Nuclear Magnetic Resonance Studies on Pyridine Dinucleotides. 6.[1a] Dependence of the ^{13}C Spin–Lattice Relaxation Time of 1-Methylnicotinamide and Nicotinamide Adenine Dinucleotide as a Function of pD and Phosphate Concentration

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Abstract: The spin–lattice relaxation times (T_1) for carbons 6 and 7 (carboxamide) in 1-methylnicotinamide and in the nicotinamide ring of NAD^+ have been determined as a function of pD and phosphate concentration. In the absence of potassium phosphate, the T_1's were found to decrease for NAD^+ but to increase for 1-methylnicotinamide when the pD was increased from 1 to 7. In the presence of phosphate, the T_1's for both compounds decreased with increasing pD. These observations suggest that the interpretation of spin–lattice relaxation times for compounds with ionizable groups when determined at high concentrations cannot be extended to predict relaxation behavior at lower concentrations. It is suggested that solute–solvent interactions may account for the behavior of 1-methylnicotinamide, but for NAD^+, internal mobility prohibits identifying such interactions exclusively. It is evident that T_1's determined under one set of solution conditions cannot be extrapolated to other solutions of differing composition.

The fundamental role of NAD^+ [2] in many biochemical systems has prompted several studies of the NMR parameters of NAD^+ and related molecules in recent years.[3–19] During this time the view of the solution conformational dynamics of these molecules has undergone considerable change. Primarily through the results of recent NOE[3a] and relaxation studies,[4] it has been demonstrated unequivocally that the pyridinium ring in NAD^+ is conformationally mobile with respect to its contiguous ribose with approximately equal populations of the so-called "syn" and "anti" conformers. Studies employing paramagnetic metal ions to probe the conformational dynamics in these systems have led to conclusions which support the conformational mobility of the nicotinamide ring, but disagree as to the relative amounts of syn and anti forms.[4l] The question of whether NAD^+ exists in a folded form and, if so, to what extent, is not an easily resolved matter. Proton relaxation and NOE studies have shown that the pyridinium and adenyl moieties do not reorient as a rate greater than the overall tumbling time of the molecule.[3b,4] Thus, there is a possibility on the molecular time scale that the bases could be proximate to one another for a time period long compared to the molecular tumbling time. A recent estimate of the percent of NAD^+ in the folded form by Hollis[13] is on the order of 10–20%. To "explain" the many observations made with respect to the NMR parameters of NAD^+, a less restrictive description of the folded form is needed. That is, the notion of the folded form need only imply that the two rings be proximate to one another rather than at some definite set of bond angles and distances described as "base stacking".

Current interest in these laboratories in enzyme–cofactor interactions has led to the investigation of the solution conformational dynamics of NAD^+ by longitudinal relaxation (T_1) measurements employing ^1H,[4] ^2H[1b], and ^{13}C[20] NMR. The observed pD dependence of the proton T_1's in the presence of buffer and the lack of such dependence in the absence of buffer led to conclusions at variance with prior studies on the pD dependence of the ^{13}C T_1's.[21] Because high concentrations are necessary for natural abundance ^{13}C NMR as used in these studies[21] and because of the ready availability of ^{13}C-enriched samples from other work in our laboratory, the pD dependence and the influence of phosphate on the ^{13}C T_1's of NAD^+-N(6)-^{13}C and NAD^+-N(7)-^{13}C, as well as 1-methylnicotinamide-6-^{13}C and -7-^{13}C at very low concentrations (10 mM) were examined in more detail.

Concurrent with these studies were several reports on the observed pD dependence of ^{13}C and ^1H T_1's[22,23] in biochemical systems at pD values in the range of 1–7. Subsequent studies have shown the observed minima in T_1 to be due to idiopathic paramagnetic impurities in commercial, "high purity" D_2O.[24,25] This is of particular concern when long T_1's are involved, e.g., carbonyl carbons. The effects of paramagnetic metal ions on T_1's are less severe at low pH (hydrated ions) and high pH (hydroxylated ions).[24] In the limit of long T_1's, paramagnetic impurities may provide the principal route for longitudinal relaxation. To avoid any such alternate paths for relaxation, various methods of sample preparation were also explored.

