^{13}C NMR Line Shapes of [2-^{13}C]ATP in Enzyme Complexes and Viscous Solutions: Glycosidic Rotation Persists at High Viscosities and Is Arrested in Enzyme Complexes†,‡

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Received July 22, 1991

Abstract: ^{13}C NMR line shapes of [2-^{13}C]ATP bound to a number of ATP-utilizing enzymes and free in solutions of varying viscosities have been investigated. The spin relaxation of this ^{13}C nucleus is mediated by ^{13}C–^{1}H dipolar interaction with the attached proton and the ^{13}C chemical shift ansiotropy (CSA). Since these are second-order tensor interactions, interference terms occur in their relaxation parameters. The line widths and other relaxation-matrix elements are derived, in Redfield notation, for a general CSA tensor and with molecular reorientation assumed isotropic. A measurement of the ^{13}C CSA tensor of [2-^{13}C]AMP was made to facilitate the analysis of the line width data. The experimentally observed line widths in the various enzyme complexes are well represented by the expressions derived, and the rotational correlation times deduced from the data correlate well with the molecular masses of the enzymes used in the study, viz., adenylate kinase, arginine kinase, 3-P-glycerate kinase, creatine kinase, pyruvate kinase, and methionyl tRNA synthetase. Adenylate kinase was also studied with [2-^{13}C]AMP. In striking contrast with the line shapes in the enzyme complexes, the ^{13}C line widths of [2-^{13}C]ATP in viscous solutions of sucrose and glycerol were virtually independent of viscosity. For an increase in the solvent viscosity by a factor of 2300, the line width increased by a factor not more than about 5, although the estimated correlation times for overall rotation are similar to, if not longer than those for the enzymes studied. A similar behavior was observed for the ^{31}P line widths. Addition of Mg(II) made no measurable difference to the viscosity insensitivity of ^{13}C and ^{31}P line widths. The results in viscous solutions are interpreted on the basis that the two large-amplitude internal motions with characteristic times in the nanosecond range, viz., the glycosidic rotation of the adenine base and the mobility of phosphate chain, persist in free ATP even at high viscosities. This reasoning is corroborated by the fact that the ^{13}C line widths of [1′-^{13}C]AMP are much larger in viscous solutions indicating that internal motions involving the ribose moiety are of lower amplitude. A corollary to this interpretation is that internal motions such as the glycosidic rotation and the phosphate chain motion in ATP, although not attenuated in viscous solutions, are nevertheless arrested in the enzyme complexes.

I. Introduction

An attractive feature of high-resolution NMR spectroscopic investigations in liquids is that in addition to acquiring useful molecular structural information it is possible to gain insight into prevailing internal motions in the molecules. The motional information is obtained through relaxation parameters such as spin–lattice relaxation time (T_1), line width (T_2), and nuclear Overhauser effect (NOE), which depend on the spectral densities of the time correlation functions of the interactions rendered random by the molecular motion and thereby mediate spin relaxation.[1] If more than one interaction contributes to spin relaxation, in addition to the correlation functions for each interaction (auto-correlation), interference (cross-correlation) effects can occur if the interactions possess similar transformation properties. If two interactions are of the same origin, e.g., two pairwise dipolar interactions in a multiple-spin system, the likeness in their transformation properties is axiomatic, and if they are distinct a detailed examination of the random time-dependent parts of the interactions should be made to determine if the interference occurs. A well-known example of the latter kind occurs for ^{13}C nuclei with protons directly bonded to them; the ^{13}C relaxation is mediated by ^{13}C–^{1}H dipolar interaction as well as the ^{13}C chemical shift anisotropy (CSA). The random time-dependent parts of both the interactions contain second-rank spherical tensors which transform similarly under rotations. The possibility of interference between these two interactions was first pointed out by Shimizu[2] in 1964, in analogy with that between g-tensor anisotropy and electron-nuclear dipolar interaction, which was theoretically predicted[3] and experimentally observed[4] in ESR spectra of free-radical solutions by that time. Although there were a number of theoretical and experimental NMR investigations since then, in which the presence of the interference effects was detected,[5] it was only during the past decade, when the use of high field NMR spectrometers became common, that the effects of this cross-correlation were being routinely observed.[6,7] At large

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† Supported in part by research grants from NSF DMB 8608185 and NIH GM43966. The NTC-300 spectrometer at IUPUI was purchased with partial support from NSF (PCM 8018725). The Purdue Biochemical Magnetic Resonance Laboratory was partially supported by NIH Grant RR01077. Collaboration between research groups at IUPUI and Max Planck Institut, Heidelberg was partially supported by a NATO travel grant. Preliminary versions of this work were presented at the Biophysical Society Annual Meetings at Phoenix, AZ (1988) and San Francisco, CA (1991) and at the XIII International Conference on Magnetic Resonance in Biological Systems at Madison, WI (1988).

‡ Abbreviations: NMR, nuclear magnetic resonance; ESR, electron spin resonance; ATP, adenosine 5′-triphosphate; AMP, adenosine 5′-monophosphate; Hepes, *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid.

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0002-7863/92/1514-1566$03.00/0

applied fields, the strength of the CSA interaction becomes proportionately larger, and the cross correlation with the dipolar interaction becomes significant. The effect is most readily detectable if the resonance of the nucleus is a multiplet due to scalar coupling with the dipolar coupled nucleus and is manifest in the form of differential relaxation parameters (e.g., line widths and T_1's) for the various transitions in the multiplet.

NMR line shapes of the proton-coupled ^{13}C doublets of [2-^{13}C]-labeled adenine nucleotides in free solutions and bound to a group of ATP-utilizing enzymes are presented in this paper. Analytical expressions inclusive of the interference terms are derived for the relaxation matrix elements of the ^{13}C–^{1}H coupled spin system with a general ^{13}C CSA tensor. Of particular interest is the striking contrast between the line shapes observed for enzyme complexes and those in highly viscous solutions in which the correlation times for overall molecular reorientation are similar to those for the enzymes. The ^{13}C CSA tensor in polycrystalline [2-^{13}C]AMP is determined to facilitate analysis of the results. The observed line shapes were analyzed in terms of these tensor elements and the ^{13}C–^{1}H dipolar interaction. The analysis allows us to draw inferences regarding the internal motions of adenine nucleotides free in viscous solutions in contrast with those in their enzyme complexes. Supporting evidence for these inferences is obtained from ^{13}P NMR measurements of ATP and ^{13}C NMR measurements on [1′-^{13}C]AMP in viscous solutions.

II. Theory

In his original paper on the effects of interference between dipolar interaction and CSA in 1964, Shimizu[2] derived the relaxation parameters for the case of an axially symmetric CSA tensor for one of the two spin $^1/_2$ nuclei. In 1984 Goldman,[8] apparently unaware of Shimizu's work, reformulated the entire problem and derived the formulae for a general CSA tensor. Goldman made elegant use of the single-transition spin operators for a weakly coupled system to demonstrate that the relaxation in the presence of two interfering mechanisms can be cast in the form of master equations for the evolution of the physical quantities of interest.[8] An alternative and more commonly used method is to calculate the relaxation matrix elements by using Redfield's formulation. Such a derivation inclusive of the interference effects in the presence of a general CSA tensor is sketched below.

