(17) J. M. Briggs, G. P. Moss, E. W. Randall, and K. D. Sales, *J. Chem. Soc., Chem. Commun.*, 1180–1182 (1972).
(18) Other positions for the lanthanide ion were tried but no solutions to the BURLESK program were found.
(19) No solutions using the C(3')-endo ribose ring conformation could be found.
(20) When averaging conformations it is necessary to average absolute shifts and not shift ratios. For a mixture of n conformations of fractional populations, α_n, the calculated chemical shifts for a proton H_1 is given by

$$\partial H_1 = K\sum_n \alpha_n f\{\theta_n(1), r_n(1)\}$$

where $f\{\theta_n(1), r_n(1)\}$ is the angle and distance term in the pseudocontact equation for proton 1 and for H_2

$$\partial H_2 = K\sum_n \alpha_n f\{\theta_n(2), r_n(2)\}$$

It is possible to compare calculated and experimental shift ratios using these values

$$\frac{\partial H_1}{\partial H_2} = \frac{\sum_n \alpha_n f\{\theta_n(1), r_n(1)\}}{\sum_n \alpha_n f\{\theta_n(2), r_n(2)\}}$$

Nuclear Magnetic Resonance Studies on Pyridine Dinucleotides. II. Solution Conformational Dynamics of Nicotinamide Adenine Dinucleotide and Nicotinamide Mononucleotide as Viewed by Proton T_1 Measurements[1]

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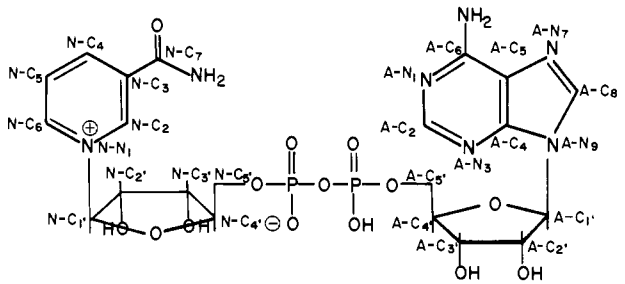
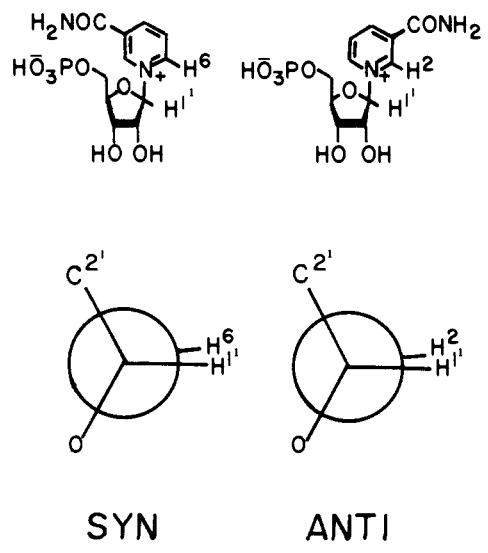
Abstract: The ^1H T_1's of NAD^+ and NMN^+ have been measured as a function of temperature. The measurements demonstrate that these ^1H T_1's are dominated by the dipole–dipole mechanism. The reorientational activation energy extracted from these measurements is 4.6 ± 0.4 kcal/mol for both NAD^+ and NMN^+. Proton and ^{13}C T_1 measurements were used to establish that the pyridyl protons T_1's can be described by a single correlation time, $\tau_c = 2.3 \times 10^{-10}$ sec (NAD^+). Proton T_1 measurements have also been made on NAD^+ deuterated at positions 2 or 6 or 2 and 6 of the pyridyl ring. Using these data and recent X-ray measurements, specific microdynamic models for the motion with NAD^+ can be made and tested. These and other measurements support the notion that the microdynamic motion of the pyridyl ring in NAD^+ can be described by a two-state model involving the rapid interconversion of syn and anti states with approximately equal populations of the syn and anti conformers. The pD dependence of the T_1's for the pyridyl ^1H's is presented. The implications of these data with regard to the "folded" structure for NAD^+ are discussed.

Since the initial ^1H NMR study of NADH[2] reported in 1962 by Mahler and coworkers,[3] there have been many papers published that discuss various aspects of the conformational dynamics of pyridine dinucleotides in solution.[3-25] With the possible exception of two of these papers,[14,20] the current hypothesis describing the solution dynamics of nicotinamide adenine dinucleotide (NAD^+, I) may be summarized in the following manner. NAD^+ is thought to exist in predominately two forms, a folded form in which the two aromatic rings are approximately parallel to each other and an extended form. Furthermore, these species are envisioned to undergo a rapid (on the NMR chemical shift time scale) interconversion. The equilibrium constant describing this process is pH dependent. At pH values greater than 4, the equilibrium is shifted in such a way that the folded form becomes more prevalent. Various estimates of the percent folded species have been made. Jardetzky,[4] using thermodynamic parameters, concluded that 20–40% of NAD^+ was folded at neutral pH and room temperature. This conclusion has been further supported by chemical evidence put forth by Oppenheimer et al.[15] from the unequal addition of cyanide to the A and B side of the pyridine ring. However, a more recent thermodynamic study by Hollis and coworkers[16] has concluded that only 15% of the folded conformer exists at any one time. For pH values less than 4, the equilibrium is such that the extended form predominates in solution. The pH dependence of the ^1H NMR of NADH and NADPH has been characterized.[4] However, because of the inherent instability of these compounds in acidic media, these systems have been primarily examined at pH levels of 7 or higher. At elevated values of pH these dinucleotides are believed to exist in the folded form.[10-13,15] The evidence used to support this conclusion is based on the appearance of an AB quartet (at 220 MHz) for the N-H_4 hydrogens of the nicotinamide portion of these dinucleotides.



I

The validity of the preceding conclusions rests on the implicit assumption that in the folded form the overall reorientational motion (tumbling) of the dinucleotide is fast compared to the internal rotation of the bases with respect to their contiguous sugars. Furthermore, it has been reported by Sarma and Kaplan[10-13] and more recently by Sarma and Mynott[22,24] that in nicotinamide mononucleotide (NMN^+, II) the nicotinamide base must be in the syn conformation with respect to its contiguous ribose. That is, the dihedral



II

angle subtended by N-C_6 and N-H_1' is approximately zero.[26]

This assertion and the preceding assumption have recently been questioned by Jacobus and coworkers.[14,20] If NAD^+, or even NMN^+, existed exclusively or nearly exclusively in a conformer which is in the syn form, then certain predications can be made concerning the magnitude of the homonuclear NOE[27] between N-H_1', N-H_2, and N-H_6. If the molecule was exclusively in the syn conformer, this would lead to the prediction that irradiation at N-H_6 would cause a large enhancement at N-H_1' relative to the enhancement which would be observed at N-H_1' caused by irradiation at N-H_2'. These authors found for NMN^+, however, that the observed enhancements were nearly equal and concluded that an exclusive, or even highly preferential, population of the syn geometry in NMN^+ is untenable. It should be noted that this conclusion was weakened because the authors were unable to predict their results due to the paucity of X-ray data giving the geometry of the ribose or nicotinamide rings.

