acquired with standard software and conditions. Carbon spectra were obtained at 62.9 MHz on the WM 250 with 16K data points over 13 000 Hz. Titrations were carried out by addition of measured quantities of pyridine-d_5 or 3,5-lutidine solution to the NMR solution.

A 500-MHz COSY spectrum of BP4 and a two-dimensional ^1H-^{13}C correlation spectrum of BP2 were obtained by Dr. W. E. Hull on AM500 and AM400 instruments at Bruker Analytische Messtechnik, Karlsruhe.

No attempt was made to remove traces of residual water from porphyrin solutions, and its presence was clearly detectable in proton spectra. Therefore we cannot exclude its participation with a ligand. However, its relatively invariant chemical shift indicates that its effect is minimal.

Computations were carried out with programs which have been described previously.[12-14,16]

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Metal-Nucleotide Interactions. 3. ^{17}O, ^{31}P, and ^1H NMR Studies on the Interaction of Sc(III), La(III), and Lu(III) with Adenosine 5′-Triphosphate[1]

Yeun-Jund Shyy, Tsun-Chung Tsai, and Ming-Daw Tsai*

Contribution from the Department of Chemistry, The Ohio State University, Columbus, Ohio 43210. *Received November 5, 1984*

Abstract: The interaction of adenosine 5′-triphosphate (ATP) with diamagnetic trivalent metal ions Sc(III), La(III), and Lu(III) was investigated by ^{17}O NMR, ^{31}P NMR, and ^1H NMR. All three techniques showed the formation of 1:2 M(III)/ATP complexes for all three metal ions. The exchange rate between free and bound ATP on the NMR time scale was fast for La(III) and Lu(III) but slow for Sc(III) ($<12\ s^{-1}$ at 30 °C). ^{31}P NMR results showed entirely different patterns of chemical shifts of ATP induced by the three metal ions. On the basis of our recent work [Huang, S. L.; Tsai, M.-D. *Biochemistry* **1982**, *21*, 951-959], the line-broadening effect in ^{17}O NMR is more reliable than the chemical shift effect in ^{31}P NMR in identifying the coordination between nucleotides and diamagnetic metal ions. The ^{17}O NMR results showed that binding of Sc(III), La(III), and Lu(III) induced a small chemical shift effect (5-15 ppm downfield shifts) and a large line-broadening effect for all of the three phosphates of ATP. Comparison of the relative magnitudes of the line-broadening effect for the α-, β-, and γ-phosphates of ATP suggested that the predominant macroscopic structure of $Sc^{III}(ATP)_2$, $La^{III}(ATP)_2$, and $Lu^{III}(ATP)_2$ is the α,β,γ-tridentate. Such a conclusion was further supported by ^1H NMR, which showed no indication of direct binding between M(III) and the adenine ring and showed significant upfield shifts for the resonances of H-2, H-8, H-1′, and others, which can be explained by the ring-current effect due to base stacking in $M^{III}(ATP)_2$.

Because of their significance in chemistry, biochemistry, and biology, the structures of metal ion-nucleotide complexes have been studied extensively by various physical techniques such as NMR (^{31}P, ^1H, ^{13}C, and ^{15}N), IR, UV, and others, as reviewed recently by Martin and Mariam.[2] However, the sites of coordination remain unresolved except for a few complexes such as $Co^{II}IMP$,[3] $Cr^{III}ATP$,[4] and $Co^{III}ATP$[4] which have been determined by X-ray crystallography. For the complexes of paramagnetic metal ions, the most widely used technique is the NMR paramagnetic relaxation method.[5] For the complexes of diamagnetic metal ions, ^{31}P chemical shifts have been used to deduce the binding of metal ions with the phosphate moiety of nucleotides,[6-10] and ^1H chemical shifts have been used to study the binding to the adenine moiety of ATP.[6,8-10]

The use of ^{31}P chemical shifts to elucidate the coordination sites of MgATP has generated controversial results.[6,7] Since ^{31}P chemical shifts of phosphate esters are very sensitive to conformation and the O-P-O bond angle,[11] there is no basis to directly correlate the metal-induced ^{31}P chemical shift to the site of coordination. As a possible substitute to the ^{31}P chemical shift method, we have shown that the diamagnetic Co(III) ion induces a large chemical shift effect and line-broadening effect on the ^{17}O NMR resonance of the directly coordinated oxygen.[12-15] The

(1) For parts 1 and 2, see ref 12 and 13, respectively. Abbreviations used: ADP, adenosine 5′-diphosphate; ATP, adenosine 5′-triphosphate; IMP, inosine 5′-monophosphate; TSP, sodium 3-(trimethylsilyl)propionate.

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Table I. ^{31}P NMR Parameters of Metal(III)–ATP Complexes at pH 8.0

complex	chemical shifts						coupling const		ref
	P_α	(ΔP_α)	P_β	(ΔP_β)	P_γ	(ΔP_γ)	$J_{\alpha\beta}$	$J_{\beta\gamma}$	
ATP	−10.6		−21.3		−5.7		19.5	19.9	
$Sc^{III}(ATP)_2$	−11.8	(−1.2)	−19.9	(+1.4)	−8.8	(−3.1)	17.0	18.7	
$La^{III}(ATP)_2$	−11.1	(−0.5)	−18.8	(+2.5)	−5.6	(+0.1)	17.4	17.7	
$Lu^{III}(ATP)_2$	−10.3	(+0.3)	−17.8	(+3.5)	−6.0	(−0.3)	15.6	17.2	
Co^{III}ATP (β,γ-bidentate)		(−0.4)		(+10.4)		(+9.9)	20	16	17
Co^{III}ATP (α,β,γ-tridentate)		(9.5)		(+12.5)		(+9.9)	17–20	16	17
Mg^{II}ATP		(+0.3)		(+2.5)		(+0.5)	15.8	15.8	8
Ca^{II}ATP		(+0.2)		(+2.0)		(+0.6)	16.6	17.2	8
Zn^{II}ATP		(+0.2)		(+1.9)		(+0.3)	16.6	15.5	8
Cd^{II}ATP		(+0.2)		(+2.1)		(+1.2)	18.0	15.8	8
Sr^{II}ATP		(+0.3)		(+1.9)		(+1.0)	17.2	17.0	8
Hg^{II}ATP		(+0.1)		(+0.1)		(0)	19.3	19.2	8
Pb^{II}ATP		(+1.5)		(+4.2)		(+2.8)	19.8	19.6	8