The use of longitudinal relaxation parameters for the study of complex molecules and their interactions in solution has been introduced only recently[26] and, consequently, little is really understood about their sensitivity to the multitude of effects possible in solution. Although the use of T_1 measurements in the investigation of the mobility of organic molecules in solution is increasing, there have been few systematic studies on the influence of solution conditions such as pH, concentration, buffers, etc., on longitudinal relaxation.[27] Recognizing the possible subtle nature of such solution effects, a study of a simple system, 1-methylnicotinamide, and a complex system, NAD^+, which is in part comprised of a nicotinamide moiety, was undertaken. All variables but two, pD and phosphate concentration, were held constant and the longitudinal relaxation times of the two analogous nicotinamide carbons were measured in both compounds. A comparison of the respective T_1's in the two systems should help to separate those effects which are solution dependent from those which are purely intramolecular in nature.

Experimental Section

Spin–lattice relaxation times were measured either on a Varian XL-100-15/VFT 100 system equipped with a VDM 620i computer and a VDM-36 disk system using the saturation–recovery/homospoil sequence[28] or on a Varian CFT-20 spectrometer with 16K of core and a Sykes Compucord cassette unit using the inversion–recovery sequence.[29] Typically, 2000 data points were acquired and zero filled

Table I. NAD^+-N^7-^{13}C Spin-Lattice Relaxation Times in D_2O at pD 7, 10° [a]

Sample preparation	T_1, s
1 mM EDTA; argon degassed	7.69 ± 0.46
1 mM EDTA; vacuum degassed	7.69 ± 0.46
Chelex 100; vacuum degassed	7.06 ± 0.50

[a] 16 mM NAD^+-N^7-^{13}C; 0.68 M potassium phosphate.

Table II. Experimental Data

Concn, mM	Phosphate concn,[a] M	pD[b]	T_1, s[c]	Method[d]
		1-Methylnicotinamide-6-^{13}C Iodide		
20	NB	1.0	1.12 ± 0.07	I
		3.3	1.15 ± 0.01	I
		7.0	1.19 ± 0.00	I
20	0.3	1.0	1.14 ± 0.04	I
		7.0	1.09 ± 0.02	I
16	0.68	1.0	1.04 ± 0.04	I
		4.0	0.91 ± 0.05	I
		7.0	0.84 ± 0.03	I
		1-Methylnicotinamide-7-^{13}C Iodide		
49	NB	1.0	57 ± 3	II
		7.0	72 ± 3	II
80	0.3	1.0	68 ± 3	II
		7.0	58 ± 3	II
		Nicotinamide Adenine Dinucleotide-N^6-^{13}C		
20	NB	1.0	0.18 ± 0.001	I
		7.0	0.16 ± 0.004	I
20	0.3	1.0	0.18 ± 0.002	I
		7.0	0.14 ± 0.002	I
16	0.68	1.0	0.19 ± 0.007	I
		7.0	0.13 ± 0.001	I
		Nicotinamide Adenine Dinucleotide-N^7-^{13}C		
20	NB	1.0	11.00 ± 0.03	II
		7.0	7.74 ± 0.06	II
20	0.3	1.0	9.84 ± 0.23	II
		7.0	7.52 ± 0.37	II
16	0.68	1.0	15.66 ± 1.00	II
		7.0	8.75 ± 0.88	II

[a] NB; no phosphate present; phosphate when used was potassium phosphate. [b] pD = pH − 0.4.[31] [c] Temperature 10.0 ± 0.5 °C. [d] I, inversion recovery sequence (CFT-20); II, homospoil sequence (XL-100).