In this study ^1H spin-lattice relaxation times are used to describe the conformational dynamics of NAD^+ in solution. In particular, T_1 experiments were designed to yield information concerning the relative populations of the syn and anti conformers of the pyridyl ring with respect to its contiguous ribose. Furthermore, a microdynamic model of NAD^+ is formulated which follows the observed syn/anti ratio.

Experimental Section

NMR Measurements. Proton spin-lattice relaxation time measurements were made on a Varian XL-100-15 NMR spectrometer operating at 100 MHz equipped with a 16K (1K = 1024 words) 620I computer with a 2 million word disk (VDM-36). The Freeman-Hill[28] inversion recovery pulse sequence was used to determine the ^1H T_1's. All samples of NAD^+ (0.005 *M*) and NMN^+ (0.005 *M*) were O_2 degassed in potassium phosphate (0.68 *M*) buffer at a pD of 7. The temperature was measured with the use of a thermocouple before and after the T_1 experiments. A given experiment was considered valid only if the temperature did not vary by more than ±0.5°.

Each τ value was a result of 100 accumulations with an acquisition time of 4 sec and a pulse delay of at least $5T_1$. To obtain the optimum reproducibility (typically less than 5% error) in the ^1H T_1 measurements, each resonance was read-out on a 50-Hz sweep width, and its area was determined with a Geotec compensating polar planimeter. For the N-H_1' and N-H_5 resonances peak heights were used to determine the T_1's due to the overlap of the resonances with other peaks in the spectrum. The order of reproducibility here was typically between 5 and 7%. The T_1 was then determined using standard least-squares techniques.

Materials and Sample Preparation. NMN^+ and NAD^+ used in these experiments were purchased from P-L Biochemicals and were used without further purification. The 99.8 and 100% D_2O were purchased from Mallinkrodt and Aldrich Chemical Co., respectively. Pig brain acetone powder NAD^+ glycohydrolase was obtained from Sigma Chemical Co.

To minimize the possibility of paramagnetic impurities in the T_1 experiments, all the glassware (NMR tubes, glass spatulas, storage containers, etc.) used in these experiments were immersed in concentrated nitric acid for at least 2 hr and then rinsed several times with glass distilled H_2O. At no time, in sample preparation, were metallic substances allowed to come in contact with the sample or solutions. The pD of the solutions was adjusted to 7 with NaOD prepared in our laboratory. Metallic sodium was distilled twice under vacuum; then the sodium was decomposed under a N_2 atmosphere with 99.8% D_2O. The pD adjusted solutions were lyophilized twice from 99.8% D_2O and then three additional times from 100% D_2O. The sample was then degassed via five freeze-pump-thaw cycles.

[2-^2H]Nicotinic Acid. 2-Chloronicotinic acid (Aldrich) was lyophilized three times from 99.8% D_2O in the presence of an equal amount of 5% paladium on charcoal. The dry material was then reduced with deuterium gas at STP with 99% trifluoroacetic acid-d_1 being used as a solvent. When the uptake of deuterium gas had ceased (10–12 hr) the reaction mixture was filtered and made basic. Proton NMR analysis indicated 95–98% [2-^2H]nicotinic acid.

Methyl 2-Deuterionicotinate. The 2-deuterionicotinic acid was dissolved in methanolic sulfuric acid (10:1) and heated under reflux for 16 hr. The solution was cooled, concentrated to one-half its volume, and neutralized with Na_2CO_3 (10% aqueous). This mixture was extracted with CH_2Cl_2 and the organic extracts were dried (Na_2SO_4), concentrated in vacuo, and distilled affording methyl 2-deuterionicotinate [80%, from the acid, bp 120° (14 mm)].

[6-^2H]Nicotinamide.[29] A suspension of deuterio-5-carbomethoxypyridine-2-carboxylic acid[30] (which had been exchanged three times with 30-ml portions of 99.8% deuterium oxide) in dry anisole (75 ml) was mixed with 10 g of 100% deuterium oxide. The D_2O was distilled into a Dean-Stark trap under a stream of nitrogen and the resulting anisole solution was maintained at reflux for 18 hr. After the volume was reduced to ~5 ml by distillation through a Holzmann column, the residue was distilled at 25 mmHg (bulb to bulb) at 100–145° giving a clear, colorless solution of methyl [6-^2H]nicotinate in anisole.

The solution of methyl nicotinate in anisole was mixed with 150 ml of water and cooled in an ice bath. Ammonia was bubbled through the mixture for 6 hr at 0° and then for 12 hr at room temperature. Ether (50 ml) was added and the layers were separated. The ethereal solution was extracted with water (25 ml). The combined aqueous portions were lyophilized leaving nicotinamide as a white solid: mp 128–130° (lit. mp 129.5–130.5°); ^1H NMR (D_2O) δ 7.50 (dd, 1, J_{45} = 8.2 Hz, J_{25} = 0.9 Hz, C_5H), 8.13 (dd, 1 H, J_{24} = 2.2 Hz, J_{45} = 8.2 Hz, C_4H), 8.78 (dd, 1 H, J_{24} = 2.2 Hz, J_{25} = 0.9 Hz, C_2H).

2-Deuterionicotinamide. Methyl 2-deuterionicotinate was converted to 2-deuterionicotinamide as described above.

[2,6-^2H]Nicotinic Acid. 2-Chloronicotinic acid (Aldrich) was lyophilized three times from 99.9% D_2O in equal proportion with a 5% mixture of palladium on carbonate (0.5 g). The lyophilized material was then reduced in 10 ml of 99.8% D_2O with deuterium gas at room temperature and pressure. When no further uptake of deuterium gas was evident (4–6 hr), the reaction mixture was filtered, and the aqueous filtrate was analyzed. Analysis by NMR revealed the product to be [2,6-^2H]nicotinic acid. The spectrum consisted of two sharp doublets corresponding to the N-H_4 and N-H_5 proton resonances. In addition, each doublet showed a weak single line at its center indicating that a small amount of deuterium incorporation had occurred in the N-H_4 and N-H_5 position of the pyridyl ring. The yield was quantitative. This acid was converted to the amide as described above.