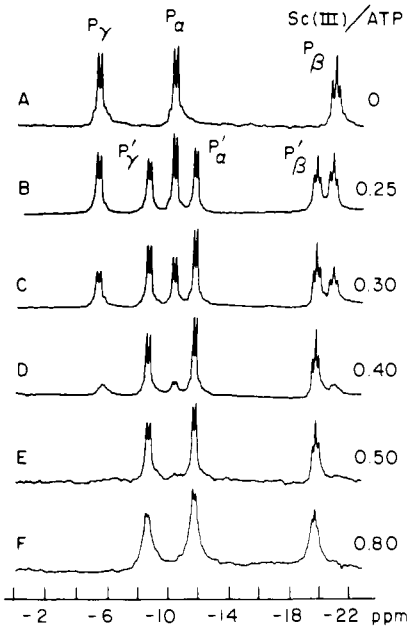

Figure 1. ^{31}P NMR (81.0 MHz) spectra of ATP (10 mM, pH 8.0) with varying concentrations of $ScCl_3$. Spectral parameters: spectral width 2500 Hz, acquisition time 1.6 s, 60° pulse, line broadening 2 Hz, 30 ± 2 °C. The signals P_α, P_β, and P_γ are due to free ATP whereas P_α', P_β', and P_γ' are due to complexed ATP.

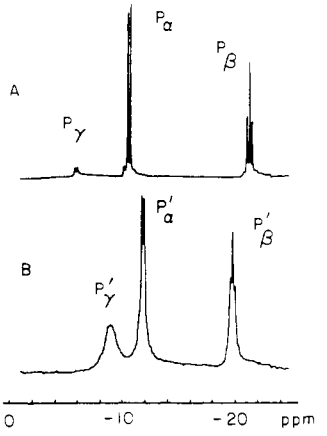

Figure 2. ^{31}P NMR spectra showing the assignment of the P_γ signal of $Sc^{III}(ATP)_2$. (A) [γ-$^{17}O_3$]ATP, 10 mM; (B) after addition of 5 mM $ScCl_3$. The sample and spectral conditions are the same as Figure 1.

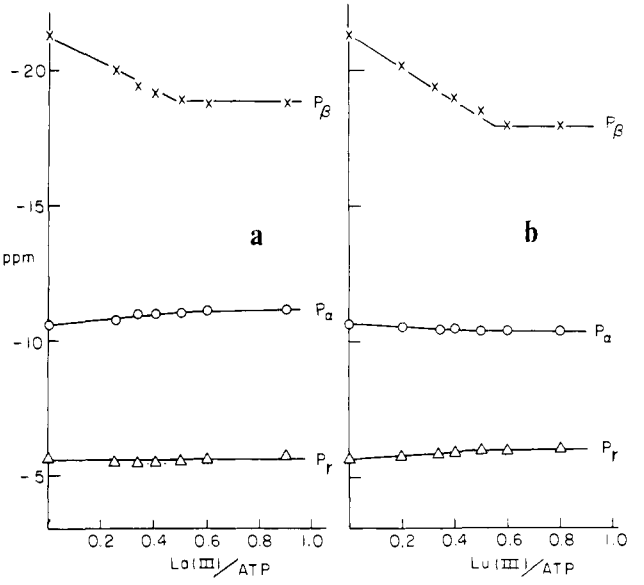

Figure 3. ^{31}P chemical shifts of ATP (10 mM) with varying concentrations of $LaCl_3$ (a) and $LuCl_3$ (b).

line-broadening effect has been used to resolve the controversy of the Mg^{II}ATP structure, even though the chemical shift effect is quite small in Mg^{II}ATP.[12,16]

As our continuing effort to develop the ^{17}O NMR technique and to understand the chemical structures of metal ion–nucleotide complexes, we now report the results of ^{17}O, ^{31}P, and ^1H NMR studies on the coordination between ATP and three trivalent metal ions, Sc(III), La(III), and Lu(III).

Results

^{31}P NMR Properties. Figure 1 shows the ^{31}P NMR spectra of ATP at different Sc(III)/ATP ratios. It is clear that Sc(III) forms a 1:2 complex with ATP at pH 8.0 and that the exchange rate between free and bound ATP is slow relative to the time scale of ^{31}P NMR. For $Sc^{III}(ATP)_2$, the triplet at −19.9 ppm can be assigned to P_β. The assignments of P_α and P_γ were based on the quadrupolar boradening of ^{31}P NMR signals by directly bonded ^{17}O. As shown in Figure 2, the ^{17}O-quenched P_γ signal of ATP is shifted to −8.8 ppm in $Sc^{III}(ATP)_2$, whereas the unlabeled P_α signal of ATP is shifted to −11.8 ppm. The ^{31}P chemical shifts and coupling constants are summarized in Table I, together with the data for ATP complexes of other divalent and trivalent metal ions (at the same pH) available in the literature.

The exchange rate of the La^{III}·ATP complex is fast relative to the time scale of ^{31}P NMR. The ^{31}P chemical shifts at varying concentrations of La(III) are shown in Figure 3a. Again, a stoichiometry of La(III)/ATP = $^1/_2$ was observed. The chemical shifts and coupling constants of $La^{III}(ATP)_2$ are also summarized in Table I. Similarly, the Lu^{III}·ATP complex shows a 1:2 stoichiometry as shown by the titration curves (Figure 3b). The large