to permit 8K transforms to be carried out. Peak areas were measured with a Geotec compensating polar planimeter. The peak areas were fitted to an exponential function with a nonlinear least-squares program on an IBM 370/168 system.[30] The precision of the T_1's reported are typically within 5%, except for the values reported for 1-methylnicotinamide-7-^{13}C. In this case the extremely long T_1's (60–70 s) and the low concentrations employed required extremely long times for data acquisition and a degradation in S/N was accepted in order to keep the time within manageable bounds (30 h). All determinations were done at 10.0 ± 0.5°. The temperature was adjusted using a Hewlett-Packard digital voltmeter and a thermocouple fixed in the center of a sample tube containing 2-propanol. The temperature was checked at the start and at the completion of each determination. The pD was adjusted using a Radiometer Model PHM 63 pH meter on a Corning Model 109 pH meter (pD = pH + 0.4)[31] with DCl or NaOD (Merck) as required. Sufficient time was allowed for equilibration of the sample and the electrode (ca. 15 to 20 min) and the calibration rechecked after adjustment was completed. The pD of the samples were checked after the T_1 determination was completed; no changes were noted.

In order to check for contamination by paramagnetic impurities and to evaluate alternative methods for removing such species, a series of T_1's were measured on 16 mM NAD^+-N(7)-^{13}C in 0.68 M potassium phosphate at pD 7.0, treated as indicated in Table I. The data indicate that solutions treated with Chelex-100 are just as free from the influence of paramagnetic metal ions as solutions containing EDTA. Samples containing 1-methylnicotinamide were treated with Chelex-100 at pH 7 for 0.5 h, pD values adjusted, degassed with five freeze–thaw cycles, and sealed in NMR tubes. The NAD^+ samples were treated with Chelex-100 under similar conditions and N_2 degassed for 0.5 h in the NMR tube, tightly capped and sealed with a tight winding of electrical tape. All glassware was soaked in nitric acid and thoroughly rinsed with glass-distilled water and dried. Caution was exercised to prevent any metallic substances from coming in contact with the glassware or with the samples.

The preparation of the labeled compounds has been reported recently.[32]

Table III. Response of T_1 for Increase in pD from 1 to 7

Carbon[a]	Solution[b]	% change in T_1[c]
C6	NB	+6
C7		+26
N6		−16
N7		−25
C6	0.3 M	−5
C7		−15
N6		−25
N7		−24
C6	0.68 M	−20
N6		−31
N7		−45

[a] C6, 1-Methylnicotinamide-6-^{13}C iodide; C7, 1-Methylnicotinamide-7-^{13}C iodide; N6, NAD^+-N^6-^{13}C; N7, NAD^+-N^7-^{13}C. [b] NB: No phosphate or potassium phosphate at concentration indicated. [c] Percent change equals = $[T_1(\text{pD1}) - T_1(\text{pD7})]/T_1(\text{pD1}) \times 100$.

Table IV. Response of T_1 to Addition of or Change in Concentration of Phosphate at Two pD's

Change in phosphate concn	Carbon[a]	%ΔT_1 pD 1.0	%ΔT_1 pD 7.0
No phosphate–0.3			
	C6	NC[b]	−7
	C7	+20	−19
	N6	NC	−12
	N7	−10	−4
0.3–0.68 M			
	C6	−9	−23
	N6	NC	−6
	N7	+59	+16

[a] Cf. Table III. [b] No change.

Results and Trends

The experimental relaxation times are presented in Table II with the conditions under which each was determined. Tables III and IV, constructed from the data in Table II, illustrate the response of the T_1's to changes in the conditions of the sample solutions, i.e., phosphate concentration and pD. The individual carbons examined manifested a wide range of T_1 values; only the direction and relative percent change are presented, since this serves to indicate the sensitivity of the relaxation time to a change in the sample conditions more effectively than would the absolute differences. For example, to compare the behavior of 1-methylnicotinamide and NAD^+ in 0.3 M phosphate when the pD is changed from 1 to 7, one notes that the T_1 for the corresponding pyridinium carbon 6 in both compounds decreases by 0.05 s. Although there is an order of magnitude difference in the T_1's between the two compounds under these conditions, the small change observed does not

illustrate the relative sensitivity to pD of the two systems. For 1-methylnicotinamide this represents only a 5% decrease in T_1, but for NAD^+ it is a 25% decrease, thus indicating that the T_1 of NAD^+ is considerably more sensitive to pD changes when phosphate is present. Table III shows the percent change in T_1 when the pD is increased from 1 to 7 at the three phosphate concentrations studied: no phosphate, 0.3, and 0.68 M phosphate. Table IV illustrates the response of T_1 at the two pD values examined when phosphate is added to the solution.

