Intermolecular Association in Adenosine 5′-Monophosphate. An ^{2}H Nuclear Magnetic Resonance Investigation

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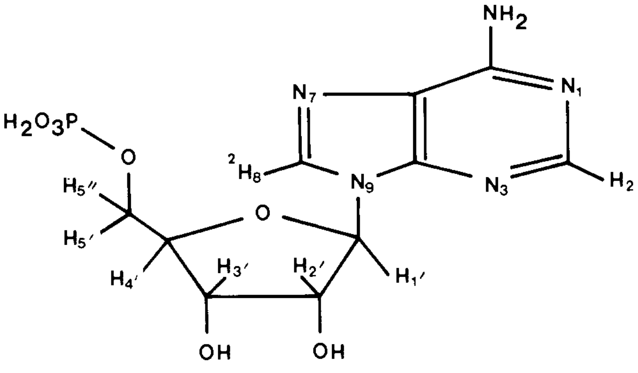
Abstract: Intermolecular association in 8-deuterioadenosine 5′-monophosphate has been investigated by deuterium magnetic resonance in the concentration range 0.01–1.0 m (pH 7.0). The dependence of the high-resolution spectral line widths on concentration provided evidence for the intermolecular association of the title compound. Analysis of the data indicates that the association may proceed past the dimer stage.

The concentration dependence of the ^{13}C nuclear magnetic relaxation times (T_1) for adenosine 5′-monophosphate (AMP) has been reported by Grant and co-workers;[2] these authors attributed the observed dependence of T_1 on concentration to the base stacking of AMP monomers.[3,4] Recently, Lüdemann and Röder[5] have criticized this interpretation and have instead noted that the T_1 concentration dependence of AMP is attributable solely to changes in solution viscosity; additionally, on the basis of rotational correlation times derived from viscosity corrected T_1 values, these authors[5] concluded that AMP is essentially monomeric over the concentration range studied, 0.2–1.0 m. The present investigation, based on ^{2}H spin lattice relaxation times for 8-deuterioadenosine 5′-monophosphate, AMP-*8-d* (**1**), provides cogent evidence for the intermolecular association of AMP as well as an explanation for these apparent discrepancies.



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Results and Calculations

High-resolution ^{2}H NMR spectra of **1** (pH 7.0, 30 °C) as a function of concentration are shown in Figure 1. A striking change in the line width at half-height ($W_{1/2}$) is seen to accompany the change in concentration, decreasing from ca. 108 Hz at 1.0 m to ca. 12 Hz at 0.01 m. A similar, though more pronounced, change is observed for **1** at 20 °C (see Table I).

Under extreme narrowing conditions, and in the absence of slow chemical exchange processes, the spin–spin and spin–lattice relaxation times are equal.[6,7] Given these circumstances, T_1 values may be taken directly from the line widths using the expression $T_1 = T_2 = (\pi W_{1/2})^{-1}$ provided the line widths can be corrected for magnetic field inhomogeneity; in the present instance this is readily accomplished using the HDO half-bandwidth as a correction factor (the natural line width for HDO is ca. 0.5 Hz). Spin-lattice relaxation times obtained for **1** in this fashion at 20 and 30 °C are presented in Table I; T_1's were also determined by the inversion recovery technique for 1.0, 0.5, and 0.25 m solutions of **1** and found to equal the values determined from the line widths, thereby demonstrating the equality of T_1 and T_2.

The Stokes–Einstein–Debye equation relates the rotational correlation time to a molecular or aggregate radius, a, and is given as

$$\tau_c = 4\pi\eta a^3/3kT \qquad (1)$$

where η is the viscosity of the medium and kT is the Boltzmann factor. Under extreme narrowing conditions, the expression for the deuterium spin–lattice relaxation rate ($\equiv 1/T_1{}^Q$) is

$$1/T_1{}^Q = \tfrac{3}{8}[e^2qQ/h]^2[1 + (\xi^2/3)]\tau_c \qquad (2)$$

where e^2qQ is the quadrupole coupling constant, ξ is the asymmetry parameter, and τ_c is the effective rotational correlation time.[6] On the basis of eq 1 and 2, it has been concluded[5] that for a nonassociating solute, the quantity ηT_1 should be concentration independent. This was not the case for AMP-*8-d*. Plots of $(\eta T_1)^{-1}$ vs. AMP-*8-d* concentration are presented in Figure 2; viscosity data are included in Table I.

