to a 3 × 25 cm DE-52 column and was eluted with a 2.2-L gradient, 0.2–0.45 M of TEA-CO_2 buffer, pH 7.6. ATP-$\beta^{18}O_3\gamma^{18}O_1$ was eluted at 0.35 M



salt. The sample was purified before storage as described above.

Conversion of ATP-$\gamma^{18}O_3$ (II) to ATP-$\beta^{18}O_3$ (VII) (One α–β Bridge, Two Nonbridge). A solution containing 20 mM Hepes buffer, pH 8.5, treated with Chelex, 8.3 mM ATP-$\gamma^{18}O_3$ (II), 2 mM valine treated with Chelex, 15 mM $MgCl_2$, and 1 mM ethylenediaminetetraacetate in 1.5 mL of 20% D_2O was incubated at 25 °C. The partial reaction shown (eq 5) was initiated by the addition of 18 μg of valine tRNA synthetase and the time course was monitored by the appearance of ^{18}O in the α-^{31}P peak. At equilibrium there are three γ-P species (0.354 $^{18}O_3{}^{18}O_1$, 0.138 $^{18}O_2{}^{16}O_2$, and 0.508 $^{16}O_4$), two α-P species (0.445 $^{18}O_1{}^{16}O_3$ and 0.555 $^{16}O_4$ and, four β-P species (0.354 $^{18}O_b$-$^{18}O_{2nb}{}^{16}O_1$, 0.092 $^{18}O_b{}^{18}O_{nb}{}^{16}O_2$, 0.046 $^{18}O_{2nb}{}^{16}O_2$, β and 0.508 $^{16}O_4$ where the subscript b indicates bridge and nb, nonbridge). The initial spectrum and the spectrum at equilibrium are shown in Figure 2.

Results

Isotopic (^{18}O) Shifts of ^{31}P Resonance. Based on the statistical distribution, for ATP-$\gamma^{18}O_3$ (II) containing 89% ^{18}O, four species exist (0.704 $^{18}O_3{}^{16}O_1$, 0.26 $^{18}O_2{}^{16}O_2$, 0.032 $^{18}O^{16}O_3$, and 0.0013 $^{16}O_4$); however, only the first two species could be

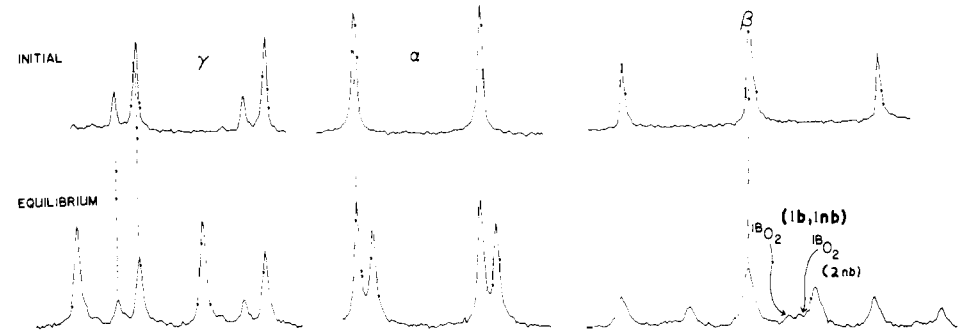
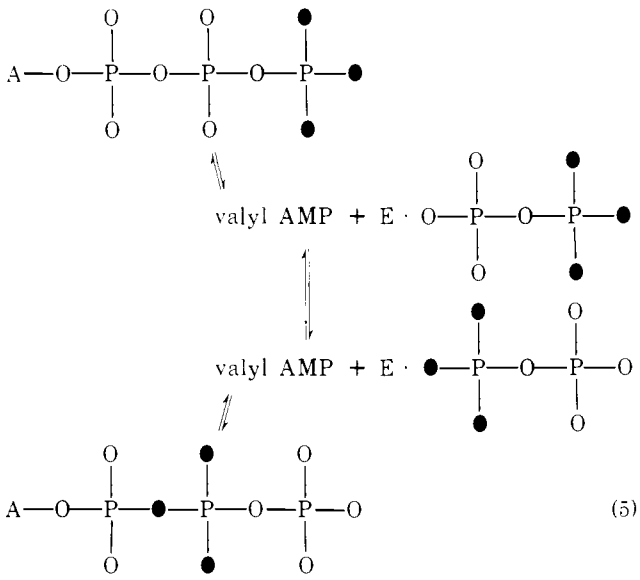