When the pD is increased from 1 to 7 (Table III), a decrease in relaxation time beyond experimental error is observed for each carbon in 1-methylnicotinamide and NAD^+ in solutions containing phosphate and for the NAD^+ carbons in a solution devoid of phosphate. Only solutions of 1-methylnicotinamide-*6*-^{13}C and -*7*-^{13}C in the absence of phosphate show an increase in T_1 with increasing pD. In the presence of phosphate, an increase in pD produces a decrease in T_1 in all cases and, as the concentration of phosphate is increased, the T_1's decrease even further. The 1-methylnicotinamide carbons are the most sensitive to the addition of phosphate when the pD is altered (Table III). For example, in the absence of phosphate the T_1's increase with increasing pD, but in the presence of phosphate the opposite behavior is noted.

Table IV shows the effect of altering the phosphate concentration at constant pD. Separate entries are made for the two pD extremes, 1 and 7. At pD 1, the pyridinium carbons in both NAD^+ and 1-methylnicotinamide are not influenced by the addition of phosphate, while at pD 7, there is generally a decrease in T_1 when phosphate is added or the concentration is increased. For NAD^+, the carbonyl carbons appear to be more sensitive to phosphate at pD 1 than at pD 7.

Discussion

Relaxation Mechanisms. The dominant relaxation mechanism for protonated carbons in compounds of moderate molecular weight has been shown to be dipolar.[33,34] For 1-methylnicotinamide-*6*-^{13}C, the NOE was found to be 2.95 at both pD 1 and pD 7 in 0.3 M phosphate, indicating a dipolar mechanism is dominant for this carbon. While the NOE was not determined for all phosphate conditions examined, there is no evidence or reason to suspect a change in relaxation mechanism when the phosphate concentration is altered. For carbonyl carbons, the dipolar mechanism is again dominant but substantially less efficient because of the much greater distances between nuclei. The longer T_1's observed coincide with the absence of any contiguous protons. For the carboxyl carbon in glycine, it has been reported[25] that the dipolar mechanism is predominant, but that other mechanisms, in particular spin rotation, have importance. Intermolecular effects appear to be more efficacious in H_2O than D_2O, at least for the carbonyl carbon of acetone[35] and the carboxyl carbon of glycine.[25] The carboxamide carbon should be somewhere between the carbonyl and carboxyl carbons in behavior, since it is more polar than the carbonyl, but unable to enter into dimers as may the carboxyl. The amide nitrogen of the nicotinamide moiety readily exchanges its protons for deuterium, further diminishing the efficiency of the dipolar mechanism and leading to even longer T_1's than those observed for the protonated species.[25] In any case, it was not anticipated that the contribution from mechanisms other than dipolar would be significant for the carbonyl carbon and this was not further explored.

The pD Dependence of the T_1's of 1-Methylnicotinamide. There is no doubt that the observed relaxation times are dependent upon pD and phosphate concentration. The principal relaxation mechanism for the pyridinium ring carbons in both 1-methylnicotinamide and NAD^+ is dipolar, as established by the full NOE's. The dipolar relaxation rate for carbon is described by the equation