The concentration dependence of $(\eta T_1)^{-1}$ displayed by **1** is most readily attributed to a change in correlation time occasioned by intermolecular association. The simplest association model which might account for the experimental results is a monomer–dimer equilibrium. Linear-least-squares analyses of $(\eta T_1)^{-1}$ as a function of concentration resulted in the following values for the equilibrium constant (K_{eq}), the ratio of correlation times ($\tau_c{}^{dimer}/\tau_c{}^{monomer}$), and $1/T_1{}^{monomer}$: at 30 °C, values of 2.1 (±0.7), 7.8 (±1.2), and 20.9 (±3.9), respectively, were obtained; at 20 °C, the corresponding values were 1.7 (±0.7), 9.7 (±1.8), and 28.3 (±6.7), respectively. The least-squares fits are shown in Figure 2.

In terms of a monomer–dimer equilibrium, the values derived for K_{eq} and $1/T_1{}^{monomer}$ are reasonable;[8] the correlation time ratios, however, are apparently too high, values of approximately 2 being expected.[9] This would indicate that a more complicated model for AMP association is operative.

We have additionally examined the solvent (H_2O) ^{1}H spin–lattice relaxation behavior as a function of AMP concentration. At 20 °C, the water relaxation time varied from ca. 1.0 s for a 1.0 m AMP solution to ca. 3.0 s for pure water; values of ηT_1, however, were virtually independent of AMP concentration; see Table II.

Discussion

The present observations and conclusions regarding the concentration dependence of ηT_1 and the intermolecular association of AMP are opposite those of Lüdemann and Röder.[5] Although based on nuclei possessing differing relaxation mechanisms, quadrupolar for deuterium and dipolar for car-

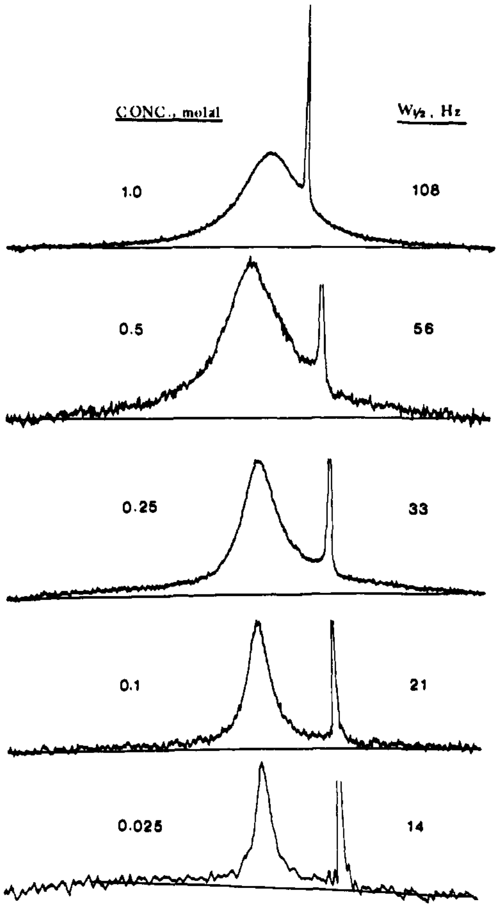

Figure 1. ^{2}H nuclear magnetic resonance spectra of **1** (pH 7.0, 30 °C) at differing concentrations (the spectrum for **1** at 1.0 m was recorded at half the scale expansion of the remaining spectra). The high-field sharp resonance is residual HDO.

bon, inspection of the theoretical expressions describing the respective relaxation rates reveals that they differ solely by a multiplicative constant[10] and accordingly these two studies should have resulted in parallel findings. This discrepancy has its primary source in the limited and high concentration range employed for the ^{13}C measurements (0.2–1.0 M). Over this range, and particularly in the region 0.5–1.0 m, the concentration dependence of ηT_1 is not especially marked (see Figure 2); had the ^{13}C measurements been extended to lower concentrations, i.e., less than 0.1 M, results and interpretations more in keeping with the present study would have undoubtedly resulted. Due to the low sensitivity of natural abundance ^{13}C NMR, however, extensions to such low concentrations are made only with great difficulty; isotopic enrichment as an alternative is, unfortunately, both difficult and expensive.