Figure 2. ^{31}P NMR spectra of the conversion of ATP$\gamma^{18}O_3$ (II), 89% ^{18}O, to ATPβ-$^{18}O_3$ (VII). The upper spectrum is the initial spectrum of a 1.5-mL solution containing 20% D_2O, 8.3 mM ATP, 2 mM valine in 20 mM Hepes buffer pH 8.8, 1 mM EDTA. The lower spectrum was recorded 7 h after the addition of 13 mM $MgCl_2$ and 18 μg of valyl tRNA synthetase of *Escherichia coli;* 40 mM EDTA was added before the spectrum was recorded. NMR parameters: 145.7 MHz, 100 scans, acquisition time 4.5 s, 45° flip angle.



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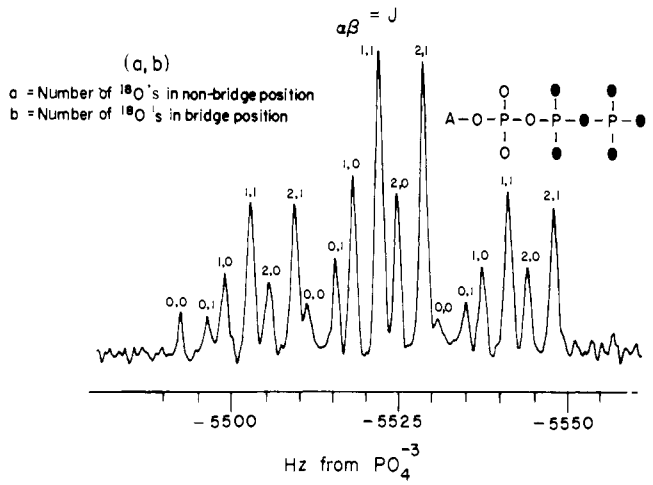

Figure 3. ^{31}P NMR spectrum of the β-P of ~63% ^{18}O ATP-β-, $\gamma^{18}O_6$ (VI) recorded at 235 MHz by correlation spectroscopy; 29 000 scans, sweep time 1 s.

determined quantitatively with the sensitivity available as shown in the upper spectrum of Figure 2. The chemical shift per ^{18}O is 0.0220 ppm (cf. Table I). Analogous spectra were obtained for all isotopically labeled nucleotides listed in Table I, two ADP species (I,β-$^{18}O_3$ and III,β-$^{18}O_4$), and five ATP species (II,γ-$^{18}O_3$, IV,γ-$^{18}O_4$, V,β-$^{18}O_3$(β-γ bridge), VI, β-γ-$^{18}O_6$, and VII,β-$^{18}O_3$(α-β bridge)). The syntheses of these compounds are described in the Experimental Section with the exceptions of ADP III, which has been previously described,[1] and ATP IV, which was synthesized chemically by Dr. M. R. Webb. The possibility of resolving peaks for the β-P with equal numbers of ^{18}O atoms differing only in the bridge vs. the nonbridge position first appeared feasible from the lower spectrum shown in Figure 2, an equilibrium mixture of ATP II and ATP VII where two overlapping ^{31}P resonances for the β-$^{18}O_2$ species were observed from ATP VII. To obtain a resolved spectrum, where the species with $^{18}O_1$ as well as $^{18}O_2$ could be observed and resolved for both β-P and γ-P of ATP, ATP VI, β-, γ-$^{18}O_6$ was synthesized with ~63% ^{18}O as described in the Experimental Section and recorded at 235 MHz. The spectrum of the β-P of ATP VI is shown in Figure 3 and all six lines due to $^{18}O_0$, two types of $^{18}O_1$, and of $^{18}O_2$ and $^{18}O_3$ are resolved; the chemical shifts are listed in Table I.