Deuterated Nicotinamide Adenine Dinucleotide. The deuterated

Table I. Spin-Lattice Relaxation Times for the Pyridyl Protons in NAD^+, [NH_2-^2H]-NAD, [NH_6-^2H]-NAD, [NH_2,NH_6-^2H]-NAD^+, and NMN^+[a]

Solutions[b]	N-H_2	N-H_4	N-H_5	N-H_6	N-H_1·
NAD^+ at pD 7.0	0.41	0.66	0.40	0.28	0.32
NAD^+ at pD 7.0[c]	0.41	0.67	0.38	0.27	0.34
NAD^+ at pD 2.0[c]	0.51	0.95	0.48	0.40	
NAD^+ at pD 7.0[d]	0.60	1.01	0.58	0.42	
NAD^+ at pD 2.0[d]	0.65	1.14	0.61	0.48	
[N-H_2-^2H]-NAD^+ at pD 7.0					0.52
[N-H_6-^2H]-NAD^+ at pD 7.0	0.41	0.67	0.70		0.42
[N-H_2,N-H_6-^2H]-NAD^+ at pD 7.0					0.75
NMN^+ at pD 7.0[c]	0.65	1.00	0.55	0.44	0.40
NMN^+ at pD 7.0	0.64	0.99	0.55	0.43	0.41

[a] All values are in seconds and the experimental error is approximately ±5% except for the NH_1· whose error is less than ±10%. [b] Each solution was 5 m*M* in NAD^+ or NMN^+, 0.68 *M* in phosphate buffer and, unless stated otherwise, 10^{-4} EDTA. The sample temperature was 5.5°. For further experimental details, see the Experimental Section. [c] EDTA was not added to these solutions. [d] EDTA and the phosphate were not added to these solutions.

Table II. Predicted Differences in the N-1' Relaxation Rates and Spin-Lattice Relaxation Times Compared to the Measured Spin-Lattice Relaxation Times for Various Isotopically Substituted NAD^+ Molecules

Conformation	Predicted GEOM I[a]	Predicted GEOM II[a]	Predicted GEOM III[a]	T_1(N-H_1·)[b]	Exptl T_1(N-H_1·)[b]
			[N-H_6-^2H]-NAD^+		
Pure syn	1.4084	1.2886	1.5409	0.596 ± 0.04	0.420 ± 0.04
Pure anti	0.0796	0.0757	0.0838	0.332 ± 0.001	0.420 ± 0.04
Free rotor	0.4741	0.4384	0.5132	0.383 ± 0.005	0.420 ± 0.04
Two state	0.6978	0.6255	0.7091	0.419 ± 0.007	0.420 ± 0.04
			[N-H_2-^2H]-NAD^+		
Pure syn	0.0883	0.0840	0.0928	0.334 ± 0.001	0.515 ± 0.05
Pure anti	1.6389	1.5098	1.7874	0.691 ± 0.068	0.515 ± 0.05
Free rotor	0.5443	0.5049	0.5888	0.393 ± 0.005	0.515 ± 0.05
Two state	0.8084	0.7449	0.8890	0.439 ± 0.014	0.515 ± 0.05
			[N-H_2,N-H_6-^2H]-NAD^+		
Pure syn	1.4967	1.3726	1.6337	0.629 ± 0.052	0.750 ± 0.08
Pure anti	1.7185	1.5855	1.8712	0.731 ± 0.078	0.750 ± 0.08
Free rotor	1.0184	0.9433	1.102	0.484 ± 0.018	0.750 ± 0.08
Two state	1.5062	1.3704	1.5981	0.633 ± 0.045	0.750 ± 0.08

[a] These numbers are the difference $1/T_1$(N-H_1· natural) − $1/T_1$(N-H_1· deuterated) for a given geometry (see text). The units are in sec^{-1}. [b] Units are in seconds.

nicotinamide analog of NAD^+ was prepared using the NAD^+ glycohydrolase exchange method described by Kaplan and Ciotti.[31] This method was modified in that thionicotinamide adenine dinucleotide was used as the starting material instead of NAD^+ and twice the units of NAD^+ glycohydrolase were employed. The purification was effected on a Dowex-1 formate column as described by Stein et al.[32] Thionicotinamide adenine dinucleotide was prepared by the method of Stein et al.[32]

Results

The measured values of the spin-lattice relaxation times (T_1) for the pyridyl ^1H's in NAD^+ and NMN^+ are summarized in Tables I and II. Each T_1 is the average of two determinations per tube for two tubes. To check against the possibility of paramagnetic impurities influencing the measured T_1's, samples of NAD^+ and NMN^+ were run in the presence of 10^{-4} *M* EDTA. For the data reported here the values of T_1 were independent of the added EDTA, and, hence, there were no paramagnetic impurities in the samples employed.

Presented in Figure 1 is a typical experimental inversion recovery plot for ^1H's N-H_2 and N-H_6 for NMN^+ at 12°. The experimental parameters are summarized in Figure 1. Figure 2 demonstrates the relative precision of a given series of T_1 experiments for N-H_2 in NAD^+ for four temperatures, 10, 27, 42, and 60°. The linearity of these plots also demonstrates that the amount of cross relaxation in this system is not sufficient to prevent precise T_1 measurements. In Figures 3 and 4 the temperature dependence of the T_1's for ^1H's N-H_2 and N-H_6 is presented for NMN^+ and NAD^+, respectively. The reported slopes are the result of standard least-squares treatments. The error bars are used to denote the maximum error (approximately 5%) in these measurements.

Discussion

Mechanisms and Correlation Times. For a nonquadrupolar nucleus, T_1^{-1} is given by

$$T_1^{-1} = T_1^{DD^{-1}}(\text{intra}) + T_1^{DD^{-1}}(\text{inter}) + T_1^{SR^{-1}} + T_1^{CSA^{-1}} + T_1^{SC^{-1}} \quad (1)$$

Here, $T_1^{DD^{-1}}$(intra), $T_1^{DD^{-1}}$(inter), $T_1^{SR^{-1}}$, $T_1^{CSA^{-1}}$, and $T_1^{SC^{-1}}$ denote the intramolecular dipole–dipole, intermolecular dipole–dipole, spin rotation, chemical shift anisotropy, and scalar coupling contributions to the observed value of T_1^{-1}, respectively. For low concentration samples containing ^1H's in oxygen degassed ^2H_2O solutions, $T_1^{-1}(i)$ for spin i is usually given by[33]

$$T_1^{-1}(i) = T_1^{DD^{-1}}(i, \text{intra}) + T_1^{SR^{-1}}(i) \quad (2)$$

The relative importance of $T_1^{SR}(i)$ to the overall value of $T_1(i)$ can be determined from the temperature dependence of $T_1(i)$. We will, at this point, treat only the dipole–dipole term and show subsequently that $T_1^{SR^{-1}}(i)$ is unimportant over the temperature range employed in this study. The dipole–dipole contribution to $T_1^{-1}(i)$ for ^1H_i is given by

$$T_1^{DD^{-1}}(i) = \tfrac{3}{2}\gamma_H^4\hbar^2\sum_j r_{H_iH_j}^{-6}\tau_{c_{ij}} \quad (3)$$

where γ_H is the magnetogyric ratio for ^1H, $r_{H_iH_j}$ is the distance between the proton of interest (i) and proton (j), and τ_{cij} is the reorientational correlation time which "best" describes the motion of all of the $r_{H_iH_j}$ vectors which contribute significantly to the value of $T_1(i)$.