We would now like to turn attention to the AMP association model. As noted in the previous section, while reasonable values of K_{eq} and $1/T_1{}^m$ were obtained using the monomer–dimer model, the derived $\tau_c{}^d/\tau_c{}^m$ ratios were greater than expected, thereby indicating association past the dimer stage and, consequently, the inadequacy of the model. Attempts to describe the concentration dependence of (ηT_1) in terms of more complex associations were not made; our reluctance to do so stemmed from two factors. The first arose from the use of concentrations instead of activities. Since it is highly unlikely that AMP concentrations and activities are equivalent, particularly for the more concentrated solutions, any resulting association model and its accompanying thermodynamic parameters would only be apparent (unless, of course, the appropriate ratios of activity coefficients were unity). The second factor concerned the viscosity correction of the relaxation times; we are not certain of the extent of adjustment that should be applied to T_1. In this regard, the independence of ηT_1 (water) is of some significance (Table II). If it were the case that the water lattice structure was unchanged by the addition of AMP, then the concentration independence of ηT_1 (water) would indicate that the AMP deuterium values ought likewise be corrected for the change in solution viscosity, as we have done (see Figure 2). However, the large viscosity changes (Table I) and other considerations[12] indicate that the water lattice structure is altered by addition of AMP; accordingly, it is not clear that the total adjustment of deuterium (or ^{13}C) relaxation times of AMP is warranted.

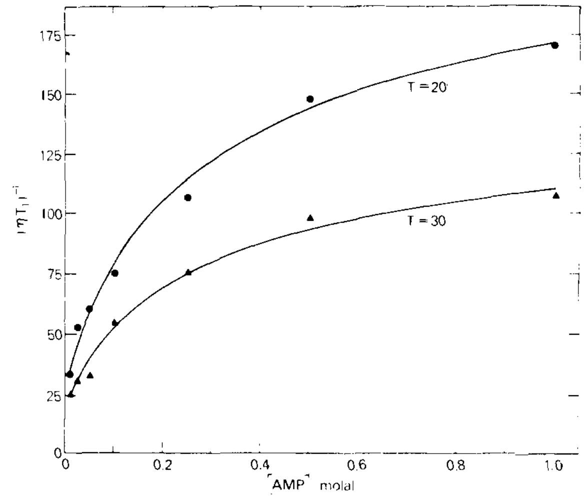

Figure 2. ^{2}H spin lattice relaxation rates (adjusted for the viscosity) as a function of AMP-*8-d* concentration and their least-squares fit to a monomer–dimer equilibrium.

Table I. Viscosities and Spectral Data for **1** (pH 7.0) as a Function of Concentration and Temperature

	$W_{1/2}$,[a] Hz T, °C		T_1,[b] ms T, °C		η[c] T, °C	
Concn of **1**, m	20	30	20	30	20	30
1.0	173	106	1.8	3.0	3.28	3.11
0.5	88	54	3.6	5.8	1.82	1.73
0.25	45	32	7.1	10.1	1.37	1.31
0.10	27	20	11.8	16.3	1.13	1.11
0.05	21	11	15.5	29.0	1.07	1.06
0.025	18	11	17.7	29.0	1.06	1.06
0.01	11	9	28.9	35.5	1.02	1.02

[a] The line widths at half-height have been corrected for magnetic field inhomogeneity; the corrected $W_{1/2}$ values are accurate to ca. ±2 Hz. [b] The T_1 values are based on the $W_{1/2}$ values given in the preceding column and have not been adjusted for the viscosity; the accuracy of the T_1 values depends on the measured line widths and varies from ca. 3% at 1.0 m AMP to ca. 10% at 0.01 m AMP. [c] Viscosities are given as ratios relative to water and have been calculated from the formula $\eta = t\eta_0\rho/(t_0\rho_0)$, where t_0 and t are the times required for water and AMP solutions, respectively, to flow out of the bulb and ρ_0 and ρ are the densities of water and AMP solution, respectively. The measured value of $\eta_0{}^{20}/\eta_0{}^{30}$ (the water viscosity ratio at 20 and 30 °C) was 1.24.

On a more positive note, *regardless of the viscosity correction,* our data clearly demonstrate the association of AMP and, furthermore, indicate that association may proceed past the dimer stage. The present findings are thus in accord with sedimentation equilibrium[13] and proton chemical shift[14] studies on AMP (due to nonideality effects, neither of these studies were able to provide a model and thermodynamic parameters for the AMP association).

Table II. Solvent (H_2O) Relaxation Data as a Function of AMP Concentration (pH 7.0, 20 °C)

Concn of **1**, m	T_1, s	ηT_1, s
1.0	0.99 (0.07)[a]	3.24 (0.23)[b]
0.5	1.76 (0.07)	3.20 (0.13)
0.25	2.16 (0.09)	2.90 (0.12)
0	3.02 (0.10)	3.0 (0.10)

[a] The error in T_1 was estimated on the basis of the minimum and maximum slopes that could be drawn through a plot of ln ($M_0 - M_t$) vs. t, where M_0 is the equilibrium magnetization and M_t is the magnetization at time t. [b] The error in ηT_1 derives essentially from the error in T_1; values for η at the appropriate concentration may be found in Table I.