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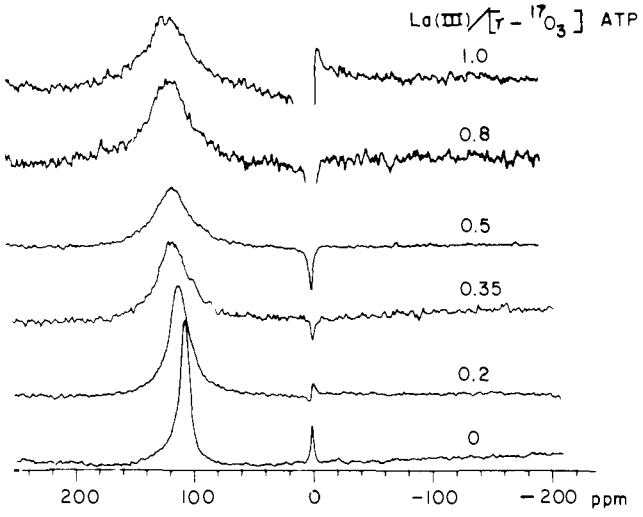
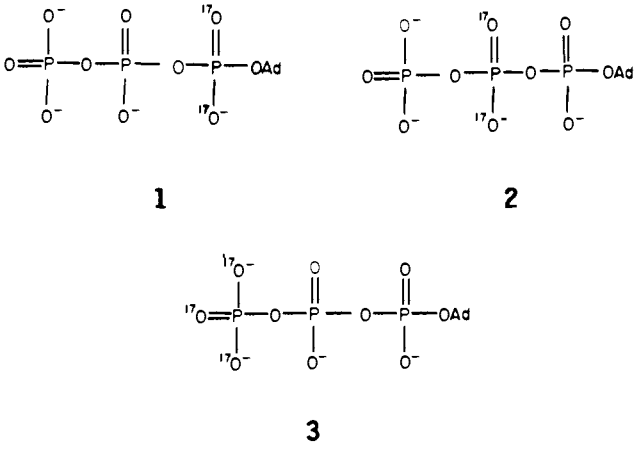


Figure 4. ^{17}O NMR spectra (40.68 MHz) of [γ-$^{17}O_3$]ATP (10 mM in ^{17}O-depleted water, pH 8.0) with varying concentrations of $LaCl_3$. Spectral parameters: spectral width 20000 Hz, acquisition time 102.4 ms, receiver gate 30 μs, line broadening 50 Hz. The T_1 inversion–recovery experiment was used for partial suppression of the solvent signal (which has longer T_1 than the sample signal), with 180° pulse, 90° pulse, and τ as 52 μs, 26 μs, and 5 ms, respectively. The delay between acquisitions was 20 ms. The number of transients varied from 5000 to 50000. The temperature was 27 ± 2 °C. No decoupling was used. The decreased signal/noise ratio when La(III)/ATP > 0.5 is partially caused by some precipitation at high La(III) concentrations.

separation between free and bound P_β signals of $Lu^{III}(ATP)_2$ (3.5 ppm) causes broadening of the P_β signal at Lu(III)/ATP ratios <0.5.

As shown in Table I, most divalent cations induce a downfield shift (+shift) in the ^{31}P chemical shifts of ATP, with the shift of P_β being the largest in all cases except Hg(II). Coordination of trivalent cation Co(III) induces + changes of 9.5–12.5 ppm.[17] The ^{31}P chemical shift changes induced by La(III) and Lu(III) are similar to those of divalent cations, i.e., a 2–4 ppm downfield shift at P_β and small shifts at P_α and P_γ. However, the result of $Sc^{III}(ATP)_2$ is quite different, with P_β shifted downfield and P_α and P_γ shifted upfield substantially. Such metal ion-induced upfield shifts have been observed only in $Al^{III}ATP$ at lower pH values.[9,10]

^{17}O NMR Properties. The effects of La(III) binding on the ^{17}O NMR properties of ATP is illustrated by [γ-$^{17}O_3$]ATP, as shown in Figure 4. It is obvious that the ^{17}O NMR signal is broadened and shifted downfield upon successive addition of $LaCl_3$. In Figure 5a, the line widths ΔO of [α-$^{17}O_2$]ATP (**1**), [β-$^{17}O_2$]ATP (**2**), and [γ-$^{17}O_3$]ATP (**3**) are plotted as a function of the [La(III)]/[ATP] ratio. The corresponding chemical shifts (δ O) are



1 **2**

3

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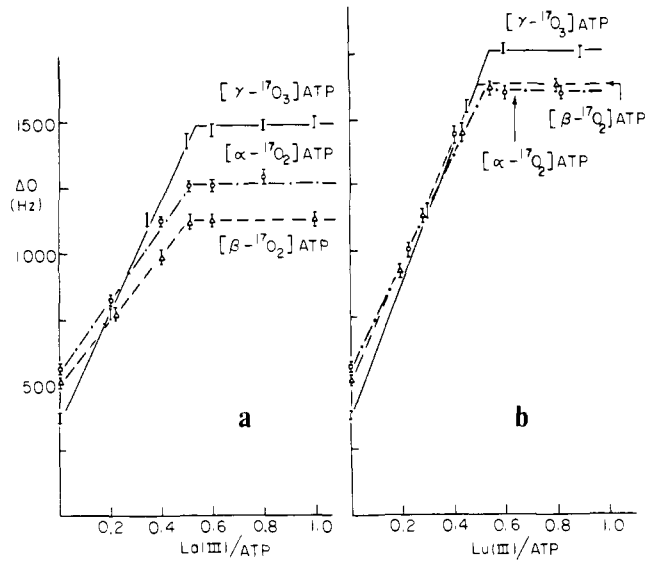

Figure 5. ^{17}O NMR line widths of [α-$^{17}O_2$]-, [β-$^{17}O_2$]-, and [γ-$^{17}O_3$]ATP (10 mM) with varying concentrations of $LaCl_3$ (a) and $LuCl_3$ (b).

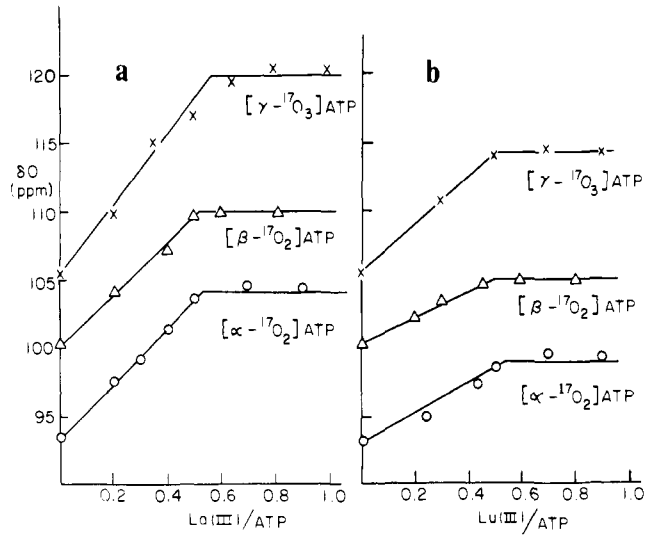

Figure 6. ^{17}O chemical shifts of [α-$^{17}O_2$]-, [β-$^{17}O_2$]-, and [γ-$^{17}O_3$]ATP (10 mM) with varying concentrations of $LaCl_3$ (a) and $LuCl_3$ (b).

plotted in Figure 6a. Both ΔO and δ O of **1**, **2**, and **3** show a linear change up to [La(III)]/[ATP] = 0.5, in agreement with a stoichiometry of La(III)/ATP = $^1/_2$ on the basis of ^{31}P NMR results.