Since molecular motion (via rotations and translation) is responsible for the magnitude of the relaxation time, studies of the solution dynamics of biological systems by NMR can be greatly facilitated by the use of T_1 measurements, due to the enhanced time resolution.[34] The time resolution of chemical shift measurements is, at best, about 10^{-3} sec, whereas the time resolution for the T_1 measurements is only limited by the magnitude of the correlation time for the system of interest (τ_c, 10^{-7}–10^{-12} sec). Although there are obvious advantages in T_1 experiments with respect to time scales, there is a potential ambiguity with regard to the definition of τ_c for ^1H T_1 measurements.

For nuclei which possess octahedral or tetrahedral symmetry in solution, a single correlation time describing this motion can be rigorously defined. However, for nuclei of lower symmetry, which can undergo anisotropic motion, the correlation time must be considered a tensor quantity which, in turn, can have its average value described by the trace of the tensor. Depending on the degree of anisotropy in the motion, one may be able to define a localized correlation time, which is adequate to describe the motion in certain regions of the molecule. Therefore, before applying eq 3 to molecules as complex as NAD^+, certain assumptions must be made about the internal motion within NAD^+.

The discussion of the correlation times will focus on the pyridyl ring portion of NAD^+ and NMN^+. To test the validity of the notion of a localized correlation time, we need

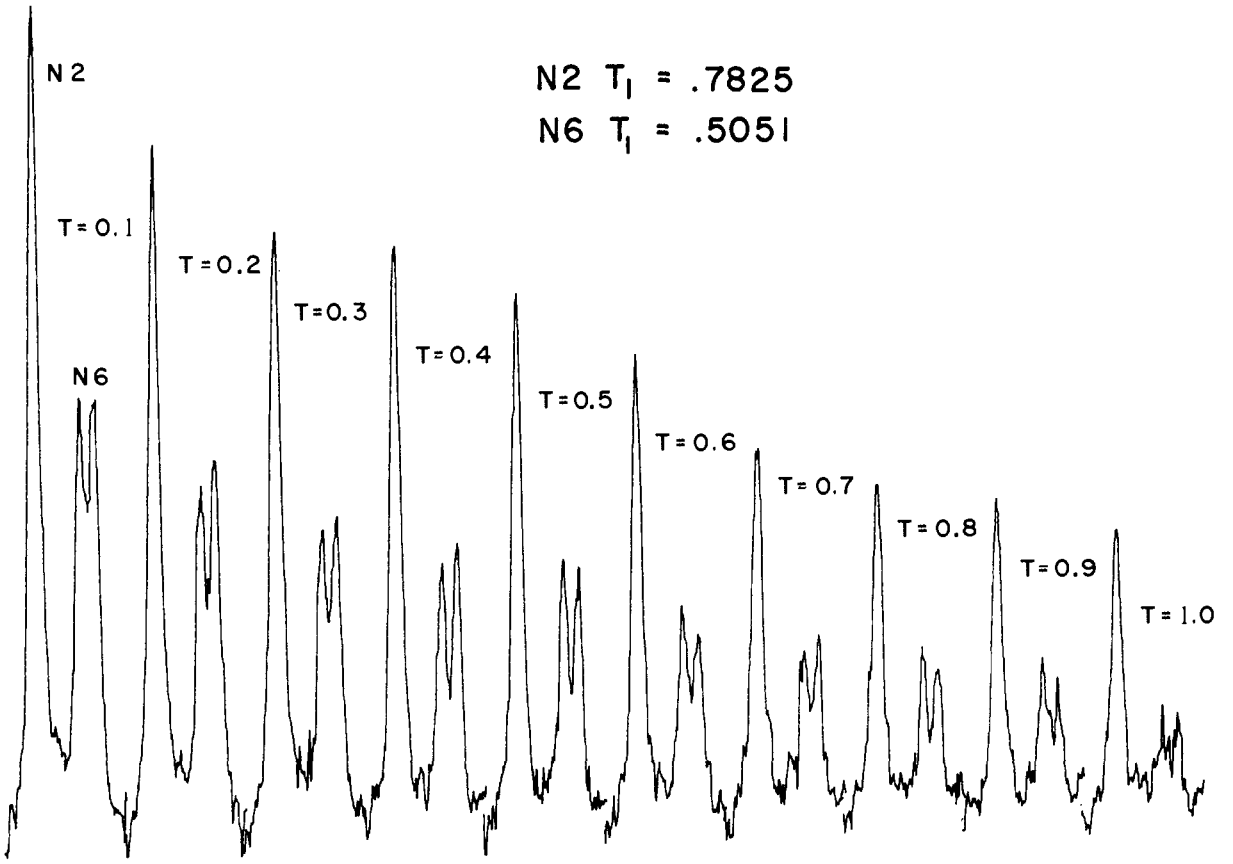


Figure 1. Typical example of a Freeman–Hill[28] inversion recovery pulse sequence on NMN^+ at 12°. The τ values are given in the figure.

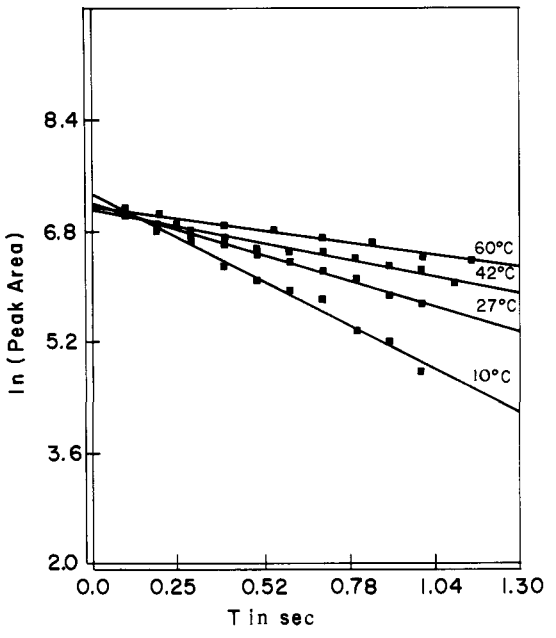


Figure 2. An illustrative example of the relative precision in the T_1 experiments discussed here. The linearity of these plots also demonstrates that over the range of τ values employed here that the amount of cross relaxation was not sufficient to prevent the precise determination of a T_1.