In Redfield's notation the elements of the relaxation matrix are given with four indices, e.g., $R_{\alpha\alpha'\beta\beta'}$, where the subscripts denote the eigenstates of the static part of the spin Hamiltonian. This theory is most convenient for calculating relaxation effects in coupled spin systems and has been described in detail in the literature.[1,9,10] The line width (full width at half-height) of a nonoverlapping resonance line $\alpha \leftrightarrow \beta$ is given by $(-2R_{\alpha\beta\alpha\beta})$, and the relaxation transition probabilities between these states is given by $R_{\alpha\alpha\beta\beta}$. A Redfield element is related to the spectral densities of the interactions mediating spin–lattice relaxation through

$$R_{\alpha\alpha'\beta\beta'} = \tfrac{1}{2}(J_{\alpha\beta\alpha'\beta'} + J_{\beta'\alpha'\beta\alpha} - \delta_{\alpha'\beta'}\sum_{\gamma} J_{\alpha\gamma\beta\gamma} - \delta_{\alpha\beta}\sum_{\gamma} J_{\gamma\alpha'\gamma\beta'}) \quad (1)$$

with

$$J_{\alpha\beta\alpha'\beta'} = \int_{-\infty}^{\infty} \langle \langle \alpha|\mathcal{H}'(t)|\beta\rangle \langle \alpha'|e^{-i\mathcal{H}_0\tau}\mathcal{H}'(t-\tau)e^{i\mathcal{H}_0\tau}|\beta'\rangle^* \rangle_{av}\, d\tau \quad (2)$$

in which the energies E_α, E_β... associated with the states α, β... satisfy the condition $E_\alpha - E_{\alpha'} - E_\beta + E_{\beta'} \approx 0$ (secular approximation), $\mathcal{H}'(t)$ is the random spin–lattice interaction consisting of the dipolar and CSA interactions given by

$$\mathcal{H}'(t) = \mathcal{H}'_D(t) + \mathcal{H}'_C(t) \quad (3)$$

$\langle\ \rangle_{av}$ represents an ensemble average, and $\mathcal{H}_0$, the static part of the spin Hamiltonian for a weakly coupled system (with eigenstates α, β, etc.), is given by

$$\mathcal{H}_0 = -\omega_C I_{zC} - \omega_H I_{zH} + J I_{zC} I_{zH} \quad (4)$$

in which ω_C and ω_H are the Larmor frequencies, $\mathbf{I}_C\hbar$ and $\mathbf{I}_H\hbar$ are spins of ^{13}C and ^{1}H, respectively, and J the indirect spin–spin coupling constant between them. All of the Hamiltonians are expressed in angular frequency units. $\mathcal{H}'_D(t)$ and $\mathcal{H}'_C(t)$ are given below in spherical tensor notation:[10,11]

$$\mathcal{H}'_D(t) = \sum_{q=-2}^{2} (-1)^q F_q^D(t) A_{-q}^D \quad (5)$$

where

$$F_q^D(t) = -\frac{\sqrt{6}\gamma_C\gamma_H\hbar}{r^3}\mathcal{D}^{(2)}_{oq}(\Omega_D) = -\sqrt{6}\chi_D\mathcal{D}^{(2)}_{oq}(\Omega_D) \quad (6)$$

and

$$A_0^D = \frac{1}{\sqrt{6}}(3I_{zC}I_{zH} - \mathbf{I}_C\cdot\mathbf{I}_H) \quad (7)$$

$$A_{\pm1}^D = \mp\tfrac{1}{2}(I_{zC}I_{\pm H} + I_{zH}I_{\pm C}) \quad (8)$$

$$A_{\pm2}^D = \tfrac{1}{2}I_{\pm C}I_{\pm H} \quad (9)$$

where γ_C and γ_H are the gyromagnetic ratios of ^{13}C and ^{1}H, respectively, and r is the distance between them. $F_q^D(t)$ acquire the stochastic time-dependence of molecular reorientation through the elements of Wigner rotation matrices,[12] $\mathcal{D}^{(2)}_{oq}(\Omega_D)$, which transform the dipolar frame (in which the dipolar vector is along the z-axis) to the laboratory frame using the Euler rotations collectively represented by Ω_D. Similarly

$$\mathcal{H}'_C(t) = -\gamma_C\mathbf{I}_C\cdot\boldsymbol{\delta}\cdot\mathbf{H} = \sum_{q=-2}^{2}(-1)^q F_q^C(t)A_{-q}^C \quad (10)$$

in which $\boldsymbol{\delta}$ is the CSA tensor of ^{13}C and the spin operators A_ξ^q (which are analogous in structure to those in eqs 7–9 with one of the spins replaced by the external magnetic field $\mathbf{H}$) may be simplified by noting that $\mathbf{H} = H_0\hat{k}$ to yield

$$A_0^C = \frac{\gamma_C}{\sqrt{6}}(3I_{zC}H_z - \mathbf{I}_C\cdot\mathbf{H}) = \frac{2}{\sqrt{6}}\omega_C I_{zC} \quad (11)$$

$$A_{\pm1}^C = \mp\frac{\gamma_C}{2}(I_{zC}H_\pm + H_z I_{\pm C}) = \mp\tfrac{1}{2}\omega_C I_{\pm C} \quad (12)$$

$$A_{\pm2}^C = \frac{\gamma_C}{2}I_{\pm C}H_\pm = 0 \quad (13)$$

$F_q^C(t)$ may be written by using coordinate transformations analogous to eq 6 in two different forms. In the first form, the lattice functions F'^C_q are evaluated in the principal axis system of the CSA tensor and transformed to the laboratory frame (analogous to eq 6) through the Euler rotations Ω_C which render $F_q^C(t)$ random and time dependent, i.e.,

$$F_q^C(t) = \sum_{q'} F'^C_{q'}\mathcal{D}^{(2)}_{q'q}(\Omega_C) \quad (14)$$

Alternatively the F_q^C may first be transformed into the dipolar frame using the time-independent Wigner rotation matrices $\mathcal{D}^{(2)}(\phi, \theta, 0)$ where θ and ϕ are the polar and azimuthal angles of the dipolar vector with respect to the principal axis system of the CSA tensor, and the resulting functions transformed into the laboratory frame by using $\mathcal{D}^{(2)}(\Omega_D)$, i.e.,

$$F_q^C(t) = \sum_{q'}\left(\sum_{q''} F'^C_{q''}\mathcal{D}^{(2)}_{q''q'}(\phi,\theta,0)\right)\mathcal{D}^{(2)}_{q'q}(\Omega_D) \quad (15)$$

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Table I. The Frequencies (ω_{qp}) Associated with the Various Terms (A_{qp}) in the Spin Operators (A_q^D and A_q^C) of the Dipolar and CSA Interactions, Respectively (See Eqs 7–9 and 11–13) as a Result of the Transformation in Eq 19

A_q	distinct terms (A_{qp})	ω_{qp}	A_q	distinct terms (A_{qp})	ω_{qp}
A_0^D	$I_{zC}I_{zH}$	0	$A_{\pm 2}^D$	$I_{\pm C}I_{\pm H}$	$\pm(\omega_H + \omega_C)$
	$I_{\mp C}I_{\pm H}$	$\pm(\omega_H - \omega_C)$	A_0^C	I_{zC}	0
$A_{\pm 1}^D$	$I_{zC}I_{\pm H}$	$\pm\omega_H$	$A_{\pm 1}^C$	$I_{\pm C}$	$\pm\omega_C$
	$I_{\pm C}I_{zH}$	$\pm\omega_C$			

$F_q^{'C}$ are the spherical tensor components of the traceless CSA tensor, δ, which has the cartesian form

$$\delta = \begin{pmatrix} \delta_{xx} & 0 & 0 \\ 0 & \delta_{yy} & 0 \\ 0 & 0 & \delta_{zz} \end{pmatrix} = \delta_{zz} \begin{pmatrix} -\frac{1}{2}(1+\eta) & 0 & 0 \\ 0 & -\frac{1}{2}(1-\eta) & 0 \\ 0 & 0 & 1 \end{pmatrix} \quad (16)$$

where the asymmetry parameter

$$\eta = \frac{\delta_{yy} - \delta_{xx}}{\delta_{zz}} \quad (17)$$

takes values $0 \le \eta \le 1$ if the principal axes are labeled such that $|\delta_{yy}| \le |\delta_{xx}| \le |\delta_{zz}|$.[11] $F_q^{'C}$ are evaluated by standard prescriptions to yield

$$F_0^{'C} = \sqrt{\frac{3}{2}}\delta_{zz} \quad \text{and} \quad F_{\pm 2}^{'C} = -\tfrac{1}{2}\eta\delta_{zz} \quad (18)$$

For weakly coupled spin systems the transformation of $\mathcal{H}'(t - \tau)$ with $e^{-i\mathcal{H}_o\tau}$ in eq 2 may be carried out by ignoring the term $JI_{zC}I_{zH}$ in $\mathcal{H}_o$ (see eq 4) leading to the general relation

$$e^{-i\mathcal{H}_o\tau}A_q e^{i\mathcal{H}_o\tau} = \sum_p e^{i\omega_{qp}\tau}A_{qp} \quad (19)$$

The subscript p is added in case different terms A_{qp} in $A_q(=\sum_p A_{qp})$ yield different ω_{qp}. Note that this problem arises only for A_0^D and $A_{\pm 1}^D$ of $\mathcal{H}_D'(t)$ and for none of the terms in $\mathcal{H}_C'(t)$. The values of ω_{qp} for the different terms in eqs 7–9 and 11–13, obtained by using standard transformations of spin operators, are given in Table I.