The effect of Lu(III) on the ^{17}O NMR properties of ATP is similar to that of La(III). The chemical shifts and line widths as a function of the [Lu(III)]/[ATP] ratio are shown in Figures 5b and 6b, respectively. The results again show a stoichiometry of Lu(III)/ATP = $^1/_2$.

The effect of Sc(III) on the ^{17}O NMR properties of ATP is illustrated by [α-$^{17}O_2$]ATP. As shown in Figure 7, , Sc(III) causes the ^{17}O NMR signal of [α-$^{17}O_2$]ATP to broaden and shift downfield. At Sc(III)/ATP ratios of 0.2 and 0.35, the signal consists of a sharp component and a broad component, which indicates a slow exchange between bound and free ATP on the ^{17}O NMR time scale. When Sc(III)/ATP = 0.53, only a broad component is observed, which is consistent with a stoichiometry of Sc(III)/ATP = $^1/_2$. Similar ^{17}O NMR properties have also been observed for the interaction of Sc(III) with [β-$^{17}O_2$]ATP and [γ-$^{17}O_3$]ATP (spectra not shown).

The ^{17}O chemical shifts of free and complexed ATP are summarized in Table II. In each case, there is a downfield shift of 5–15 ppm. The effect seems to follow the orders La(III) > Sc(III) > Lu(III) and γ-O > α-O ≈ β-O. As noted in the Discussion

Table II. Summary of ^{17}O NMR Results

	$\Delta 0_f$ or $\Delta 0_b$, Hz[a]			R values			δ 0, ppm[b]		
	α	β	γ	α	β	γ	α	β	γ
ATP	480	430	290				93.3	100.3	105.5
$Sc^{III}(ATP)_2$	1620	1420	1680	2.4	2.3	4.8	102 (+9)	106 (+6)	118 (+13)
$La^{III}(ATP)_2$	1180	1040	1400	1.5	1.4	3.8	104 (+11)	110 (+10)	120 (+15)
$Lu^{III}(ATP)_2$	1530	1550	1680	2.2	2.6	4.8	99 (+6)	105 (+5)	114 (+9)

[a] The estimated error is ±5% for $\Delta 0_f$ and ±10% for $\Delta 0_b$. [b] The estimated error is ±0.5 ppm for free ATP and ±1.5 ppm for complexed ATP. The numbers in parentheses are changes from free ATP.

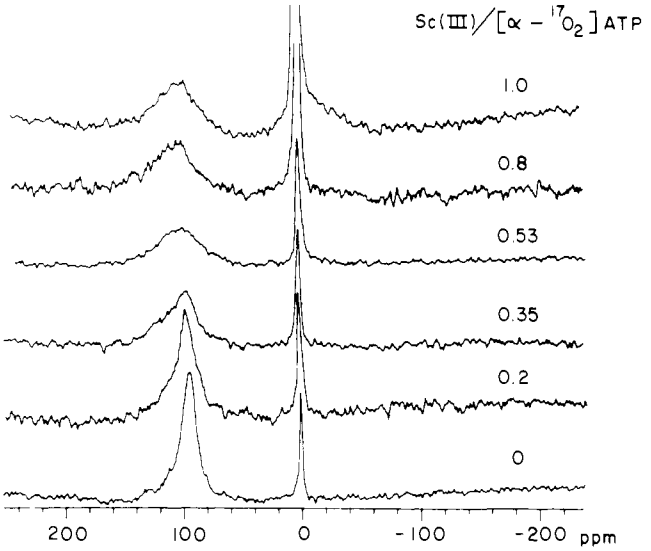

Figure 7. ^{17}O NMR spectra of [α-$^{17}O_2$]ATP with varying concentrations of $ScCl_3$. The sample and spectral conditions are the same as Figure 4, except the use of single-pulse experiments without solvent suppression.

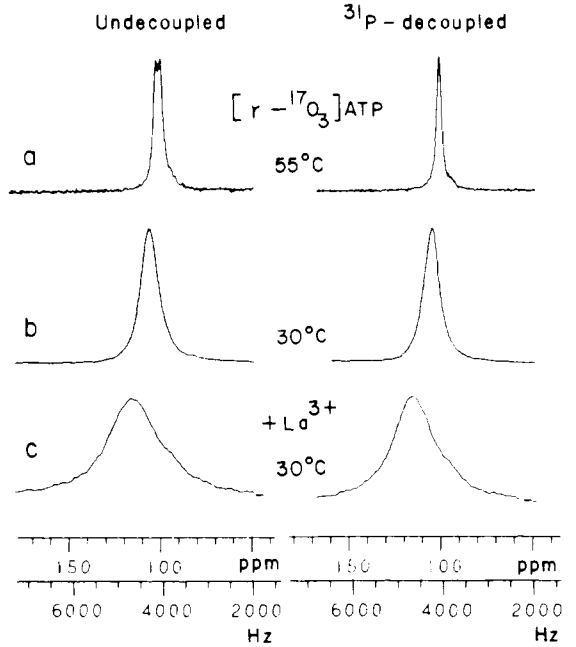

Figure 8. Undecoupled and ^{31}P-decoupled ^{17}O NMR spectra of [γ-$^{17}O_3$]ATP (10 mM, pH 8.0): (a) 55 °C; (b) 30 °C; (c) 30 °C, with addition of 5 mM $LaCl_3$. The spectral parameters are the same as Figure 4, except the number of transients (1000, 2000, and 18 000 for a, b, and c, respectively) and the line broadening (2 Hz for a, 50 Hz for b and c).

section, the chemical shift changes cannot be quantitatively correlated with the extent of coordination.