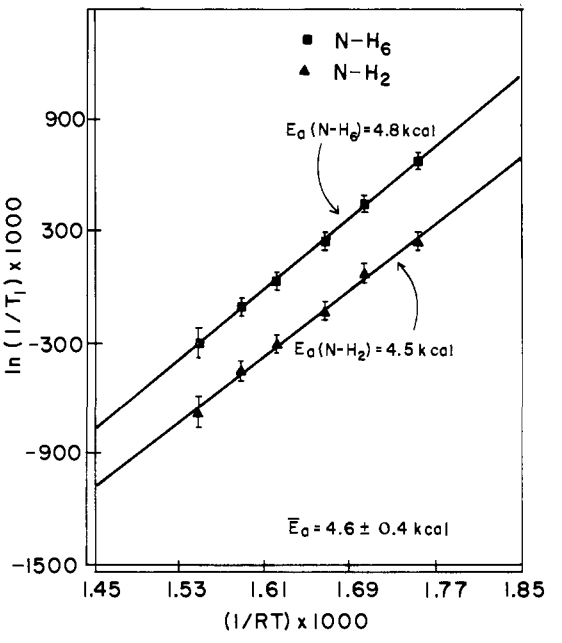


Figure 3. An Arrhenius plot for the relaxation rate for hydrogens N-H_6 and N-H_2 in NMN^+.

only consider two extremes in the molecular motion: (1) the overall motion of NAD^+ or NMN^+ is fast compared to the rotation of the pyridyl ring with respect to its contiguous sugar, and (2) the rotational motion of the pyridyl ring is fast compared to the overall motion of either NAD^+ or NMN^+. From the number of cross-ring $r_{H_iH_j}$ vectors which can contribute to the relaxation of ^1H_i and the fact that the pyridyl ring is a rigid ring system, it seems reasonable that in either extreme, each proton of the pyridyl ring can be described by a single correlation time τ_c. In this case τ_c will represent the overall motion of the set of all $r_{H_iH_j}$ vectors which contribute to the T_1 for ^1H_i.

This assumption can be tested by measuring the correlation time from two independent experiments. These experi-

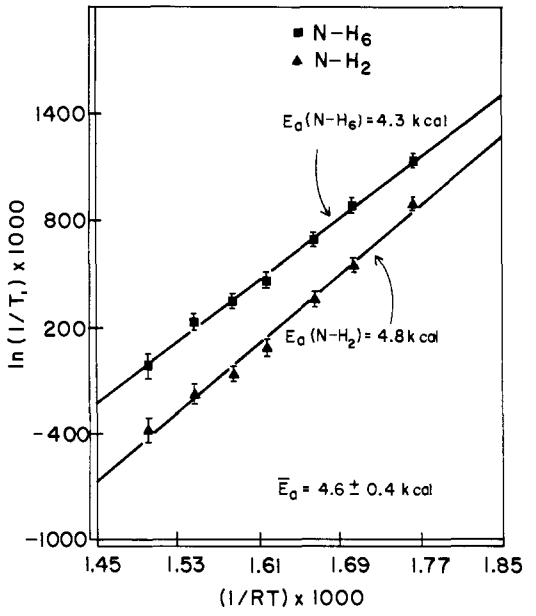


Figure 4. An Arrhenius plot for the relaxation rate for hydrogens N-H_6 and N-H_2 in NAD^+.

ments involve the measurements of ^1H and ^{13}C T_1's. The first experiment involves examination of the ratio of T_1 for N-H_4 [$T_1(4)$] to the T_1 for N-H_5 [$T_1(5)$] in NMN^+ and NAD^+, i.e., from eq 3 we have

$$T_1(4)/T_1(5) = \sum_j r_{H_5H_j}^{-6} / \sum_j r_{H_4H_j}^{-6} \quad (4)$$

Using the H–H distances extracted from the recent X-ray data on NAD^+ model compounds by I. C. Paul and coworkers,[35-37] the ratio can be calculated. The calculated ratio is 1.78 compared to the experimental ratio of 1.82 for NMN^+ and 1.76 for NAD^+. The excellent agreement between the calculated results and experimental data strongly supports the notion of using a single correlation time to describe the pyridyl ring ^1H T_1's. Furthermore, it demonstrates that the dominant mechanism for spin-lattice relaxation of the pyridyl ^1H's is the dipole–dipole process. A summary of the ^1H spin-lattice relaxation times of NAD^+ and NMN^+ is presented in Table I.

With knowledge that we can apparently use a single correlation time to describe the T_1's for the pyridyl ^1H's, eq 3 can be employed to calculate τ_c. From the data in Table I for protons N-H_4 and N-H_5 we calculate τ_c to be approxiately 2.3×10^{-10} sec for NAD^+ and 1.5×10^{-10} sec for NMN^+. A correlation time of this magnitude is representative of molecules in the extreme narrowing region, $\tau^2\omega^2 \ll 1$. For ^1H at 100 MHz, this condition is just met. It should be noted that at higher frequencies this condition, in all probability, would not be met.

However, this calculated value for τ_c can be checked from an independent ^{13}C T_1 experiment. From NOE measurements on 5 m*M* solutions of ^{13}C-enriched NAD^+, at N-C_6, it may be concluded that the ^{13}C T_1 for N-C_6 was dominated by the dipole-dipole mechanism, i.e.

$$T_1^{-1} = (\hbar^2\gamma_C^2\gamma_H^2/r_{CH,}^6)\tau_c \quad (5)$$

In eq 5, τ_c is the same reorientational correlation time used to describe the motion of H-H vectors in eq 3. From known X-ray geometries and a knowledge of the ^{13}C T_1 we can extract a value of τ_c from eq 5. The value obtained from such analysis is 1.9×10^{-10} sec. Furthermore, the same value of the ^{13}C T_1 was obtained in two different buffer systems, 0.68 and 0.24 *M* HPO_4^{-2}. These results seem to indicate there is not a strong dependence of τ_c on the buffer concentration. Considering the error in the two T_1 experiments and the X-ray measurement of r_{CH}, the values are in excellent agreement. Hence, one can use a single effective correlation time to describe the ^1H T_1's in the pyridyl ring of NMN^+ and NAD^+.

By investigating the temperature dependence of the ^1H T_1's, one can experimentally test the assertion that the ^1H T_1's in NMN^+ and NAD^+ are dominated by the dipole–dipole mechanism. Furthermore, such an experiment allows the determination of the reorientational activation energy for the motion described by τ_c. The temperature dependence of the correlation time, τ_c, can be expressed in the following form[38]

$$\tau_c = \tau_0 \exp[E_a/RT] \quad (6)$$

where E_a represents the reorientational activation energy. Hence, from eq 3, a plot of ln $[1/T_1(i)]$ vs. $1/RT$ should yield a straight line with slope equal to E_a, if T_1 is dominated by dipole–dipole processes. The results of the temperature dependence of the T_1 for N-H_2 and N-H_6 for NAD^+ and NMN^+ are summarized in Figures 3 and 4, respectively. These plots clearly indicate that the T_1's for these ^1H's are dominated by the dipole–dipole mechanism. Similar behavior is observed for the remaining pyridyl hydrogens.