We now have expressions for all the terms required for the evaluation of $J_{\alpha\beta\alpha'\beta'}$ in eq 2. The algebra is tractable with the assumption of a single correlation time τ_c to characterize the molecular reorientation (i.e., isotropic rotation) and can proceed along the lines described in literature. With the secular approximation[1] the correlation functions of $F_{qp}(t)$ become

$$\langle F_{qp}(t)F_{q'p'}^*(t-\tau)\rangle_{av} = \delta_{qq'}\delta_{pp'}\langle|F_{qp}|^2\rangle_{av}e^{-|\tau|/\tau_c} \quad (20)$$

and thereby lead to Lorentzian spectral densities through

$$\tfrac{1}{2}\int_{-\infty}^{\infty} d\tau e^{-|\tau|/\tau_C}e^{i\omega_{qp}\tau} = \frac{\tau_c}{1+\omega_{qp}^2\tau_c^2} = J(\omega_{qp}) \quad (21)$$

It is important to recognize that $F_q(t)$ in eq 20 may consist of similarly transforming terms from different relaxation mechanisms, and in such cases $\langle|F_{qp}|^2\rangle_{av}$ on the right-hand side of eq 20 will have quadratic terms from each mechanism as well as cross terms between different mechanisms. The cross terms are precisely the interference terms which are of interest in this calculation. With the above requirements, eq 2 may be rewritten as

$$\begin{aligned}\tfrac{1}{2}J_{\alpha\beta\alpha'\beta'} = &\sum_{q=-2}^{2}\sum_p J(\omega_{qp}(\mathrm{D}))\langle|F_{qp}^D|^2\rangle_{av}\langle\alpha|A_{-qp}^D|\beta\rangle\langle\alpha'|A_{-qp}^D|\beta'\rangle^* + \\ &\sum_{q=-1}^{1} J(\omega_q(\mathrm{C}))\langle|F_q^C|^2\rangle_{av}\langle\alpha|A_{-q}^C|\beta\rangle\langle\alpha'|A_{-q}^C|\beta'\rangle^* + \\ &\sum_{q=-1}^{1}\sum_{p'} J(\omega_q(\mathrm{C}))\langle|F_{qp'}^D F_q^{C*}|\rangle_{av}\langle\alpha|A_{qp}^D|\beta\rangle\langle\alpha'|A_{-q}^C|\beta'\rangle^* + \\ &\sum_{q=-1}^{1}\sum_{p'} J(\omega_{qp'}(\mathrm{D}))\langle|F_q^C F_{qp}^{D*}|\rangle_{av}\langle\alpha|A_{-q}^C|\beta\rangle\langle\alpha'|A_{-qp}^D|\beta'\rangle^* \quad (22)\end{aligned}$$

The first two terms in eq 22 are respectively due to the dipolar and CSA interactions acting alone, and the remaining two terms represent the effect of interference between them. The sum over subscript p' in the interference part singles out the terms among $A_{-qp'}^D$ that have the same ω_{qp} as A_{-q}^C as required by the secular approximation,[1] e.g., if A_{-q}^C is I_{-C}, $A_{-qp'}^D$ is $I_{-C}I_{zH}$. The ensemble averages $\langle\ \rangle_{av}$ in eq 22 simplify through the orthogonality relations of the $\mathcal{D}$ matrices[12]

$$\frac{1}{8\pi^2}\int_\Omega \mathcal{D}_{q_1q_2}^{(l)*}(\Omega)\mathcal{D}_{q_3q_4}^{(l')}(\Omega)d\Omega = \frac{1}{2l+1}\delta_{ll'}\delta_{q_1q_3}\delta_{q_2q_4} \quad (23)$$

Averages of the two quadratic (auto-correlation) terms in eq 22 may be readily evaluated; from eqs 6 and 23

$$\langle|F_{qp}^D|^2\rangle_{av} = \tfrac{6}{5}\chi_D^2 \quad (24)$$

for all q and p, and similarly from eqs 14, 18, and 23

$$\langle|F_q^C|^2\rangle_{av} = \frac{3\delta_{zz}^2}{10}\left[1 + \frac{\eta^2}{3}\right] \quad (25)$$

For the evaluation of the averages in the interference terms in eq 22, it is convenient to use eqs 6 and 15, respectively, for $F_q^D(t)$ and $F_q^C(t)$ since they both contain $\mathcal{D}^{(2)}(\Omega_D)$ and eq 23 may be applied to the average of the product. Thus, we get

$$\langle|F_{qp}^D F_q^{C*}|\rangle_{av} = \langle|F_q^C F_{qp}^{D*}|\rangle_{av} = -\frac{\sqrt{6}}{5}\chi_D\sum_{q''}F_{q''}^{'C}\mathcal{D}_{q''}^{(2)}{}_0(\phi,\theta,0) \quad (26)$$

By substituting for $F_q^{'C}$ from eq 18 and noting that

$$\mathcal{D}_{00}^{(2)} = \tfrac{1}{2}(3\cos^2\theta - 1) \quad \text{and} \quad \mathcal{D}_{\pm 2,0}^{(2)} = \sqrt{\frac{3}{8}}\sin^2\theta e^{\mp i2\phi} \quad (27)$$

Equation 26 simplifies to yield

$$\langle|F_{qp}^D F_q^{C*}|\rangle_{av} = \langle|F_q^C F_{qp}^{D*}|\rangle_{av} = -\frac{3}{10}\chi_D\omega_C\delta_{zz}[(3\cos^2\theta - 1) - \eta\sin^2\theta\cos 2\phi] \quad (28)$$

All the quantities entering eq 22 are now evaluated except the matrix elements of the spin operators A_q in $\mathcal{H}_D'(t)$ and $\mathcal{H}_C'(t)$. For a weakly coupled two spin $-^1/_2$ system the eigenstates of $\mathcal{H}_o$ (see eq 4) are simply the spin product states which may be designated as (1) $|++\rangle$, (2) $|+-\rangle$, (3) $|-+\rangle$, and (4) $|--\rangle$ where the first spin is ^{1}H and the second, ^{13}C. In this basis the matrix elements of A_{qp}^D in eqs 7–9 and A_q^C in eqs 11–13 may be written down essentially by inspection.