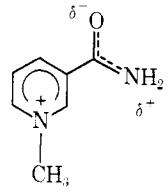
$$R^{DD} = (T_1^{DD})^{-1} = \gamma_H^2\gamma_C^2\hbar^2 \sum_{i=1}^{N} r_{CH_i}^{-6}\tau_c \qquad (1)$$

where γ_C and γ_H are the magnetogyric ratios of carbon and hydrogen, respectively; r_{CH_i} is the interatomic distance between the relaxing carbon and the interacting dipole, and τ_c is the effective reorientational correlation time. From eq 1 it is evident that the distance between the relaxing nucleus and the interacting dipole will be the major influence on the relaxation time because of the inverse 6th power relation. For carbon this is inevitably a contiguous hydrogen when one is present; consequently, carbons without directly bonded hydrogens have much longer T's than carbons with bound hydrogens and contributions from nonbonded protons will be smaller due to the inherently greater distances involved. The aromatic nicotinamide-6 carbon has a proton attached and the possibility that mesomeric effects might be reflected by a change in C–H bond distance, i.e., changing the length of the effective dipole, can be raised. A quick calculation shows that this is not plausible: assuming no change in correlation time and a C–H bond length of 1.00 Å; to account for an increase in T_1 of only 0.07 s, the C–H bond distance would have to increase by 0.025 Å, which is energetically highly unfavorable. Consequently, changes in T_1 can not be explained in terms of a change in C–H bond distances induced by a change in pH alone and, most likely, seldom even in part. To support this conclusion, the J_{CH} for 1-methylnicotinamide was measured at pD 1 and 7 and was found to be independent of pD in the presence of phosphate.

Spin–lattice relaxation is an enthalpy process in which energy is transferred from the spin system to the surroundings, the lattice, here principally comprised of the solvent molecules. The actual transfer of energy occurs via fluctuating magnetic fields arising from molecular motions. These fields have components over a wide range of frequencies, but only those components near the Larmor frequency can interact with the relaxing nucleus. To describe these molecular motions in terms of Brownian motion, a correlation time, τ_c, is introduced,[33] which denotes the average time that two nuclei remain in a given relative orientation with respect to one another.

The rate of relaxation, then, will depend very critically on the microviscosity of the medium and the size, shape, and polarity of both the solute and solvent molecules. These factors are reflected through the correlation time describing the molecular motions. To understand the observed changes in T_1, it is necessary to consider solvent–solute interactions and their effects on molecular motion; i.e., the correlation time. Where solvent effects have been examined,[26] it has been observed that highly polar media (strong solvation) inhibit interionic interactions resulting in augmented motions (longer T_1's). On the other hand, a less polar medium (weak solvation) permits stronger interionic interactions between molecular ions (i.e., ion pairs), which will result in an attenuation of molecular motion and, hence, a shorter T_1.

The increase in T_1 for 1-methylnicotinamide when the pD is raised from 1 to 7 in the absence of phosphate must result from additional molecular mobility and, hence, cause a reduction in the correlation time. The positive charge on the pyridinium nitrogen and the highly polar carboxamide group suggest the possibility of association with solvent molecules. There is evidence from Raman data[36,37] and protein structure studies[38] that the carboxamide group has substantial double bond character between the carbonyl carbon and the amide nitrogen (**1**). At low pD, it would be expected that the carboxamide group would be tightly solvated by the large excess of hydronium ions, producing a more bulky system with its associated longer correlation time. The interaction in neutral D_2O appears to be weaker than the interactions at low pD



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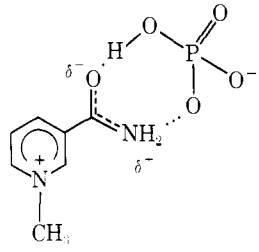
values, as indicated by the longer T_1. It has been reported that there is preferential hydrogen bonding to the carbonyl oxygen of amides rather than other water molecules,[39] thus supporting our contentions of a strong solvent–solute interaction.

If 1-methylnicotinamide reoriented isotropically as a single molecule with no internal degrees of freedom, the observed change in T_1 for both the carbonyl carbon and carbon 6 would be the same. Reference to Table III clearly indicates that this is not the case. Therefore, the carboxamide group must have a substantially different reorientational correlation time than that of the pyridyl ring itself. Hence, the smaller change in T_1 for carbon 6 is in all probability a reflection of the increased solvation of the carboxamide group, which, in turn, alters the overall mobility of the nicotinamide molecule as a whole.