It is interesting to note that the activation energies derived from the plots summarized in Figures 3 and 4 are equal, $E_a = 4.6 \pm 0.04$ kcal/mol, for NAD^+ and NMN^+. The value of E_a is composed of at least two terms: the energy required to change solution structure upon reorientation and the component of motion which reflects the notation of N-H_2 and N-H_6 about the base to ribose linkage. From solution physical chemistry considerations for molecules as large as NAD^+ and NMN^+, it is expected that energy required to change the solution structure upon reorientation would be equal for these systems.[39] Furthermore, whatever the barrier to rotation is for the base to rotate against the ribose, one would expect that the value would be approximately the same in both NMN^+ and NAD^+.

Pyridyl Base to Ribose Conformational Dynamics. The use of spin-lattice relaxation times provides a unique opportunity to study the conformational dynamics of a base with respect to its contiguous ribose. By conformational dynamics we mean an approximate knowledge of the spatial relationships of the minimum number of pairs of atoms needed to establish the conformation as a function of time. The conformational dynamics for most monocyclic mononucleotides or dinucleotides can be specified by varying amounts of the syn and anti conformation. For molecules of this type, one can establish the conformational dynamics by following only one pair of atoms. The atoms of choice (for ^1H T_1 measurements) are the anomeric hydrogen (1′) and either hydrogen 2 or 6 on the contiguous base. Equation 3 provides the basis for this new technique.

Consider the expanded form of eq 3, where *i* is the anomeric proton, N-$H_{1'}$, i.e.

$$T_1^{-1}(\text{N-H}_{1'}) = \tfrac{3}{2}\gamma_H^4\hbar^2[\langle r_{1'2}^{-6}\rangle\tau_c + \langle r_{1'6}^{-6}\rangle\tau_c + \langle r_{1'2'}^{-6}\rangle\tau_c' + \langle r_{1'3'}^{-6}\rangle\tau_c'' + \ldots] \quad (7)$$

In expanding eq 3 we have used the concept of a single correlation time for any vector connecting a spin on the ribose to a spin on the base. The value of the correlation time is the same as the correlation time describing base–base vectors. The use of this correlation time implies that the ring rotationl motion with respect to its contiguous ribose is not significantly faster than the overall motion of the molecule. It is likely that this rotational motion is, in fact, slower than the overall motion.[40] Hence, it appears that this portion of the NAD^+ molecule reorientates isotropically. The notation $\langle r_{ij}^{-6}\rangle$ denotes the "conformationally averaged" value for

r_{ij}^{-6}. To focus selectively on particular $1'$ j pairs, one need only to measure the spin-lattice relaxation of N-$H_{1'}$ for molecules with atom(s) j specifically deuterated. For example, if one measured the T_1 for $NH_{1'}$ in [N-H_2-^{2}H]-NAD$^+$ and then subtracted that result from eq 7, one would obtain

$$1/T_1(\text{N-H}_{1'}\text{ natural}) - 1/T_1(\text{N-H}_{1'}\text{ deuterated}) = {}^3/_2\gamma_H{}^4\hbar^2\langle r_{1'2}{}^{-6}\rangle\tau_c - {}^8/_3\gamma_D{}^2\gamma_H{}^2\hbar^2\langle r_{1'2}{}^{-6}\rangle\tau_c \quad (8)$$

The second term in eq 8 arises because the ^{2}H can also provide, an albeit significantly reduced, dipole-dipole contribution when the $r_{1'j}$ distance is at a minimum. Generally, this contribution can be assumed not to be significant at other distances. Equation 8 now allows for a direct determination of the relative importance of atom j to the T_1 of N-$H_{1'}$. Furthermore, if one also has a value of τ_c and an X-ray geometry (or a reasonable estimate), eq 8 can be used to predict the importance of atom j to the relaxation of N-$H_{1'}$ under various models for the conformational dynamics. Therefore, this method can be used to formulate microdynamic models for the base to contiguous ribose motion. The conformational models for the pyridyl ring to be tested here are pure syn, pure anti, a free rotor, and a librating two-state model. Furthermore, this method can be extended to determine the stereochemistry of enzyme substrate complexes. Work on this facet of the technique will be reported later.[41]

In order to study the conformational dynamics of the pyridyl base with respect to its contiguous ribose, we have prepared the deuterated forms of NAD$^+$ and measured the T_1 for N-$H_{1'}$ in [N-H_2-^{2}H]-NAD$^+$, [N-H_6-^{2}H]-NAD$^+$, and [N-H_2,N-H_6-^{2}H]-NAD$^+$. The results of these experiments are summarized in Tables I and II. Furthermore, with the aid the structural models for NAD$^+$ provided by I. C. Paul and coworkers,[35-37] and the correlation time measurements discussed previously, we have been able to predict the relative importance of these atoms to the relaxation of N-$H_{1'}$. These calculations were done using four conformational averaging schemes and three geometries. The results of these predictions are presented in Table II. In addition to the geometry of the pyridyl ring used by Paul et al., we considered the possibility of an error in CCH angles used to define hydrogens N-H_2 and N-H_6. This error was estimated to be ±2.0°. This was done to establish the sensitivity of the predicted results to the input geometries. The "normal" geometry is denoted by GEOM I. The geometry where N-H_2 and N-H_6 have been moved closer to N-C_5 and N-C_3, respectively, by 2° is referred to as GEOM II, and where the hydrogens have moved closer to N-1 by 2° is represented by GEOM III.

If the pyridyl ring within NAD$^+$ is predominantly syn, as has been discussed by previous workers,[22,24] or if the ring is predominantly anti, definite prediction can be made concerning these assertions from the T_1 measurements. If the pyridyl ring is predominantly syn, then one would predict that there would be a large change in the T_1 for N-$H_{1'}$ in going from isotopically natural NAD$^+$ to [N-H_6-^{2}H]-NAD$^+$. Similarly, one would also predict that there would be little or no change in the T_1 for N-$H_{1'}$ in going from isotopically natural NAD$^+$ to [N-H_2-^{2}H]-NAD$^+$. These predictions are summarized in Table II; however, they do not correspond with the experimental data. That is, being predominantly syn leads to the prediction that the T_1 for N-$H_{1'}$ would be 0.596 and 0.334 sec for [N-H_6-^{2}H]-NAD$^+$ and [N-H_2-^{2}H]-NAD$^+$, respectively. The experimentally determined T_1's are 0.420 and 0.515 sec, respectively. Hence, the notion that the pyridyl ring within NAD$^+$ is predominantly or exclusively syn is not consistent with the experimental data. Similarly, the data indicate that the pyridyl ring cannot be exclusively in the anti conformation.