Line-Broadening Effect in ^{17}O NMR. For small molecules in solution, the ^{17}O NMR line width, ΔO, can be expressed by[15]

$$\Delta O \simeq \frac{12\pi}{125}\left(1 + \frac{\eta^2}{3}\right)\left(\frac{e^2qQ}{h}\right)\tau_r \qquad (1)$$

where $(e^2qQ)/h$ is the nuclear quadrupolar coupling constant, η is the asymmetry parameter, and τ_r is the rotational correlation time. The "line-broadening effect" induced by metal coordination has been defined as [12,15]

$$R = \frac{\Delta O_b - \Delta O_f}{\Delta O_f} \qquad (2)$$

where ΔO_f and ΔO_b represent the line widths of free and bound nucleotides, respectively. The values of ΔO_f and ΔO_b can be obtained from the observed line widths (i.e., Figures 5 and 7) by correcting for the contribution from exponential multiplication (50 Hz), field inhomogeneity (20–30 Hz for horizontal, nonspinning samples), and other factors such as spin–spin coupling.

Although the ^{17}O NMR spectra were measured in H_2O (^{17}O-depleted), the phosphates are in the deprotonated state at pH 8.0. Thus, all ^{17}O NMR spectra were obtained without ^1H decoupling to avoid heating of samples.

The ^{17}O–^{31}P spin–spin coupling constant, J_{P-O}, was in the order of 105–120 Hz for ATP measured at elevated temperatures.[18] The coupling constants of metal ion–ATP complexes should be similar based on our recent work.[13] Thus, it seems that either the observed ΔO should be corrected for 105–120 Hz of J_{P-O} as was done in our study with MgATP[12] or the ^{17}O spectra should be measured with ^{31}P decoupling. However, we have found that either procedure will simply introduce larger errors. Figure 8 shows the undecoupled and ^{31}P decoupled ^{17}O NMR signals of [γ-$^{17}O_3$]ATP at 55 °C (a), 30 °C (b), and in the presence of La(III) at 30 °C (c). At 55 °C, the signal was split, with J_{P-O} = 105 Hz, which collapsed upon ^{31}P decoupling. At 30 °C, the free ATP and $La^{III}(ATP)_2$ signals were narrowed by 40 and 200 Hz, respectively. Such narrowings were found to be mainly caused by a 5–10 °C increase in the actual sample temperature due to the decoupler power, even though the meter reading remained at 30 °C. Thus, the P–O coupling contributes significantly to the ^{17}O line width only when the coupling is partially resolved. When the signal is broad, it can contribute no more than 10–20 Hz, which is within the experimental error of measurements.

For the above reasons, the ^{17}O NMR spectra were measured without P–O decoupling. The ΔO_f and ΔO_b, as listed in Table II, were obtained from these spectra by correcting for exponential multiplication and field inhomogeneity, but not for ^{17}O–^1H or ^{17}O–^{31}P spin–spin coupling. The actual sample temperature was 27 ± 2 °C. The R values are also summarized in Table II. The estimated errors in ΔO_f and ΔO_b are ±5% and ±10%, respectively.

On the basis of our previous model study with Co(III) complexes of ADP and ATP,[12,13] the fact that R values are substantially greater than zero suggests that all three M(III) ions interact with α-, β-, and γ-phosphates of ATP. In the case of Mg^{II}ATP, we reported R values of 0.7–1.1, 1.4–2.0, and 1.8–2.5 for the α-, β-, and γ-^{17}O of ATP under various conditions.[12] This was used to conclude that Mg(II) interacts with all the α-, β-, and γ-phosphates of ATP and that the extent of α coordination may be smaller than the β and γ coordination (which implies that MgATP could be a mixture of α,β,γ-tridentate and β,γ-bidentate

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Table III. Proton NMR Results of Metal(III)–ATP Complexes at pH 8.0[a]

complex	chemical shifts, ppm							coupling const (Hz)
	H8	H2	H1′	H2′	H3′	H4′	H5′5″	$J_{1'2'}$
ATP	(8.545)	(8.248)	(6.136)	(4.812)	(4.639)	(4.390)	(4.24)	(6.1)
$Sc^{III}(ATP)_2$	−0.445[b]	0[b]	−0.156	−0.062	−0.109			0
$La^{III}(ATP)_2$	−0.175	−0.158	−0.136	−0.122	−0.099	−0.04	+0.03	−0.8
$Lu^{III}(ATP)_2$	−0.225	−0.178	−0.166	−0.142	−0.129	−0.04	+0.06	−0.5

[a] The assignments of free ATP signals were based on ref 8. The assignments of $La^{III}(ATP)_2$ and $Lu^{III}(ATP)_2$ complexes were based on succesive changes of the signals upon titrating with the metal ions. For the slow-exchange complex $Sc^{III}(ATP)_2$, the assignments were confirmed by homonuclear decoupling experiments. [b] Alternative assignments are −0.297 ppm for H8 and −0.148 ppm for H2.

macroscopically). It was also pointed out that "whether the R value or the absolute ΔO_b is a better reflection of binding remains to be established".

For $Sc^{III}(ATP)_2$, the R values of α- and β-^{17}O are the same, which is a notable difference from $Mg^{II}ATP$. Although the R value of γ-^{17}O is twice as large, the ΔO_b values of α-, β-, and γ-^{17}O are within ±9% of one another (the error for ΔO_b is ±10%). Furthermore, only a single species is observed in ^{31}P and ^{17}O NMR, even though the complex is in slow exchange. Thus, it is most reasonable to conclude that $Sc^{III}(ATP)_2$ is predominantly α,β,γ-tridentate.

The two lanthanide(III) ions could have different coordination properties from Sc(III). However, $Lu^{III}(ATP)_2$ has ΔO_b and R values comparable to $Sc^{III}(ATP)_2$ and thus is also likely to be α,β,γ-tridentate predominantly.

The structure of $La^{III}(ATP)_2$ could be more complicated. The ΔO_b and R values are smaller than those of $Sc^{III}(ATP)_2$ and $Lu^{III}(ATP)_2$, and the ΔO_b of γ-^{17}O is larger than that of α- and β-^{17}O. The data could be interpreted as either a tridentate with a stronger γ coordination, or a mixture of α,β,γ-tridentate (ca. 75%) and γ-monodentate (ca. 25%).

^{1}H NMR Properties. ^{1}H NMR has been used to study the interaction of metal ions with the adenine ring of ATP. A downfield shift of H-8 has been used as evidence for metal ion binding with N-7, as in cases of $Zn^{II}ATP$ and $Cd^{II}ATP$.[8,19,20,26] Since the ^{17}O NMR results suggest that Sc(III), La(III), and Lu(III) all coordinate with α-, β-, and γ-phosphates of two ATP molecules, there should be no direct binding with the adenine ring if the complexes are octahedral. Such a prediction has been supported by ^{1}H NMR.