Discussion

The observable chemical shift of the ^{31}P resonances due to ^{18}O substitution in the phosphate group is a powerful tool to solve many mechanistic aspects of phosphate reactions.[1,3,8-11] However, the shift can be determined directly at high frequency only, e.g., at 145.7 MHz or even more readily at 235 MHz. Lower frequency instruments (40.5 MHz) may be used in conjunction with a curve analyzer.[3] Some problems emerge at high frequency which do not occur at low frequency. In particular, chemical exchange effects among various species cause greater line broadening. For example, the exchange rate between various metal chelates of ATP or with free ATP will affect the line width not only of the γ-P, but also of the α- and β-P. With the most commonly used metal ion, Mg^{2+}, the line width of the ^{31}P resonance at 24.3 MHz is 0.9 Hz for both MgATP and free ATP. At 145.7 MHz, the line width of metal free ATP is 1.2 Hz, but, after addition of a saturating amount of $MgCl_2$ (99.999% pure), the line width of the α-P increased to 2 Hz and the line width of β- and γ-P increased to 4 Hz undoubtedly due to exchange among the various MgATP species in solution. If Mg^{2+} is substituted by Ca^{2+}, with exchange rates ~1000 times faster than those of Mg^{2+}, no such line broadening is observed.

For the most part, the magnitude of the ^{18}O shift on the ^{31}P resonance correlates well with the amount of double-bond character of the bond as indicated in Table II, the difference being greatest between the shifts of β-P of ATP due to the nonbridge oxygens (0.0285 ppm) and the α-β or β-γ bridge oxygen (~0.0167 ppm). A similar difference between the effect due to bridge and nonbridge oxygen has been reported for [$\alpha\beta$-^{18}O]ATPαS and [α-^{18}O]ATPαS.[12] However, the shift on β-P due to the two nonbridge oxygens of the β-phosphate of ATP shows a considerable deviation from the calculated value. The differences due to the bridge oxygen between the pairs ADP$\beta^{18}O_3$-ADP$\beta^{18}O_4$ and ATP$\gamma^{18}O_3$-ATP$^{18}O_4$ are apparently too small to detect at 145.7 MHz. It is somewhat

Table II. ^{31}P Chemical Shift per ^{18}O as a Function of P-O Bond Type, N[a]

source of ^{18}O	N	shift, ppm calcd[b]	shift, ppm obsd (av)
phosphoanhydride bridge (ADP, ATP)	1.00		0.0166[c]
phosphate anion	1.25	0.0208	0.0206
terminal-$P^{18}O_3$ anion (ADP, ATP)	1.33	0.0221	0.0220
ATP β-nonbridge ^{18}O	1.50	0.0249	0.0285

[a] $N = \sum(1a + 2b)/(a + b)$ where a = number of single bonds and b = number of double bonds. [b] Calculated from the value observed for $N = 1$. [c] Average of ^{31}P shifts due to the α-β bridge ^{18}O on α-^{31}P and β-^{31}P and to the β-γ bridge ^{18}O on β-^{31}P.

surprising, however, that even at 235 MHz where the β-γ bridge ^{18}O and nonbridge ^{18}O species on β-P of ATPβ,γ-$^{18}O_6$ are well resolved (see Figure 3), the spectrum of the γ-P($^{18}O_4$) gave no indication of any nonequivalence of the shifts due to the nonbridge ^{18}O as compared to the β-γ bridge ^{18}O. A simple five-line pattern corresponding to $^{18}O_0$, $^{18}O_1$, $^{18}O_2$, $^{18}O_3$, and $^{18}O_4$ was observed for each of the two regions of the γ-P doublet of the 63% ^{18}O sample at 235 MHz. The equivalence within experimental error of the chemical shift of the bridge and nonbridge oxygens on the γ-phosphate of ATP and the nonequivalence for the β-phosphate of ATP may be related to the axial symmetry in the chemical-shift tensors of the dianionic forms of mononucleotides and the axial asymmetry for the anionic form of diesters.[14] Since the chemical-shift tensor of the acid form of mononucleotides is no longer axially symmetric, it would be of interest to examine ATP $^{18}O_4$ in the acid form.