In the presence of phosphate, the T_1's decrease in all cases when the pD is raised, indicating that molecular motion has been constrained. Typically, the presence of exogenous salts disorder solvent systems, a result which should permit increased molecular motion with concomitant longer T_1's. This is observed at pD 1 but not at pD 7, where there is a decrease in T_1 upon addition of phosphate (Table IV). This suggests a specific solvent–solute interaction at pD 7 such as portrayed in **2**, which is not present at pD 1. The large additional bulk



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added to the system by interaction with phosphate would decrease the mobility of 1-methylnicotinamide, resulting in a decrease in T_1.

When the pD is lowered, the phosphate equilibrium shifts in the direction of phosphoric acid. The phosphate solvation effects would then decrease, permitting the 1-methylnicotinamide greater freedom of motion and, hence, a longer T_1. The further addition of phosphate (to 0.68 M) brings the phosphate concentration near saturation. The decrease in T_1 for carbon 6 can now be attributed to an increase in microviscosity as well as a stronger phosphate–carboxamide interaction, which is less effective at pD 1 than at pD 7. Table IV illustrates this point: at pD 1, the addition of phosphate (to 0.3 M) has no effect on carbon 6 and causes only a small decrease in T_1 when raised to 0.68 M. This implies that only a small decrease in overall molecular motion has occurred. The carbonyl carbon, however, shows a large increase in T_1 when phosphate is added at pD 1, indicating a large gain in molecular mobility. Here the effect of the phosphate, predominantly as phosphoric acid, is to disrupt the solvent ordering and to attenuate the hydrogen bonding. In contrast to this behavior at pD 1, the addition of phosphate at pD 7 produces a large decrease in T_1 for the carbonyl due to the phosphate–carboxamide interaction, which is also reflected by a small decrease in the T_1 for carbon 6.

pD Dependence of the T_1's of NAD^+. The effect of solution conditions, i.e., phosphate concentration and pD, on the T_1's of NAD^+ are complicated by the presence of the charged pyrophosphate linkage and the adenine 1-nitrogen ($pK_a = 3.88$), as well as by the fact that NAD^+ can exist in a multiplicity of conformations depending on pD.[3,32] At low pD (below 4) where the adenine 1-nitrogen is protonated, the set of energetically allowed conformations is not the same as that above pD 4. Consequently, the nature of the solute–solvent interactions may undergo drastic changes with changes in pD and, moreover, compensating effects may not be detectable. For instance, one conformational form which is not tightly solvated may have the same T_1 as another form which is tightly solvated. It is now generally accepted that pH is the controlling factor in determining the set of allowed conformations; however, this assumes that the concentration is low enough to avoid significant solute–solute interactions from occurring. To minimize the possibility of these effects, NAD^+ samples at 20 mM were examined where it has been shown previously that solute–solute interactions are small.[14,32]

The data in Table III indicate that the pD dependence of the T_1's of NAD^+ in solutions devoid of phosphate is very much like that in solutions with phosphate, contrary to the behavior of 1-methylnicotinamide under similar conditions. This suggests that NAD^+, via the pyrophosphate linkage and the ionization of the adenine 1-nitrogen, may influence solution structure in a way similar to phosphate.

When the behavior of NAD^+ at constant pD and different phosphate concentrations is examined (Table IV), there is no change in T_1 for carbon 6 when the phosphate concentration is increased from 0 to 0.3 to 0.68 M, paralleling 1-methylnicotinamide and illustrating the insensitivity of carbon 6 of NAD^+ to the presence of phosphate at low pD. At pD 7, however, an overall decrease in T_1 is observed. The lower molecular mobility under these conditions must be due to a more tightly solvated system or an increase in microscopic solution viscosity.