Figure 9 shows the ^{1}H NMR spectra (low field region) of ATP in the presence of varying concentration of Sc(III). The chemical shifts and coupling constants are summarized in Table III. It seems that Sc(III) induces a large upfield shift (0.46 ppm) on one of the ring protons (H-8) but has little effect on the other (H-2). An alternative interpretation is that H-8 is shifted to coincide with the resonance of H-2, whereas H-2 is shifted to 8.10 ppm. In addition, the ribose protons are all shifted upfield to varying degrees, as summarized in Table III. Such a result is best explained by the ring current effect due to stacking of bases from the two molecules of ATP in a $Sc^{III}(ATP)_2$ complex. The small shift of H-2′ (0.062 ppm) sets the upper limit of the exchange rate of $Sc^{III}(ATP)_2$ at 12 s^{-1}.

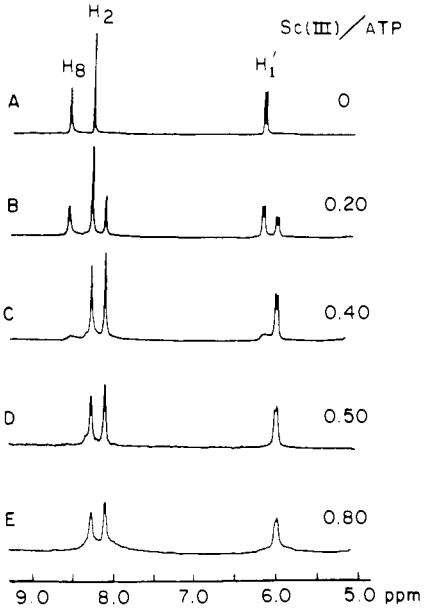

Figure 9. ^{1}H NMR (200 MHz) spectra of ATP (20 mM in D_2O, pD 8.0) with varying concentrations of $ScCl_3$. Spectral parameters: spectral width 2000 Hz, acquisition time 4.1 s, line broadening 0.4 Hz, 30 ± 2 °C. H-2, H-8, and H-1′ are signals of free ATP.

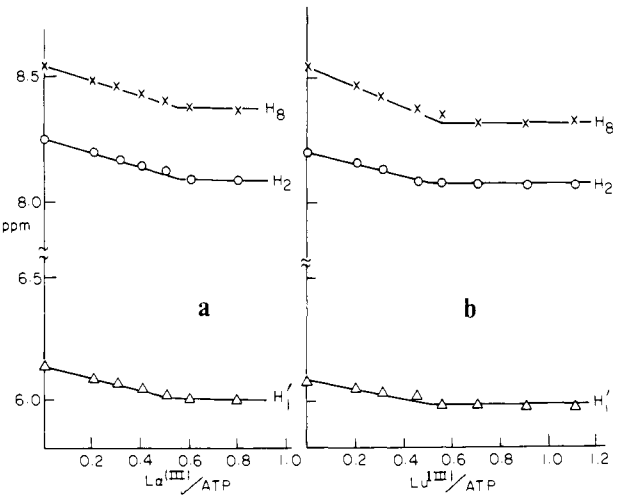

Figure 10. ^{1}H chemical shifts of H-8, H-2, and H-1′ of ATP (20 mM, pD 8.0) with varying concentrations of $LaCl_3$ (a) and $LucL_3$ (b).

La(III) also induces upfield shifts of most ^{1}H signals, as shown in Figure 10a and Table III. The exchange rate of La(III)/ATP is fast on the ^{1}H NMR time scale and the changes level off at [La(III)]/[ATP] = 0.5, in agreement with ^{17}O and ^{31}P NMR results. The effect of Lu(III) on the ^{1}H NMR properties of ATP is comparable to that of La(III), as shown by Figure 10b and Table III.

Because H-8 is shifted upfield in all cases, binding of M(III) with N-7 of the adenine ring is not supported by ^{1}H NMR results. The exchange rate, the stoichiometry, and the base stacking as

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revealed by ^1H NMR results all support the results of ^{17}O NMR and the formation of α,β,γ-tridentate $M^{III}(ATP)_2$ for all three metal ions.

Discussion

Stoichiometry of ATP Complexes of Sc(III), La(III), and Lu(III). The stoichiometry of all three complexes is clearly 1:2. Such a stoichiometry has also been observed for ATP complexes of Mn(II), Sn(II), Zn(II), and Cd(II).[8,21] However, in those cases the 1:2 complexes were identified only when ATP concentration was kept high relative to the metal ion. The 1:1 complex seems to be the favorable structure when the M(II)/ATP ratio is kept at 1:1 at low concentrations, except for the case of $Sn^{II}ATP$, for which a 2:2 complex has been proposed.[8,20,21] In the present cases, the 1:2 complex seems to be the most favorable structure. Addition of excess M(III) ions to the 1:2 complexes caused slight broadening of NMR signals and enhanced decomposition of ATP but did not seem to convert the 1:2 complexes to 1:1 complexes. The well-defined 1:2 stoichiometry, and the possible interference by excess M(III) ions, cautions the use of 1:1 $M^{III}ATP$ complexes in biochemical studies,[22-24] as well as the determination of the dissociation constants of lanthanide(III)–ATP complexes assuming a 1:1 stoichiometry.[24,25] Recently it has been shown that some paramagnetic lanthanide(III) ions also form 1:2 complexes with ATP.[5m,26]