Another alternative to the microdynamic behavior of the pyridyl ring is that it is a free rotor, i.e., all states (or positions) of the ring are equally probable with respect to its contiguous ribose. Such a model leads to the prediction that in going from isotopically natural NAD$^+$ to [N-H_2-^{2}H]-NAD$^+$ to [N-H_6-^{2}H]-NAD$^+$ to [N-H_2,N-H_6-^{2}H]-NAD$^+$ that the T_1 for $NH_{1'}$ would change in the following manner, 0.383, 0.393, and 0.484 sec, respectively. Whereas, experimentally we find 0.420, 0.515, and 0.750 sec, respectively. Therefore, one must conclude that this model is also *not* consistent with the experimental observations.

Yet another model, which perhaps is more reasonable, is that the pyridyl ring librates about either the syn or anti conformation and then jumps over a small barrier to the other conformation. Such a system could be called a librating two-state model. If we assume that the populations of the syn and anti conformations are nearly equal, then upon going from isotopically normal NAD$^+$ to [N-H_6-^{2}H]-NAD$^+$, we predict that the T_1 of N-$H_{1'}$ would become 0.419 sec. Experimentally the T_1 for N-$H_{1'}$ in [N-H_6-^{2}H]-NAD$^+$ is 0.420 ± 0.04 sec. The agreement is encouraging. Similar arguments for the [N-H_2-^{2}H]-NAD$^+$ yield the prediction that the T_1 for N-$H_{1'}$ would change to 0.439 sec. The experimental value for N-$H_{1'}$ in [N-H_2-^{2}H]-NAD$^+$ is 0.515 ± 0.05 sec. The difference between experiment and theory is just outside the experimental error. This difference may be due in part to a slight preference of the anti conformer as opposed to the syn.[42] However, the combination of our experimental error and the uncertainty in the X-ray data does not allow for an unambiguous conclusion at this time. These data, in total, do support the conclusion that the pyridyl ring within NAD$^+$ is librating between two (syn and anti) with *nearly* equal populations. Furthermore, if the populations are not equal, the difference would favor the anti form slightly.

pD Dependence of the ^{1}H Spin-Lattice Relaxation Times of NAD$^+$. It is generally agreed that NAD$^+$ undergoes a conformational change when the pD is lowered from a value of 7 to below 4. A more detailed discussion of the origin of the conformational change will be presented in the next section. This conformational change could be used to gain more insight into the various kinds of motion that are described by the reorientational correlation time, τ_c, for the pyridyl protons.

There are at least two ways that the ^{1}H T_1's can be pD dependent. If the τ_c, measured from the pyridyl protons, represents the correlation time for reorientation of the molecule as a whole *and* NAD$^+$ undergoes a significant change in its molecular shape, then the ^{1}H T_1's will be pD dependent. The pD dependence could also arise via a change in the solution structure surrounding the NAD$^+$ molecule.

The data summarizing the pD dependence of the ^{1}H T_1's are presented in Table I. The ^{1}H T_1's were determined in two different solutions: one with phosphate buffer and one without any added buffer. The relaxation rates for the buffered solutions indicate that, within experimental error, the correlation time, τ_c, has increased by approximately 30 ± 10% in going from pD 7 to 2. Whereas, in the solutions where the buffer was absent, the observed change in ^{1}H T_1's is barely evident. If NAD$^+$ underwent a significant change in molecular shape upon lowering the pD of the solution, one would expect to observe a significant change in T_1 in *both* buffered and unbuffered solutions. Two tentative conclusions can be drawn from these observations. First, the pD dependence of the T_1's for the pyridyl ^{1}H's in buffered NAD$^+$ solutions is probably due to a solution structure change brought about by the drastic charge change that the buffer undergoes in going from pD 7 to 2. Secondly, the conformational change that NAD$^+$ undergoes upon lower-

ing the pD does *not* also produce a significant change in molecular shape. To further check this latter conclusion we investigated the ^{13}C T_1's of *N*-methylnicotinamide.

While this work was in progress Hamill, Pugmire, and Grant[43] reported the pD dependence the ^{13}C T_1's of NAD^+. They concluded that the unfolding of NAD^+ upon protonation of the adenine ring was responsible for the pyridyl carbon T_1 pD dependence. Because of the high concentration of NAD^+ they employed in their work, and our own interest in the solution dynamics of NAD^+, we decided to examine the pD dependence of the T_1 at 5 m*M* with NAD^+ ^{13}C enriched at N-C_6 position. In addition, we also obtained the pD dependence of the ^{13}C T_1's of ^{13}C(N-C_6)-enriched *N*-methylnicotinamide. These and other experiments will be discussed in a separate publication.[44] However, the results of the *N*-methylnicotinamide experiments are germane to the present discussion. If the conclusion of Hamill et al. was correct, then one would predict that the T_1's for *N*-methylnicotinamide would be independent of pD. This would be due to the *lack* of intramolecular interactions in *N*-methylnicotinamide which are presumably present in NAD^+. We found that the T_1 for N-C_6 in the presence of HPO_4^{2-} buffer is a function of pD and the T_1 changes by approximately 50% in going from pD 1 to 7. Without the added buffer the change in T_1 is less than 10%. Similar studies without buffer on the ^{13}C T_1 for N-C_6 in NAD^+ at 20 m*M* and the same pD range show a change in T_1 of approximately 10%. Therefore, these T_1's are *not* changing because of any intramolecular process. Hence, the ^{13}C T_1 measurements may reflect a solution structure change and not an intramolecular conformational change.

These results do not suggest in any way that NAD^+ does not undergo a conformational change as a function of pD. However, these data suggest that there is no gross change in molecular shape when this conformational change occurs.

Summary and Conclusions

From the T_1 experiments discussed above, it is apparent that there are potential problems in the interpretation of the pH dependence of ^1H chemical shifts in terms of conformer populations. A representative example is the recent "explanation" of the pH dependence of the ^1H NMR of N-H_2 and N-H_6 in NMN^+.[22] The data showed clearly that ^1H chemical shift of N-H_2 was following the ionization of the phosphate in NMN^+, whereas N-H_6 was not. From these data, Sarma and Mynott[22] concluded that NMN^+ must exist in the syn conformation, i.e., N-H_2 pointed toward the phosphate group. However, Blumenstein and Raftery[25] have reported and we have subsequently verified in our own laboratory that carbon N-C_6 follows the ionization of the phosphate group, whereas carbon N-C_2 does not! These data illustrate the danger in trying to imply conformer populations from the pH dependence of chemical shifts. A more detailed discussion of these problems will be presented elsewhere.[45]

It is pertinent at this point to see if we can gain some insight into the nature of the so-called "folded" form of NAD^+ from the T_1 data presented here. From the experiments on the various deuterated NAD^+'s we can conclude that the pyridyl ring within NAD^+ is conformationally mobile and with approximately equal populations of syn and anti conformers. Furthermore, if there is any conformational preference, it would be slightly to the anti form. From the analysis of the conformational dynamics within NAD^+ and other nucleotides,[40] one can conclude that in all probability the nicotinamide ring rotation with respect to its contiguous ribose is not faster than the overall motion of the NAD^+ molecule. Hence, the time scales could be such to allow for a folded form of NAD^+ to exist for at least a couple of molecular tumbling times.