The presence of phosphate causes a decrease in T_1 at both pD 1 and 7 for the carbonyl carbon of NAD^+, with the greater change at pD 1. Since interaction with phosphate is not likely at low pH, this must be a reflection of an increase in microviscosity, which is less severe at pD 7 as indicated by the smaller decrease in T_1. When the phosphate concentration is raised to 0.68 M, the carbonyl T_1's show a large increase in T_1 at both pD's, with the change at pD 1 being much greater than that at pD 7. This indicates a major change in the behavior of the carbonyl carbon at high levels of phosphate and suggests a major change in the solution behavior of the carboxamide group. Since an increase in T_1 indicates an increase in mobility, the conclusion which must be drawn is that some major interaction between the carbonyl and another part of the NAD^+ molecule is disrupted by high levels of phosphate or that a major disordering of the solution surrounding NAD^+ has occurred and, despite the large increase in viscosity, the carbonyl is now more free to move. The latter possibility is preferred since it has been shown[40] that trends in the ^{13}C chemical shifts as a function of pH at 0.68 M phosphate can *not* be accounted for in terms of hydrogen bonding to the carboxamide oxygen.

Conclusion

It is evident that the interpretation of longitudinal relaxation times in terms of molecular dynamics is neither simple nor straightforward. To understand the microdynamic behavior of a molecule in solution will require much greater knowledge of the properties of solutions, and, especialy, how these properties change as the composition of the solution is varied. Certainly it will be very difficult to use or compare T_1 data unless completely identical conditions are employed, although it is conceivable that the presence of buffers at low concen-

trations may have less profound effects. The measurement of T_1's on high concentrations of sample, such as commonly used in natural abundance ^{13}C studies, may introduce aberrations due to intermolecular interactions and thus invalidate extrapolation to lower concentrations. When pH dependent studies are involved, complex changes in solution structure are likely which, in turn, will be reflected by a different behavior of the molecule.

As has been illustrated in this work, the behavior of a complex, nonrigid molecule such NAD^+ may be very different from that of a small molecule such as 1-methylnicotinamide, even though identical solution conditions were maintained.

Acknowledgment. The authors gratefully acknowledge financial support from the American Cancer Society, Grant BC-111, and the National Cancer Institute of the National Institutes of Health through Grant CA 15645, and helpful discussions with Dr. A. P. Zens.

References and Notes

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(2) Abbreviations: NAD^+: nicotinamide adenine dinucleotide; NAD^+-N^6-^{13}C, NAD^+-N^7-^{13}C: nicotinamide adenine dinucleotide with the carbon-13 label in the nicotinamide ring at position 6 or 7 (carbonyl), respectively.
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Optical Properties of Sugars. 3. Circular Dichroism of Aldo- and Ketopyranose Anomers[1a]

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Abstract: Techniques have been developed for measuring the circular dichroism spectrum of an individual sugar anomer. Vacuum ultraviolet spectra are presented for α- and β-D-glucose, α- and β-D-galactose, and α-D-xylose in D_2O to 165 nm. These spectra confirm our original suspicion that it is not meaningful to compare the circular dichroism spectra of sugars at anomeric equilibrium, even when the equilibrium mixtures have the same anomeric composition, because either anomer may contribute the bulk of the intensity to the spectrum of an equilibrium mixture. Instead, spectra of corresponding anomers should be compared. In addition, circular dichroism spectra of three ketopyranoses which do not undergo appreciable anomerization (α-D-sorbose, α-D-tagatose, and α-D-*manno*-heptulose) are presented to 165 nm. Difference circular dichroism spectra reveal similar contributions to the spectra of homomorphic and epimeric pairs when near-neighboring groups are the same. When near-neighboring groups are different, so are the difference circular dichroism spectra demonstrating the sensitivity of the technique to configuration and conformation. The low-energy band in the spectra of the aldohexoses is apparently due to the presence of the hydroxymethyl group. It is suggested that in solution, water structure about the sugar molecule may be more important than minor electrostatic interactions in determining the rotamer population of the hydroxymethyl group.

One expects the diverse biological activities of the carbohydrates and glycoproteins to be mediated by their conformation, in a manner analogous to nucleic acids and proteins. We have begun circular dichroism (CD) studies in the vacuum ultraviolet to try to understand the effects of configuration, sequence, and environment on carbohydrate conformation. In