Macroscopic Structures of $Sc^{III}(ATP)_2$, $La^{III}(ATP)_2$, and $Lu^{III}(ATP)_2$. The coordination of M(II) or M(III) with the triphosphate moiety of ATP is of vital importance in the reactions catalyzed by phosphotransferases. Recently Viola et al.[23] observed that the inhibition constant K_i of $M^{III}ATP$ in the reaction catalyzed by yeast hexokinase increases as the ionic radius of M(III) increases. Since the β,γ-bidentate of $Cr^{III}ATP$ has a much lower K_i (0.069 μM) than the α,β,γ-tridentate of $Cr^{III}ATP$ does (120 μM),[27] they suggested that the binding strength of $M^{III}ATP$ complexes with hexokinase might relate to the different proportions of bidentate isomers present in solution, which could in turn relate to the ionic radius with 0.88 Å being the critical size. Thus, on the basis of their results, $La^{III}ATP$ (ionic radius 1.06 Å, K_i = 174 ± 8 μM at pH 8) should have a higher percentage of tridentate, whereas $Sc^{III}ATP$ (ionic radius 0.73 Å, K_i = 8.0 ± 2.9 μM at pH 6, 14.7 ± 1.6 μM at pH 7) and $Lu^{III}ATP$ (ionic radius 0.85 Å, K_i = 0.84 ± 0.36 μM at pH 8) should have higher percentages of the β,γ-bidentate. Our conclusion that tridentate is the predominant structure of the $M^{III}(ATP)_2$ complexes is not fully compatible with the interpretation of Viola et al.,[23] but it cannot be ruled out that β,γ-bidentates exist in small percentage under their experimental conditions or in the active site of hexokinase. Tanswell et al.[28] suggested, on the basis of lanthanide(III)-induced pseudocontact shifts in ^1H and ^{31}P NMR of ATP, that Pr(III), Nd(III), Eu(III), and Yb(III) bind predominantly to the β- and γ-phosphates of ATP. Direct comparison of their results with this work is difficult because they assumed 1:1 stoichiometry, and our methods only apply to diamagnetic ions.

The ^1H NMR results indicate "base stacking" in the $M^{III}(ATP)_2$ complexes. The base stacking in $Zn^{II}ATP$ and $Cd^{II}ATP$ has been related to *inter*molecular phosphate $\cdots M^{II}\cdots$N-7 interaction.[20] However, there is no evidence of $M^{II}\cdots$N-7 interaction in the present cases. The most reasonable macroscopic structure for $Sc^{III}(ATP)_2$ seems to be that the six phosphates from the two ATP molecules occupy the six ligand sites of an octahedral structure, with the two adenine rings partially stacked. The structures of $La^{III}(ATP)_2$ and $Lu^{III}(ATP)_2$ could be similar to that of $Sc^{III}(ATP)_2$, but it is possible for the lanthanide(III) ions to have higher coordination numbers[30] by additional coordination of water ligands.

After submission of this paper, a report on the multinuclear NMR study of the triphosphate complexes with lanthanide(III) ions appeared.[29] The stoichiometry was found to be 1:2, in consistence with our results on ATP complexes. The triphosphate was suggested to coordinate to the lanthanide ion via two oxygens of one PO_3 group, one oxygen of the other PO_3 group, and one oxygen of the PO_2 moiety.

Effect of Metal Coordination on ^{17}O Chemical Shifts. In the complexes of Co(III) with ATP and ADP, $O{=}P{-}{}^{17}O^{-}\cdots Co^{III}$ and $^{17}O{=}P{-}O^{-}\cdots Co^{III}$ signals were shifted upfield by 180–200 ppm and downfield by 1–9 ppm, respectively relative to free nucleotides.[12,13] In the complexes of Mg(II) with ADP and ATP, the ^{17}O NMR signal is an average of $O{=}P{-}{}^{17}O^{-}\cdots Mg^{II}$ and $^{17}O{=}P{-}O^{-}\cdots Mg^{II}$ but is shifted upfield only by <6 ppm.[12] In the present case, the ^{17}O NMR signal is also an average of $O{=}P{-}{}^{17}O^{-}\cdots M^{III}$ and $^{17}O{=}P{-}O^{-}\cdots M^{III}$. However, the signal is shifted downfield by 5–15 ppm. The chemical shift change seems to depend on the electronic structure of the coordinating metal ion, but the detailed mechanism is awaiting further investigation.

Another question is whether, within the same metal–ATP complex, the relative magnitudes of ^{17}O chemical shift changes reflect the relative extent of interaction for α-, β-, and γ-phosphate. Although such a correlation has been well established in the β,γ-bidentate of $Co^{III}ATP$,[12] the quantitative application to the present systems is difficult due to the small magnitudes of shifts (5–15 ppm) and the large error in the chemical shifts of broad ^{17}O signals (±1.5 ppm). Qualitatively, the data in Table II indicate that the magnitudes of changes in δ O fall in the order γ-^{17}O > α-$^{17}O \approx \beta$-^{17}O.

Microscopic Structures of $M^{III}(ATP)_2$. The distances between the metal ion and oxygen atoms, the conformation of the ribose moiety, and the distance between the two stacking adenine rings all require further investigation by use of various spectroscopic techniques. These problems are further complicated by the fact that there are four possible diastereomers for the α,β,γ-tridentate of $M^{III}(ATP)_2$. These diastereomers should have distinctly different ^{31}P and ^{17}O chemical shifts, as in the Co(III) complexes of ADP and ATP.[12,13,17] However, only one set of ^{31}P NMR and ^{17}O NMR signals was observed for $Sc^{III}(ATP)_2$, even though its exchange rate is slow in the NMR time scale (<12 s^{-1} at 30 °C). Two possible explanations are (a) only one diastereomer is formed specifically and (b) the exchange between diastereomers is a distinct process from the exchange between free and bound ATP and is a faster process.

Explanation a is chemically unlikely. Explanation b is not only consistent with all the spectral data, but also precedented by $Co^{III}(NH_3)_4ADP$.[13] By use of stereospecifically labeled Δ and Λ isomers of $Co^{III}(NH_3)_4(S_p)$-[α-$^{17}O_1$]ADP, we have demonstrated by ^{17}O and ^{31}P NMR that interconversion between the two diastereomers occurs faster than dissociation of the complexes, though both are slow on the NMR time scale.[13] In the case of $Sc^{III}(ATP)_2$, the interconversion of diastereomers is apparently a fast process.

It is not known whether such a rapid *intramolecular* interconversion also occurs between tridentate, bidentate, and monodentate isomers. If not, the $Sc^{III}(ATP)_2$ should consist of α,β,γ-tridentate as the only species since the complex is in slow exchange (with free ATP) on the NMR time scale, and only a single set of spectra is observed in ^1H NMR, ^{31}P NMR, and ^{17}O NMR. On the other hand, if such an interconversion is a fast process, the microscopic structure of $Sc^{III}(ATP)_2$ could actually be a mixture of several species, e.g., tridentate, bidentate (α,β; β,γ; α,γ), and monodentate (α; β; γ). In any case, the spectral data in Tables I–III represent the average of several microscopically different species (diastereomers and/or positional isomers), the "exchange rate" represents the rate of exchange between free ATP and an average of several species of complexed ATP, and the conclusion that α,β,γ-tridentate is the predominant species represents only the macroscopic view of the structure of the $M^{III}(ATP)_2$ complexes.