Substituting from eqs 24, 25, and 28 into eq 22 along with the matrix elements of the spin operators leads to the following expressions for the line widths of the ^{1}H-coupled ^{13}C transitions (1 ↔ 2 and 3 ↔ 4)

$$-2R_{1212} = (\mathrm{LW})_{12} = \mathrm{LW(DD)} + \mathrm{LW(CSA)} - \mathrm{LW(INT)} \quad (29)$$

$$-2R_{3434} = (\mathrm{LW})_{34} = \mathrm{LW(DD)} + \mathrm{LW(CSA)} + \mathrm{LW(INT)} \quad (30)$$

with

$$\mathrm{LW(DD)} = \tfrac{3}{10}\chi_D^2[\tfrac{4}{3}J(0) + \tfrac{1}{3}J(\omega_H - \omega_C) + J(\omega_C) + J(\omega_H) + 2J(\omega_H + \omega_C)] \quad (31)$$

$$\mathrm{LW(CSA)} = \tfrac{3}{10}\omega_C^2\delta_{zz}^2\left(1 + \frac{\eta^2}{3}\right)[\tfrac{4}{3}J(0) + J(\omega_C)] \quad (32)$$

and

$$\mathrm{LW(INT)} = \tfrac{3}{10}\chi_D\omega_C\delta_{zz}[(3\cos^2\theta - 1) - \eta\sin^2\theta\cos 2\phi][\tfrac{4}{3}J(0) + J(\omega_C)] \quad (33)$$

where $(\mathrm{LW})_{\alpha\beta}$ represents full width at half-height of the transition $\alpha \leftrightarrow \beta$, LW(DD) and LW(CSA) are the line widths arising

exclusively from the dipolar and CSA interactions, respectively, and LW(INT) is the line width from the interference term which adds to the line width of the transition 3 ↔ 4 and subtracts from that of the transition 1 ↔ 2. All the line widths in eqs 31–33 are expressed in the units rad s^{-1}. Similarly the reciprocal T_1 type of Redfield elements $R_{\alpha\alpha\beta\beta}$ for the two ^{13}C transitions show differential effects as given by

$$R_{1122} = J_{1212} = {}^3/_{20}J(\omega_C)\{\chi_D^2 + \omega_C^2\delta_{zz}^2 - \chi_D\omega_C\delta_{zz}[(3\cos^2\theta - 1) - \eta\sin^2\theta\cos 2\phi]\} \quad (34)$$

and

$$R_{3344} = J_{3434} = {}^3/_{20}J(\omega_C)\{\chi_D^2 + \omega_C^2\delta_{zz}^2 + \chi_D\omega_C\delta_{zz}[(3\cos^2\theta - 1) - \eta\sin^2\theta\cos 2\phi]\} \quad (35)$$

The line widths of the proton transitions (1 ↔ 3 and 2 ↔ 4) are given by

$$-2R_{1313} = -2R_{2424} = {}^3/_{10}\chi_D^2[{}^4/_3J(0) + {}^1/_3J(\omega_H - \omega_C) + J(\omega_H) + J(\omega_C) + 2J(\omega_H + \omega_C)] + {}^3/_{10}\omega_C^2\delta_{zz}^2\left[1 + \frac{\eta^2}{3}\right]J(\omega_C) \quad (36)$$

and are unaffected by the interference terms. The remaining $R_{\alpha\alpha\beta\beta}$-type of terms are given by

$$R_{1133} = R_{2244} = {}^3/_{20}\chi_D^2J(\omega_H)$$

$$R_{1144} = {}^3/_5\chi_D^2J(\omega_H + \omega_C)$$

$$R_{2233} = {}^1/_{10}\chi_D^2J(\omega_H - \omega_C) \quad (37)$$

It may be noted that the Redfield elements have the properties[9,10]

$$R_{\alpha\alpha'\beta\beta'} = R_{\beta\beta'\alpha\alpha'} = R_{\alpha'\alpha\beta'\beta} = R_{\beta'\beta\alpha'\alpha} \quad (38)$$

$$\sum_\beta R_{\alpha\alpha'\beta\beta} = 0 \quad (39)$$

which may be utilized to write all the nonvanishing elements of the relaxation matrix for this spin system.

It is appropriate to comment on how the expressions given in eqs 31–37 compare with those existing in literature. These expressions agree with those published by Shimizu,[2] if η is set equal to zero, the extreme narrowing condition is invoked, and a few typographical errors are corrected.[13] On the other hand eqs 31–37 (with $\eta = 0$) do not agree with those given by Farrar's group[7a] because the expressions given by them are in error. If their expressions are used as given, they lead to the anomalous result that the contribution to the Redfield elements exclusively due to CSA (not the interference terms) depends on the angle between the dipolar vector and the symmetry axis of the CSA tensor.[7b] Goldman's expressions[8] can be shown to be algebraically equivalent to eqs 31–33, although they may not appear transparent for the analysis of experimental data. For example, the terms containing the elements of the CSA tensor corresponding eq 32 are given as

$$(\sigma_{x'}^2 + \sigma_{y'}^2 + \sigma_{z'}^2 - \sigma_{x'}\sigma_{y'} - \sigma_{y'}\sigma_{z'} - \sigma_{z'}\sigma_{x'}) \quad (40)$$

which after some algebra may be shown to be simply equivalent to

$$2\delta_{zz}^2\left(1 + \frac{\eta^2}{3}\right) \quad (41)$$

Similarly, the interference terms contain the expression

$$\sigma_{x'}(3\cos^2\theta_{x'z} - 1) + \sigma_{y'}(3\cos^2\theta_{y'z} - 1) + \sigma_{z'}(3\cos^2\theta_{z'z} - 1) \quad (42)$$

which can be simplified to the expression

$$\frac{3\delta_{zz}}{4}[(3\cos^2\theta - 1) - \eta\sin^2\theta\cos 2\phi] \quad (43)$$

In Goldman's notation[8] $\sigma_{x'}$, $\sigma_{y'}$, and $\sigma_{z'}$ are the principal values of the CSA tensor, and the angles $\theta_{x'z}$, $\theta_{y'z}$, and $\theta_{z'z}$ are the direction cosines of the dipolar vector with respect to the CSA principal axes.

III. Materials and Methods

Hepes, dithioerythritol, and dithiothreitol were from Research Organics. Glycerol, DEAE Sephadex, Dowex-1, ATP, AMP, bovine γ globulin, and P-enolpyruvate triscyclohexylammonium salt were from Sigma. Ultrapure sucrose was a gift from Dr. Franklyn G. Prendergast. Chelex-100 was from BioRad. Raney nickel was from Aldrich. [^{13}C]-Formaldehyde was from Cambridge Isotope Laboratories. All other chemicals were of analytical reagent grade.

Labeled Compound Syntheses. [2-^{13}C]AMP was formed from AMP by a series of steps with intermediate chromatographic purifications over Dowex-1 (formate). AMP was initially oxidized to the 1-oxide with monopermaleic acid.[14] This was followed by ring opening to the carboxamidoxime, reduction over Raney nickel to give the carboxamidine, and ring closure with [^{13}C]formaldehyde to give the labeled AMP.[15] [1′-^{13}C]AMP was supplied to us by the NIH Stable Isotope Resource of the Los Alamos National Laboratory, Los Alamos, NM.

[2-^{13}C]ATP was synthesized by enzymatic phosphorylation of [2-^{13}C]AMP in a 20 mM pH 8.2 potassium Hepes buffered reaction mixture at 30 °C of 50 μM ATP, 2 mg/mL bovine γ globulin, 10 U adenylate kinase, 50 U pyruvate kinase, 50 mM potassium acetate, 40 mM magnesium acetate, 10 mM dithioerythritol, 60 mM P-enolpyruvate triscyclohexylammonium salt, and 25 mM [2-^{13}C]AMP. Progress of the reaction was monitored by ^{31}P NMR. At completion, Mg(II) was removed from the reaction mixture by chromatography over 5 mL of Chelex-100. The reaction mixture was then loaded onto a 2.5 × 30 cm column of DEAE Sephadex (carbonate) equilibrated with 10 mM pH 8 ammonium bicarbonate buffer. This column was developed with a linear gradient from 10 to 75 mM pH 8 ammonium bicarbonate to remove residual AMP, ADP, proteins, and salts. [2-^{13}C]ATP was then eluted with 1 M pH 8 ammonium bicarbonate. The absorbance at 254 nm was continuously monitored during column loading, development, and ATP elution. Fractions containing [2-^{13}C]ATP as identified by absorbance were combined and dried under rotary evaporation. Residual ammonium bicarbonate was removed by repeated rotary evaporation with methanol. The remaining solid was resuspended in a small volume of water, neutralized to pH 8.2 by addition of solid potassium carbonate, filtered through a 0.45 micron Gelman acrodisk, chromatographed over 1.5 mL of Chelex-100 equilibrated with water, and lyophilized. The lyophilizate was resuspended in water and examined for purity by UV absorbance and ^{31}P, ^{13}C, and ^1H NMR spectroscopy. Yields of [2-^{13}C]ATP at 97% isotopic abundance were between 90 and 95%.