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References and Notes

(1) Cohn, M.; Hu, A. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 200–203.
(2) Lutz, O.; Nolle, A.; Staschewski, D. *Z. Naturforsch. A* **1978**, *33*, 380–382.
(3) Van Etten, R. L.; Risley, J. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, 4784–4787.
(4) Cohn, M.; Drysdale, G. *J. Biol. Chem.* **1955**, *216*, 831–846.
(5) The following abbreviations are used: PEP, phosphoenolpyruvate; TEA, triethylamine; PEI, polyethylenimine; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.
(6) Mokrosch, L. C.; Caravaca, J.; Grisolia, S. *Biochim. Biophys. Acta* **1960**, *37*, 442–447.
(7) Metzenberg, R. L.; Marshall, M.; Cohen, P. P.; Miller, W. G. *J. Biol. Chem.* **1959**, *234*, 1534–1537.
(8) Brown, F. F.; Campbell, I. D.; Henson, R.; Hurst, C. W. J.; Richards, R. E. *Eur. J. Biochem.* **1973**, *38*, 54–58.
(9) Webb, M. R.; McDonald, G. G.; Trentham, D. R. *J. Biol. Chem.* **1978**, *253*, 2908–2911.
(10) Bock, J. L.; Cohn, M. *J. Biol. Chem.* **1978**, *253*, 4082–4085.
(11) Lowe, G.; Sproat, B. S. *J. Chem. Soc., Perkin Trans. 1* **1979**, 1622–1630.
(12) Jordan, F.; Patrick, J. A.; Salamone, S., Jr. *J. Biol. Chem.* **1979**, *254*, 2384–2386.
(13) Jarvest, R. L.; Lowe, G. *J. Chem. Soc., Chem. Commun.* **1979**, 364–366.
(14) Terao, T.; Matsui, S.; Akasi, K. *J. Am. Chem. Soc.* **1977**, *99*, 6136–6138.

Nucleoside Complexing. A Raman and ^{13}C NMR Spectroscopic Study of the Binding of Hard and Soft Metal Species

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Abstract: The influence of a wide variety of metal salts and complexes on the Raman and ^{13}C NMR spectra of three of the four common nucleosides (uridine, adenosine, and cytidine) in Me_2SO has been determined. Several related molecules also studied included deoxycytidine, 5-methyldeoxycytidine, 8-bromoadenosine, 6-dimethylaminopurine-9-riboside, and N^4,N^4-dimethylamino-1-methylcytosine. Inorganic species utilized included *cis*-$[Pt(Me_2SO)_2Cl_2]$, $HgCl_2$, $Zn(NO_3)_2$, $Cd(NO_3)_2$, $Pb(NO_3)_2$, $Pb(ClO_4)_2$, $PbCl_2$, $Mg(NO_3)_2$, $Ca(NO_3)_2$, $Sr(NO_3)_2$, $Ba(NO_3)_2$, $BaCl_2$, $La(NO_3)_3$, $Pr(NO_3)_3$, $Lu(NO_3)_3$, $Lu(ClO_4)_3$, $Ga(NO_3)_3$, $LiClO_4$, NEt_4Cl, and NEt_4NO_3. The ^{13}C NMR shift dependencies on metal concentration were used to calculate formation constants for selected purine and pyrimidine derivatives. Constants obtained with $HgCl_2$ agreed well with literature values. However, our studies suggest that alkaline earth metal ions have a lower affinity for nucleosides than that suggested previously. Of the common nucleosides, cytidine has the greatest affinity for alkaline earth metal ions and these appear to bind to O-2. Raman and ^{13}C NMR studies with N^4,N^4-dimethylamino-1-methylcytosine are presented which support the O-2 binding mode. The only electrophile capable of interacting with N-3 of this pyrimidine is the H^+ ion. Conclusions drawn about binding sites to other nucleosides are in agreement with previous studies. A summary table specifying the importance of anion and cation binding to the four common nucleosides is included. This summary provides a rationale for all of the literature observations and should help to minimize the confusion currently surrounding the nature of the interactions of inorganic species with nucleosides in Me_2SO.

Introduction

Growing recognition of the importance of metal ion interactions with nucleic acids and nucleotides has stimulated an explosive growth in the number of studies devoted to understanding the chemistry of the complexes formed. This interest has led to at least seven recent reviews of this topic.[1-7]

The great promise of platinum(II) metalloantineoplastic

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