It should be emphasized at this point that our relaxation data provide no information about the mechanism (hydrogen bonding or stacking) and geometry of the AMP association. A model for the association, based on proton relaxation rates, has been furnished by Gueron et al.[15] who concluded that AMP undergoes a head-to-tail stacking. Although there is no reason to assume the contrary, the geometry of the associated species responsible for the relaxation effects observed by Gueron et al.[15] is not necessarily the same as that primarily responsible for the observed change in correlation time.

From the viscosity corrected ^{13}C relaxation times, and assuming isotropic reorientation, Lüdemann and Röder[5] were able to determine a reorientational correlation time for AMP. Substitution of this value into the Stokes-Einstein-Debye equation yielded an average molecular radius of ca. 4.5 Å, a value which these authors offered as evidence for the monomeric nature of AMP. The present study therefore serves as a caution against the use of the Stokes Einstein-Debye equation for the calculation of *absolute* radii, particularly when small molecules are involved.[16]

Experimental Section

8-Deuterioadenosine 5′-monophosphate (**1**) was prepared according to the procedure of Bullock and Jardetzky;[17] the resulting material (ca. 85% deuterium incorporation as judged by its proton NMR spectrum) was treated with Chelex-100 ion-exchange resin to remove paramagnetic ion impurities, followed by lyophilization from deionized water (four times) to remove exchangeable N-D and O-D deuterons. Solutions of **1** (in H_2O) at various concentrations were prepared and the pH adjusted to 7.0 ± 0.1.

High-resolution ^2H NMR spectra were recorded at 15 MHz on a Varian XL-100-15 spectrometer operating in the fourier transform mode. The deuterium T_1 measurements[18] by the inversion recovery technique [(d-180-τ-90) pulse sequence where d is a delay time greater than $5T_1$] were made on a Bruker BK-322s pulse spectrometer operating at 14 MHz. The solvent (H_2O) T_1 values were determined by the inversion recovery technique using a modified Spin Lock CPS-2 pulse spectrometer operating at 30 MHz. Sample temperatures were maintained at the desired value by a stream of precooled or -heated nitrogen. Solution viscosities were determined at 20 and 30 °C by standard procedure using an Ostwald viscometer.[19]

Analysis of the (ηT_1, [AMP]) data sets in terms of a monomer-dimer equilibrium was accomplished by the method of least squares utilizing the MLAB facilities of the NIH.[20] In terms of the concentration of AMP monomer, {m}, and the total AMP concentration, C_{tot}, the observed T_1 value is given as

$$1/T_1^{\text{obsd}} = \frac{\{\text{m}\}/C_{\text{tot}}}{T_1^{\text{m}}} + \frac{(C_{\text{tot}} - \{\text{m}\})/C_{\text{tot}}}{T_1^{d}}$$

where T_1^{m} and T_1^{d} are the monomer and dimer T_1 values; {m} is given as

$$\{\text{m}\} = \frac{1 - (1 + 8K_{\text{eq}}C_{\text{tot}})^{1/2}}{-4K_{\text{eq}}}$$

The analysis assumes fast (relative to T_1) exchange between monomer and dimer so as to assure that

$$1/T_1^{\text{obsd}} = p_{\text{m}}/T_1^{\text{m}} + p_{\text{d}}/T_1^{\text{d}}$$

where p_{m} and p_{d} are the fractional population of monomer and dimer, respectively.

References and Notes

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(8) Comparable equilibrium constants for purine and pyrimidine nucleosides and nucleotides have been reported (see ref 4); $1/T_1^{\text{monomer}}$ was calculated to be ca. 32 s^{-1} from eq 2 assuming a value of the quadrupolar coupling constant of 185 kHz (the value found for benzene[11]), setting $\xi = 0$, and using and average molecular radius of 4 Å.

(9) See, for example, J. H. Noggle and R. E. Schirmer, "The Nuclear Overhauser Effect: Chemical Applications", Academic Press, New York, N.Y., 1971, pp 26–31, and references cited therein.

(10) This statement refers strictly only to the ratio of $^2H(8)$ of **1** to $^{13}C(8)$ of AMP; see ref 11.

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(12) See ref 4, pp 375–386, and references cited therein.

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