Enzymes. Lobster muscle arginine kinase,[16] rabbit muscle creatine kinase,[17] and yeast 3-phosphoglycerate kinase[18] were purified and assayed as described previously. *Escherichia coli* adenylate kinase was a gift from Professor Paul Rösch, Universität Bayreuth, Bayreuth, Germany, partially deuterated adenylate kinase was a gift from Dr. Reuben Leberman, EMBL Out Station, Grenoble, France, and a tryptic fragment of *Escherichia coli* methionyl tRNA synthetase was a gift from the late Dr. Guy Fayat at École Polytechnique, Plaiseau, France. Rabbit muscle pyruvate kinase was purchased from Boehringer.

High-Resolution NMR Measurements. ^{13}C NMR measurements at 75 MHz and ^{31}P NMR measurements at 120 MHz were made on a NT-300 wide-bore NMR spectrometer equipped with a 12-mm multinuclear probe, a 293C pulse programmer, a Nicolet 1280 computer, and a variable temperature controller. For the kinases, a typical sample consisted of ~1.7 mL of the enzyme in 50 mM K-Hepes buffer at pH 8.0 concentrated to 5–6 mM (sites) with 4–5 mM [2-^{13}C]ATP in a 12-mm NMR sample tube. For methionyl tRNA synthetase and pyruvate kinase enzyme (sites) concentrations of 1.5 and 5.5 mM were used, respectively, with 1.4 and 2 mM [2-^{13}C]ATP. In all cases, D_2O for

(13) The following corrections should be made in the Redfield elements given in Table I of Shimizu's paper:[2] (1) In R_{1133} and R_{2244}, α^2 should be $2\alpha^2$ and the terms following τ_c should be in parentheses. (2) In R_{1212} and R_{3434}, $3\alpha^2$ should be $6\alpha^2$ and $3\delta_0$ should be $3\delta_0^2$. (3) In R_{1313} and R_{2424}, $11\alpha^2$ should be $14\alpha^2$. These corrections were verified with the author (Shimizu, H. Private communication, 1988).

(14) Mantsch, H. H.; Gola, I.; Kezdi, M.; Bârzu, O.; Dânsoreanu, M.; Jebeleanu, G.; Ty, N. G. *Biochemistry* **1975**, *14*, 5593.
(15) Meyer, R. B., Jr.; Wong, C. G. *J. Lab. Comp. Radiopharm.* **1981**, *18*, 1119.
(16) Jarori, G. K.; Ray, B. D.; Nageswara Rao, B. D. *Biochemistry* **1989**, *28*, 9343.
(17) Jarori, G. K.; Ray, B. D.; Nageswara Rao, B. D. *Biochemistry* **1985**, *24*, 3487.
(18) Ray, B. D.; Nageswara Rao, B. D. *Biochemistry* **1988**, *27*, 5574.

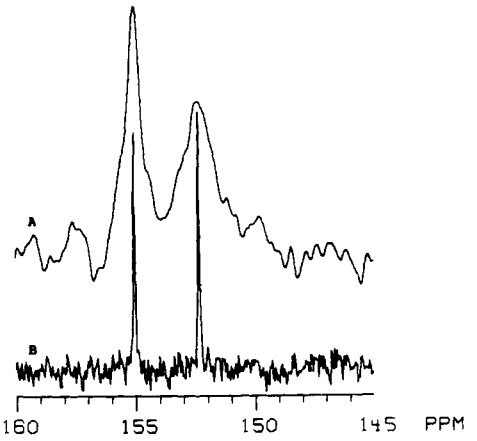

Figure 1. (A) 75-MHz ^{13}C NMR spectrum of [2-^{13}C]ATP bound to 3-P-glycerate kinase. Sample conditions: 3.3 mM [2-^{13}C]ATP, 5.4 mM 3-P-glycerate kinase. Spectral conditions: 60° pulse, 8K data points, 4076 acquisitions, 20 Hz line broadening, 5 °C. (B) 75-MHz ^{13}C NMR spectrum of 5 mM [2-^{13}C]ATP, free in aqueous solution at pH 8.0. Spectral conditions: same as A, except 2048 acquisitions, 2 Hz line broadening, 20 °C.

field-frequency lock was contained in a concentric 5-mm NMR sample tube. Measurements at 50 and 119 MHz for ^{13}C were made on NT-C-200 and NTC-470 spectrometers at the Purdue University Biochemical Magnetic Resonance Laboratory. ^{13}C measurements at 125 MHz were made at the Max Planck Institut für Medizinsche Forschung, Heidelberg, Germany on a Bruker AM-500 spectrometer. In every case, proton-coupled spectra were acquired with a simple one-pulse sequence with a 60° flip angle. Line widths were determined by appropriate curve fitting routines in the NMR software.

^{13}CSA Tensor Measurements. The principal elements of ^{13}C CSA tensor of [2-^{13}C]AMP were determined with the help of Dr. T. Klutz in the laboratory of Professor U. Haeberlen, at the Max Planck Institüt für Medizinische Förschung at Heidelberg, Germany. The measurements were made on a polycrystalline sample by analyzing the sideband intensities obtained at intermediate magic-angle spinning speeds as proposed by Herzfeld and Berger.[19] This method is particularly useful for CSA tensors that are not axially symmetric as is the case in the present work. Proton cross polarization was used to enhance the sensitivity of the measurement.

IV. Results and Analysis

^{13}C Line Shapes in Enzyme Complexes. The proton-coupled ^{13}C doublets of [2-^{13}C]ATP bound to 3-P-glycerate kinase are shown in Figure 1 in juxtaposition with that in simple aqueous solution. There is no significant chemical shift between the centers of the two spectra, nor is there a measurable difference in the doublet separation in the two cases. The ^{13}C–^{1}H spin–spin coupling constant is $(J/2\pi) = 204 \pm 2.5$ Hz. It is clear that while the two transitions in the doublet of free ATP are of nearly equal line widths, those of enzyme-bound ATP exhibit an unmistakable differential broadening. This differential effect for the enzyme-bound [2-^{13}C]ATP is ascribed to the interference between ^{13}C–^{1}H dipolar and ^{13}C CSA interaction which simultaneously contribute to ^{13}C line widths. The interpretation was corroborated by the field dependence of the line widths. Similar line shapes were observed for ATP bound to a number of kinases and methionyl tRNA synthetase. For adenylate kinase, the measurements were made with enzyme-bound AMP as well. The line width data obtained, along with the field-dependence, wherever such measurements were made, is compiled in Table II. For all the measurements with enzyme complexes, the enzyme concentrations were chosen to be sufficiently in excess of those of the nucleotides so that the bound fractions of nucleotide estimated on the basis of known dissociation constants exceeded 90%.

The line widths of [2-^{13}C]ATP and [2-^{13}C]AMP bound to *E. coli* adenylate kinase were also measured using a protein that has all the amino acids except the phenylalanines, threonines, and leucines deuterated, in order to determine the extent of the contribution from the protein protons to the dipolar interactions experienced by the ^{13}C. There was no measurable difference in the line widths observed with the deuterated protein and those with the normal protein.