Conclusion. We have employed ^{17}O NMR, along with ^{31}P NMR and ^1H NMR, to establish that Sc(III), La(III), and

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Lu(III) form 1:2 complexes with ATP, by coordinating with α,β,γ-phosphates of two ATP molecules. The complexes are mixtures of rapidly exchanging diastereomers, and the detailed microscopic structures of the complexes remain to be established by further investigation.

Experimental Section

Materials. The $H_2{}^{17}O$ (51.0 atom % ^{17}O, 38.6 atom % ^{18}O) was obtained from Monsanto. The ^{17}O-depleted water (0.00338 atom % ^{17}O, 0.00135 atom % ^{18}O) was obtained from Yeda Stable Isotopes. The metal oxides Sc_2O_3, La_2O_3, and Lu_2O_3 were of the puratronic grade (99.999%e from Alfa. Unlabeled ATP was obtained from Sigma.

^{17}O-Labeled ATP. The [α-$^{17}O_2$]ATP (38 atom % ^{17}O) and [γ-$^{17}O_3$]ATP (42 atom % ^{17}O) were synthesized by combined chemical and biochemical procedures as described previously.[12,16] The [β-$^{17}O_2$]ATP was synthesized from [β-$^{17}O_3$]ADP (39 atom % ^{17}O) (prepared as described in ref 12) according to the procedure of Wehrli.[31,32] All three labeled samples were newly prepared for this work. The atom % ^{17}O enrichments were determined by the integration method of Tsai et al.[16] on the basis of the quadrupolar effect of ^{17}O in ^{31}P NMR.

Sample Preparations. Stock solutions of $M^{III}Cl_3$ were prepared by dissolving the metal oxides in concentrated HCl upon gentle heating, followed by repetitive rotary evaporation to remove excess HCl. After redissolving in triple-distilled water, the concentration of M(III) was determined by passing the $M^{III}Cl_3$ solution through a cation-exchange column (Dowex 50W-X8, H^+ form, Bio-rad) followed by titrating the released H^+ ions with standardized NaOH. The results were reproducible within ±2% in three independent determinations.

The nucleotides were first converted to sodium salts by passing through a sp-Sephadex C-25 column (Pharmacia). The solution was then passed through a small column of Chelex-100 (Bio-Rad), lyophilized, redissolved in ^{17}O-depleted water, quantified by UV absorption at 259 nm, and used as a stock solution. NMR samples were prepared by mixing proper amounts of $M^{III}Cl_3$ and nucleotide stock solutions (usually in <100-μL quantities) in ^{17}O-depleted water (for ^{17}O NMR), in H_2O/D_2O (3:1 v/v) (for ^{31}P NMR), or in 99.8% D_2O (for ^1H NMR), followed by adjusting to pH 8.0 (direct reading from the pH meter) with NaOH/HCl or NaOD/DCl. In ^{17}O NMR and ^{31}P NMR the experiments were usually begun by taking the spectrum of the free nucleotide as a control (for purity, homogeneity, etc.) followed with successive titration with $M^{III}Cl_3$ (pH was adjusted at each titration). In cases where decomposition of ATP occurred (hydrolysis to ADP and AMP), a new sample was prepared at the later part of the titration. In most cases, <5% decomposition occurred within 3-5 h. One set of experiments usually took 5-8 h. For ^1H NMR, multiple samples of different M(III)/ATP ratios were prepared, lyophilized, dried under vacuum, and redissolved in 99.996% D_2O. Such a process resulted in 10-20% decomposition in the ATP complexes of La(III) and Lu(III).

NMR Methods. ^1H and ^{31}P NMR spectra were obtained from a Bruker WP-200 NMR spectrometer, with deuterium lock, at ambient temperature (30 ± 2 °C). The chemical shifts were referenced to external TSP and 85% H_3PO_4, respectively, with + signal indicating a downfield shift. Homonuclear ^1H-decoupling experiments were performed to aid peak assignments when necessary.

^{17}O NMR spectra were measured on a GE-300 widebore NMR spectrometer. A horizontal, nonspinning probe (10-mm outer diameter, 2-mL sample size) was used for most experiments. The ^{31}P-decoupled ^{17}O NMR experiments were carried out on a spinning horizontal probe (20-mm outer diameter, 4.5-mL sample size). Chemical shifts were referenced to H_2O, with + signal indicating a downfield shift. ^{17}O-depleted water was used in all experiments. In most cases, the T_1 inversion recovery experiment was used to partially suppress the solvent signal on the basis of different relaxation times between the solvent signal and the nucleotide signal.

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Uncovering Remote Nuclear-Spin Connectivities by Relayed Zero and Double Quantum Coherence

Luciano Müller* and Arthur Pardi†

Contribution from Smith Kline & French Laboratories, 1500 Spring Garden Street, Philadelphia, Pennsylvania 19101. *Received September 26, 1984*

Abstract: A novel method is described which allows identification of spin–spin coupling networks in complex spin systems. The method is a two-dimensional time-domain experiment in which double (and zero) quantum states are excited and allowed to evolve for a period t_1 after which signals from these excited states are converted into transverse magnetization. This magnetization is then relayed to remote spins and subsequently recorded in the detection period t_2. The method is applied to assign the proton spectrum of the arginine residue in a vasopressin analogue.

NMR has been shown to be a powerful tool for structure determination of complex molecules such as polypeptides and small proteins in solution.[1] This structural information is obtained from two-dimensional nuclear Overhauser effect (2DNOE) experiments where through-space connectivities between remote nuclear spins are detected.[2] In order to make use of these through-space connectivities the resonance assignments of the spins in the molecule must first be made. The inability to make specific resonance assignments in complex molecules is often a major limitation in how much information can be extracted from the NMR experiment. Thus, procedures for simplifying resonance assignments are of prime importance for studies of complex

† Present address: Rutgers University, Department of Chemistry, Busch Campus, Piscataway, NJ 08954. A novel method is described which allows identification of spin–spin coupling networks in complex spin systems. The method is a two-dimensional time-domain experiment in which double (and zero) quantum states are excited and allowed to evolve for a period t_1 after which signals from these excited states are converted into transverse magnetization. This magnetization is then relayed to remote spins and subsequently recorded in the detection period t_2. The method is applied to assign the proton spectrum of the arginine residue in a vasopressin analogue.

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