^{13}C CSA Tensor for [2-^{13}C]AMP. Measurements of the principal elements of the ^{13}C CSA tensor, as outlined above, yielded the results

$$\delta_{xx} = -84.6, \quad \delta_{yy} = -13.8, \text{ and } \quad \delta_{zz} = 98.3 \text{ ppm} \qquad (44)$$

These chemical shift values are given with reference to the position of the isotropic signal, and the experimental error in these values is about ±2 ppm. The average value in the solid state is equal to the isotropic shift in the liquid state.[20] On the basis of generalizations made in the literature, the three principal directions (x, y, z) were assumed to be the C–H bond direction, the direction tangential to the ring in its plane, and the direction perpendicular to the plane of the adenine ring respectively.[20,21a] We shall further make the assumption that the CSA tensor is the same for AMP and ATP and is not altered by the binding of the nucleotides to the various enzymes. The data relevant for eqs 32 and 33 are thus given by

$$\theta = \frac{\pi}{2}, \quad \phi = 0, \quad \delta_{zz} = 98.3 \text{ ppm, and } \quad \eta = 0.72 \qquad (45)$$

Analysis of Line Width Data for the Enzyme Complexes. For all the enzymes considered, the rotational correlation time is expected to be larger than 10 ns. Therefore, all the spectral density terms other than $J(0)(=\tau_c)$ in eqs 31–33 are negligible. Furthermore, as may be expected on the basis of the distances of closest approach and as corroborated by the measurements with deuterated *E. coli* adenylate kinase, the dipolar interaction experienced by the ^{13}C nucleus arises almost entirely from the attached proton.[21b] Thus, using eqs 6 and 45 along with standard values of γ_C and γ_H and setting $r = 0.109$ nm gives

$$\text{LW(DD)} = \tfrac{2}{5}\chi_D^2\tau_c = 8.579 \times 10^9\tau_c \text{ rad s}^{-1} \qquad (46)$$

$$\text{LW(CSA)} = \tfrac{2}{5}\omega_C^2\delta_{zz}^2\left[1 + \frac{\eta^2}{3}\right]\tau_c = 4.533 \times 10^{-9}\omega_C^2\tau_c \text{ rad s}^{-1} \qquad (47)$$

$$\text{LW(INT)} = \tfrac{2}{5}\chi_D\omega_C\delta_{zz}\tau_c[(3\cos^2\theta - 1) - \eta\sin^2\theta\cos 2\phi]$$
$$= 9.901\omega_C\tau_c \text{ rad s}^{-1} \qquad (48)$$

and therefore the difference between the two line widths of the ^{13}C doublet (ΔLW) as a fraction of ω_C is given by

$$(\Delta\text{LW}/\omega_C) = 19.802\tau_c \qquad (49)$$

The experimentally measured line widths presented in Table II for the various enzyme–nucleotide complexes are readily analyzable in terms of eqs 46–49. The value of τ_c obtained by using eq 49 with the data for each complex is also given in Table II, along with the molecular masses of these enzymes. The experimental error in the line widths is significant, about ±10%, because of the problems associated with recording broad and partly overlapping resonances (see Figure 1) from enzyme bound complexes. Nevertheless, there is an approximately linear relationship between the molecular masses and the corresponding values of τ_c. In the case of ATP bound to the tryptic fragment of methionyl tRNA synthetase the value of τ_c obtained appears to correspond to an aggregated form of this protein. Previous light scattering experiments[22] provide evidence for aggregation of this protein in the absence of salt (KCl) concentrations of at least 0.1 M, as was the case here. Thus, it is reasonable to conclude that the ^{13}C line

(19) Herzfeld, J.; Berger, A. E. *J. Chem. Phys.* **1980**, *73*, 6021.

(20) Mehring, M. *Principles of High-Resolution NMR in Solids*; Springer: New York, 1983; p 251.

(21) (a) Kempf, J.; Spiers, H. W.; Haeberlin, V.; Zimmermann, H. *Chem. Phys. Lett.* **1972**, *17*, 39; *Chem. Phys.* **1974**, *4*, 269. (b) Contributions to the line width due to scalar coupling with ^{14}N nuclei at the neighboring 1 and 3 positions on adenine modulated by the ^{14}N quadrupolar relaxation are negligible.

(22) Gulik, A.; Monteilhet, C.; Dessen, P.; Fayat, G. *Eur. J. Biochem.* **1976**, *64*, 295.

Table II. ^{13}C Line Widths of [2-^{13}C]ATP Bound to Various ATP Utilizing Enzymes (and [2-^{13}C]AMP Bound to Adenylate Kinase)[c]

enzyme complex	^{13}C freq (MHz)	line widths (Hz) left	line widths (Hz) right	τ_c (ns)	molecular mass
adenylate kinase·AMP	75	21	39	10	24 000
	125	12	45		
adenylate kinase·ATP	75	34	49		
	125	12	51		
3-phosphoglycerate kinase·ATP	50	45	68	28	47 000
	75	60	100		
	118	36	117		
arginine kinase ATP	50	58	78	21	38 000
	75	39	73		
creatine kinase·ATP	75	62	131	42	81 000
	125	58	151		
methionyl tRNA synthetase·ATP	75	66	222	104	67 000[a]
pyruvate kinase·ATP[b]	75	115	412	119[b]	238 000

[a] This is the monomer molecular weight. The correlation time calculated appears to correspond to an aggregated form of this protein (see text). [b] Line width data are rough estimates because lines are extremely broad. [c] Measurements were made at 20 °C and at several spectrometer frequencies for some of the enzymes. The experimental error is ±10%. Left and right represent low field and high field transitions of the proton-coupled ^{13}C doublet, respectively. Average correlation times τ_c calculated from the data for different enzymes using eq 49 are given along with the molecular masses of the enzymes.

widths of [2-^{13}C]ATP (and AMP) in all the enzyme complexes studied are adequately described by the theoretical formalism presented above and by the formulae in eqs 46–49.

^{13}C Line Shapes of [2-^{13}C]ATP in Viscous Solutions. As may be seen in Figure 1, the two ^{13}C transitions of [2-^{13}C]ATP free in aqueous solutions are sharp resonances consistent with the estimate that the reorientational correlation time for a molecule of the size of ATP is about 0.1 ns. One might, therefore, expect that line broadening as well as the associated differential effects observed for [2-^{13}C]ATP in the various enzyme complexes will also be observed for free [2-^{13}C]ATP in viscous solutions in which the correlation time for overall rotation of the molecule is similar to that for the enzyme complexes. In order to verify this prediction, the ^{13}C line widths as well as T_1's were measured for [2-^{13}C]ATP dissolved in aqueous solutions containing varying concentrations of sucrose for the purpose of changing the viscosity of the solution. The line widths (and T_1's) observed were surprisingly insensitive to the variation in viscosity (see Figure 2). The line widths at different viscosities,[23] given in Table IIIA, show that for an increase in viscosity by a factor of about 2300, the experimental line widths increase by no more than a factor of about 4. (The increase in the true line width is by a somewhat larger factor since the inhomogeneity contributes 0.5–1.0 Hz to these line widths). Further evidence for the insensitivity of the ^{13}C line widths to viscosity was obtained by repeating the measurements with [2-^{13}C]MgATP. The results, given in Table IIIB, show a similar behavior. ^{13}C line widths of [2-^{13}C]ATP were also measured using glycerol as a solvent which allows the viscosity to be increased by a factor of about 1000 over that of water.[23] Once again the results were similar to those in Figure 2 and Table IIIA. Also included in Table IIIC are theoretical line widths calculated by using eqs 31–33 with τ_c = 0.1 ns for η_s/η_w = 1 and assuming a linear relationship between η_s and τ_c. (η_s and η_w are solvent and water viscosities, respectively.) We thus have a striking contrast between the line widths of ATP bound to the various ATP-utilizing enzymes and those of ATP in viscous solutions in which the overall rotational correlation time is similar to those of the enzymes.

Additional Line Width Measurements in Viscous Solutions. In order to investigate further the insensitivity of the line widths of free ATP to viscosity, two other types of measurements were made.

(23) Viscosity data for sucrose solutions was obtained: ISCO *Tables*; 1982; pp 15–16. The data for glycerol: *Lange's Handbook of Chemistry*, 13th ed.; McGraw-Hill: New York, 1985; pp 10–97.

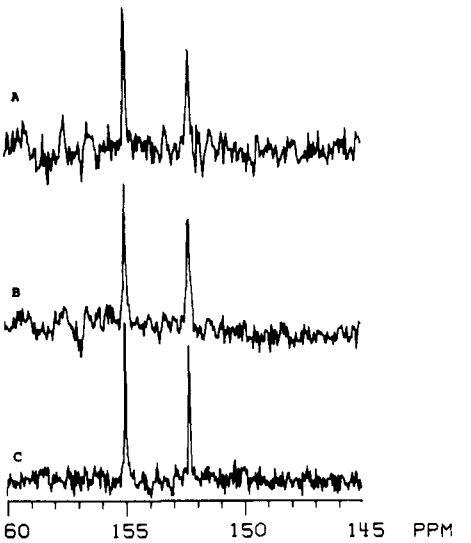

Figure 2. 75-MHz ^{13}C NMR spectra of 5 mM [2-^{13}C]ATP at pH 8.0 and varying sucrose concentrations: (A) 75% sucrose, viscosity of 2320 cP, (B) 46% sucrose, viscosity of 10 cP, and (C) 0% sucrose, viscosity 1 cP. Spectral conditions: 60° pulse, 8K data points, 2048 acquisitions, 2 Hz line broadening, 20 °C.

Table III. ^{13}C Line Widths of ATP Measured at 75 MHz and 20 °C in Viscous Solutions[a]

η_s/η_w	τ_c, ns	line width in Hz left	line width in Hz right
	A. [2-^{13}C]ATP		
1.0		2.5	2.7
10		2.8	2.9
99		6.0	6.8
182		7.3	9.3
288		7.2	8.6
481		7.1	7.6
2320		7.2	7.7
	B. [2-^{13}C]MgATP		
1.0		5.4	5.8
10		4.8	6.0
182		4.5	5.8
288		4.8	7.2
2320		6.3	10.0
	C. Theoretical		
1.0	0.1	0.45	0.7
10	1.0	1.9	4.3
100	10	8.1	23.6
1000	100	78.1	227.5
	D. [1′-^{13}C]ATP		
1.0		2.0	2.0
248		28	29
544		40	40
1060		57	76

[a] The experimental error is ±10%. The viscosity of the solution, η_s, obtained from published data,[25] is given as a multiple of water viscosity (η_w). Left and right represent the low field and high field transitions of the proton-coupled ^{13}C doublet, respectively: A. data for [2-^{13}C]ATP, B. data for [2-^{13}C]MgATP, C. theoretical line widths for [2-^{13}C]ATP on the basis of eqs 46–48, assuming a linear relationship between η and the relaxation time τ_c, and that τ_c = 0.1 ns for η_s/η_w = 1.0, D. data for [1′-^{13}C]ATP.

First, the ^{31}P line widths were also measured in the same sucrose solutions of [2-^{13}C]MgATP used for the ^{13}C measurements described above. These results are qualitatively shown in Figure 3. The ^{31}P line widths are also insensitive to the large change in viscosity. Second, the ^{13}C line widths of [1′-^{13}C]AMP were measured in solutions of glycerol. (Sucrose solutions could not be used because of overlap in the ^{13}C signals of the solvent and solute.) The results are presented in Table IIID and Figure 4.

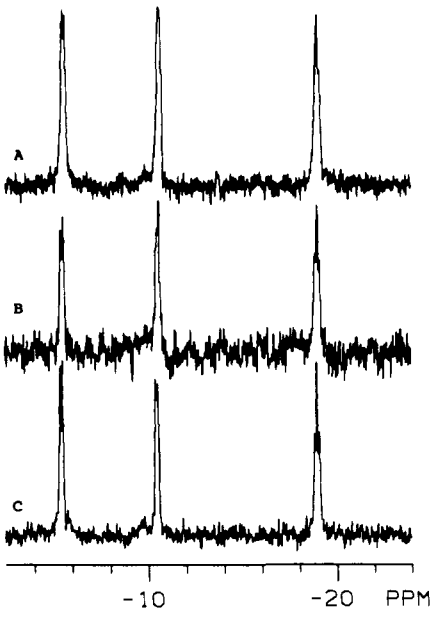

Figure 3. 120-MHz ^{31}P NMR spectra of [2-^{13}C]MgATP at pH 8.0 and varying sucrose concentrations. Sample conditions: 5 mM [2-^{13}C]ATP and 12 mM $MgCl_2$, (A) 75% sucrose, viscosity of 2320 cP, (B) 46% sucrose, viscosity of 10 cP, and (C) 0% sucrose, viscosity of 1 cP. Spectral conditions: 60° pulse, 4K data points, 256 acquisitions, 2 Hz line broadening, 20 °C.

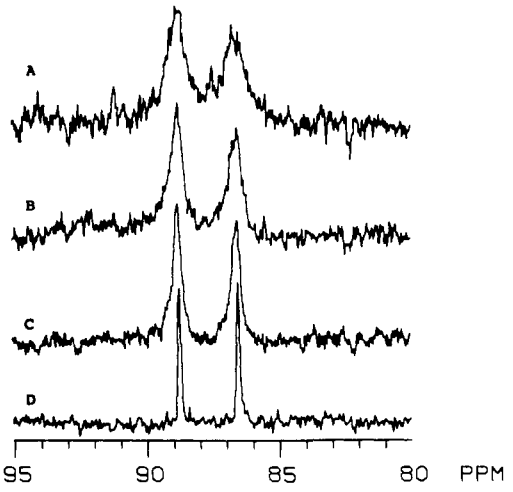

Figure 4. 75-MHz ^{13}C NMR spectra of 5 mM [1′-^{13}C]ATP at varying glycerol concentrations and temperatures: (A) 98.4% glycerol, 20 °C, viscosity of 1060 cP, (B) 95% glycerol, 20 °C, viscosity of 544 cP, and (C) 95% glycerol, 30 °C, viscosity of 248 cP, and (D) 0% glycerol, 20 °C, viscosity of 1 cP. Spectral conditions: 60° pulse, 4K data points, 6000 acquisitions, 2 Hz line broadening.

These line widths show a significant increase with increasing viscosity in contrast to the ^{13}C and ^{31}P line widths of [2-^{13}C]-MgATP. The line width increases by a factor of about 40–50 for an increase of η_s/η_w by about 1000. Although this increase does not reflect a linear relationship with viscosity, it is significantly larger than the virtual independence on viscosity exhibited by the ^{13}C and ^{31}P line widths of [2-^{13}C]ATP.

Interpretation of the Line Width Data in Viscous Solutions. An examination of eqs 31–33 and the derivation that preceded these equations suggest a possible explanation for the contrasting line width behavior observed for the enzyme complexes and viscous solutions. It must be recognized that eqs 31–33 are derived by assuming isotropic reorientation (vide infra). The conformity of the observed differential line width patterns for the enzyme complexes with these equations indicates that the motion of ATP bound to any of the enzymes is reasonably well-represented by isotropic reorientation. The numerical data that enter eqs 31–33 are all known except for τ_c. If these equations are valid for $\tau_c \geq 10$ ns the line widths should increase in a linear fashion with the increase in τ_c (see eqs 46–48). The explanation for the fact that the line width data in viscous solutions do not conform to eqs 31–33 must, therefore, lie in the assumption of isotropic reorientation implicit in these equations.

As a Dreiding model of the molecule would readily indicate ATP has considerable internal mobility. There are three basic internal motions: the rotation of the adenine moiety with respect to the glycosidic bond that attaches it to the ribose, the mobility of the phosphate chain, and the ribose pucker. Of these, the first two motions are large-amplitude motions which make the molecule rather floppy. These ideas about the internal mobility are based on molecular models in vacuum. There is no a priori knowledge regarding such motions in the presence of large concentrations of solvent molecules such as sucrose or glycerol which make the solution highly viscous and thereby restrict the overall reorientation of ATP. When several motions contribute to relaxation, the faster motions dominate the spectral densities (see eq 21). If the internal motions are slower than the overall rotation at all viscosities, i.e. they are characterized by larger correlation times, the observed line widths will be primarily governed by the overall rotation and are therefore expected to increase with viscosity as indicated in Table IIIC. Such a behavior has not been observed for the ^{13}C (and ^{31}P) line widths of [2-^{13}C]ATP. A comparison of the data in Table IIIA and the theoretical line widths in Table IIIC indicates that the effective correlation time increases with viscosity for low viscosities until it reaches a value of a few ns for $\eta_s/\eta_\omega \sim 30$ and that there is no further increase with viscosity. A simple explanation for this variation is that while the overall rotation is faster than the internal motion at low viscosities, as the viscosity is increased, the overall rotation slows down compared to the internal motions. The crossover point is in the neighborhood of a few ns. One then infers that these internal motions in the nanosecond range are not attenuated by further increase in the viscosity of the solvent, and that the linewidths are therefore viscosity independent although the correlation time for the overall rotation continues to increase.

The above discussion offers a qualitative explanation for the narrow ^{13}C and ^{31}P line widths of [2-^{13}C]ATP and [2-^{13}C]MgATP on the basis of the persistence of the glycosidic rotation and the phosphate chain mobility at high viscosities. A quantitative evaluation of these line widths in the presence of such motions is formidable and intractable because of the need to consider anisotropic rotational models along with dipolar and CSA tensor interactions as well as the interference effects between them.[24] However, within the framework of the qualitative picture of the internal motions it may be noted that while the glycosidic rotation and the phosphate chain motion occur with appreciable amplitude, the ribose moiety appears, in contrast, to be less mobile and the ribose pucker is not likely to make an important contribution to line narrowing. Thus it may be expected that line widths of nuclei in the ribose moiety show a markedly greater sensitivity to the attenuation of the overall rotation of the molecule at high viscosities. This reasoning is corroborated by the ^{13}C line width data of [1′-^{13}C]AMP in glycerol shown in Table IIID.

V. Discussion

The possibility of interference effects between relaxation mechanisms that transform in a similar manner is an unavoidable feature of relaxation theories in the liquid state and should therefore be examined when several mechanisms contribute to relaxation. In particular with the advent of high field NMR, CSA becomes a significant relaxation mechanism for nuclei such as ^{13}C, ^{31}P, and ^{15}N, and with dipolar interactions being pervasive and even dominant when there are directly bonded protons with these nuclei, interference effects of the type considered in this paper are likely to occur in a number of NMR spectra. It should be noted that the differential interference effects are prominent if

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the resonance of the nucleus is a resolved spin-coupled multiplet and tend to cancel out if the resonance is a single line or a closely spaced multiplet due to a small coupling constant. The derivation of relaxation-matrix elements presented in this paper for the $^{13}C-^{1}H$ spin-coupled system, in the presence of a general ^{13}C CSA tensor and $^{13}C-^{1}H$ dipolar interactions, generates expression in Redfield notation that makes them directly applicable to the analysis of experimental data. The calculational procedure is quite general and may be readily adapted to relaxation-matrix elements for strongly coupled spin systems as well as to interference effects involving other relaxation mechanisms.

The fact that the ^{13}C line widths of [2-^{13}C]ATP are fairly small even at solvent viscosities a factor of 2300 larger than that of water, is a rather unexpected result. At such high viscosities the overall reorientational time is estimated to be over 100 ns, which is slower than the rotational correlation times of some of the enzymes used (see Table II). The narrow ^{13}C line widths observed in [2-^{13}C]ATP may be qualitatively understood if the glycosidic rotation of the adenine base persists with a characteristic time in the nanosecond range virtually independent of viscosity. This interpretation is based on the reasoning that in the presence of fast internal motions and overall rotations the effective correlation time for an interaction is determined by the faster of the motions. The analysis is perforce qualitative because an analytical formulation of the spectral densities in the relaxation theory for a molecule as floppy as ATP is quite difficult even in the absence of solvent–solute interactions. The ^{31}P line widths also display a similarly negligible viscosity dependence which may be ascribed to the internal motions involving the phosphate chain of ATP. Just as in the case of the ^{13}C line widths, the ^{31}P line widths of ATP bound to the various enzymes[25] are much larger than those of ATP in the viscous solutions studied here. As is readily apparent from a molecular model, the glycosidic rotation of the adenine and the phosphate chain mobility occur with little hindrance in ATP. The measurements presented here show that high viscosity does not attenuate these motions. In comparison, the motion of the ribose moiety of ATP should be of low amplitude, and the fact that the ^{13}C line widths of [1′-^{13}C]AMP are indeed much larger than those of [2-^{13}C]ATP at similar viscosities corroborate the interpretation. The line widths observed for nuclei located at different parts of the ATP molecule are thus qualitatively consistent with the persistence of two large-amplitude nanosecond-range internal motions viz. the glycosidic rotation and the phosphate chain mobility. It is interesting that the addition of Mg(II) does not make a measurable difference to this pattern of internal motions and their insensitivity to viscosity.

The persistence of internal motions at high viscosities is not without precedence. Fast nanosecond motions of low activation energy that are insensitive to solvent viscosity were observed for the rotation of the phenyl group of ethidium bromide in glycerol solutions by means of picosecond time-resolved fluorescence spectroscopy.[26] It was suggested that the internal motion is not controlled by the static viscosity of the solvent but by the high-frequency components of the frictional forces of the viscous solvent. A similar process may be operative for the nucleotides in the high-viscosity solutions studied here.

In sharp contrast with the ^{13}C line widths in viscous solutions, those of [2-^{13}C]ATP bound to the various ATP-utilizing enzymes are reasonably well represented by the formulae in eqs 46–48 which are derived by assuming a single correlation time τ_c which describes isotropic molecular reorientation. Furthermore, the correlation times deduced not only are of the appropriate magnitudes but also bear an approximately linear relationship with the molecular masses. Therefore, one is led to the conclusion that in its enzyme-bound form the internal motions of ATP discussed above do not occur to any appreciable extent. Thus these internal mobilities which are negligibly influenced by the solvent packing around ATP are arrested by the ATP-utilizing enzymes. It may be argued on general grounds that the enzymatic action of cleaving and transferring a moiety of the ATP molecule (such as the terminal phosphoryl group in the case of the kinases, and the adenylyl moiety in the case of the aminoacyl tRNA synthetases) is most efficient if the molecule does not have appreciable internal mobility. It is, therefore, reasonable to suppose that large-amplitude internal mobilities such as the glycosidic rotation and the phosphate chain mobility will be attenuated in the enzyme complexes. The fact that these motions are not easily influenced by nonspecific solvent interactions even at exceedingly high solvent concentrations and viscosities suggests that the specific chemical architecture of the enzymatic active-site contains features that accomplish the purpose of arresting these motions and facilitate efficient catalytic action.

Acknowledgment. We are grateful to Dr. T. Klutz and Professor U. Haeberlen, Max Planck Institüt für Medizinische Förschung, Heidelberg, Germany for the measurement of the ^{13}C CSA tensor in [2-^{13}C]AMP. Thanks are due to Professor Paul Rösch, Universität Bayreuth, Bayreuth, Germany for continued collaboration on the work with adenylate kinase, to Dr. Reuben Leberman, EMBL, Grenoble, France, for supplying partially deuterated adenylate kinase, to the late Dr. Guy Fayat, Ecole Polytechnique, Palaiseau, France, for providing us with the tryptic fragment of methionyl tRNA synthetase, to Professor Hiroshi Shimizu, University of Tokyo, Tokyo, Japan for the correspondence regarding his original paper on differential broadening, to Professor Wilmer Fife and Ms. Marie-Ann Bertrand, Chemistry Department, IUPUI, for the synthesis of [2-^{13}C]AMP, to the Stable Isotope Resource of Los Alamos, National Laboratory, Los Alamos, NM, for providing us with [1′-^{13}C]AMP, to Dr. N. Murali for checking the theory section, and to Ms. Margo Page for typing the manuscript.

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