What is needed is a less restrictive description of the "folded" form. An estimate of the percent "folded" form of NAD^+ has been made by Hollis and coworkers. They concluded from thermodynamic considerations that NAD^+ would be approximately 15% "folded". To "explain" the previous experimental observations concerning NAD^+, the folded form need only mean that the two rings are proximate to one another, rather than at some very specific set of bond angles and distances described as "base stacking".

The pH dependence of the ^{13}C and ^1H NMR of NAD^+ can be explained in terms of simple electrostatic considerations.[46] *Because of the mutual charge repulsion in the protonated form of NAD^+, the set of all allowed conformations in this molecule will not be congruent with the set of allowed conformations of the unprotonated form of NAD^+.* Hence, the *time averaged* chemical shift for a given nucleus will not be necessarily the same in the two environments. The pH dependence can then be rationalized in terms of pH-dependent statistical weighting factors for the populations of the large number of allowed conformations.

Another set of experimental observations used to support the presence of a folded form in reduced pyridine dinucleotides derives from ultraviolet spectral data[47] and fluorescence energy transfer from the adenine ring to the reduced pyridine ring.[48-50] The ultraviolet data cannot be used to support or disprove statements of conformational dynamics derived from NMR measurements due to the large difference in their respective time scales, i.e., electronic correlation times are more than two orders of magnitude faster than rotational correlation times.[51] For all practical purposes the molecule is almost stationary during the transition and, therefore, these data could be consistent with a specific conformation. Fluorescence experiments are not an unequivocal statement of conformational dynamics because of the inability to predict the fluorescence efficiency as a function of the relative position of the two rings.

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References and Notes

(1) Part I: P. D. Ellis, R. R. Fisher, R. B. Dunlap, A. P. Zens, T. A. Bryson, and T. J. Williams, *J. Biol. Chem.*, **248**, 7677 (1973).
(2) Abbreviations used: NAD^+, nicotinamide adenine dinucleotide; NADH, 1,4-dihydronicotinamide adenine dinucleotide; NMN^+, nicotinamide mononucleotide; $TNAD^+$, thionicotinamide adenine dinucleotide. To denote a particular atom within NAD^+ *or* NMN^+, we use the nomenclature summarized in I, e.g., the 1′ hydrogen of the sugar bound to nicotinamide ring is represented as N-$H_{1'}$.
(3) W. L. Meyer, H. R. Mahler, and R. H. Baker, Jr., *Biochem. Biophys. Acta*, **64**, 353 (1962).
(4) O. Jardetzky and N. G. Wade-Jardetzky, *J. Biol. Chem.*, **241**, 85 (1966).
(5) R. H. Sarma, V. Ross, and N. O. Kaplan, *Biochemistry*, **7**, 3052 (1968).
(6) W. A. Catterall, D. P. Hollis, and C. F. Walter, *Biochemistry*, **8**, 4032 (1969).
(7) D. P. Hollis, *Org. Magn. Reson.*, **1**, 305 (1969).
(8) R. H. Sarma and N. O. Kaplan, *Biochem. Biophys. Res. Commun.*, **36**, 780 (1969).
(9) D. J. Patel, *Nature (London)*, **221**, 1239 (1969).
(10) R. H. Sarma and N. O. Kaplan, *Biochemistry*, **9**, 539 (1970).
(11) R. H. Sarma and N. O. Kaplan, *Biochemistry*, **9**, 557 (1970).
(12) R. H. Sarma and N. O. Kaplan, *Proc. Natl. Acad. Sci. U.S.A.*, **67**, 1375 (1970).
(13) R. H. Sarma, M. Moore, and N. O. Kaplan, *Biochemistry*, **9**, 549 (1970).
(14) J. Jacobus, *Biochemistry*, **10**, 161 (1971).
(15) N. Oppenheimer, T. Arnold, and N. O. Kaplan, *Proc. Natl. Acad. Sci. U.S.A.*, **68**, 3200 (1971).
(16) G. McDonald, B. Brown, D. P. Hollis, and C. Walter, *Biochemistry*, **11**, 1920 (1972).
(17) R. H. Sarma and R. J. Mynott, *Org. Magn. Reson.*, **4**, 577 (1972).

(18) M. Blumenstein and M. A. Raftery, *Biochemistry*, **11**, 1643 (1972).
(19) B. Birdsall, N. J. M. Birdsall, and J. Feeney, *J. Chem. Soc., Chem. Commun.*, **1972**, 316 (1972).
(20) W. Egan, S. Forsen, and J. Jacobus, *J. Chem. Soc., Chem. Commun.*, **1972**, 42 (1972).
(21) R. H. Sarma and R. J. Mynott, *J. Chem. Soc., Chem. Commun.*, **1972**, 975 (1972).
(22) R. H. Sarma and R. J. Mynott, *J. Am. Chem. Soc.*, **95**, 1641 (1973).
(23) R. H. Sarma, R. J. Mynott, F. E. Hruska, and D. J. Wood, *Can. J. Chem.*, **51**, 1843 (1973).
(24) R. H. Sarma and R. J. Mynott, *J. Am. Chem. Soc.*, **95**, 7470 (1973).
(25) M. Blumenstein and M. A. Raftery, *Biochemistry*, **12**, 3585 (1973).
(26) An equivalent, but less covenient, definition of the syn and anti forms has been put forth by M. Sundaralingam, *Biopolymers*, **7**, 821 (1969).
(27) J. H. Noggle and R. E. Schirmer, "The Nuclear Overhauser Effect Chemical Applications", Academic Press, New York, N.Y., 1971.
(28) R. Freeman and H. D. W. Hill, *J. Chem. Phys.*, **53**, 5103 (1970).
(29) T. A. Bryson, J. C. Wisowaty, R. B. Dunlap, R. R. Fisher, and P. D. Ellis, *J. Org. Chem.*, **39**, 3436 (1974).
(30) K. Isagawa, M. Kawai, and Y. Fushizaki, *Nippon Kagaku Zasshi*, **88**, 553 (1967).
(31) N. O. Kaplan and M. M. Ciotti, *J. Biol. Chem.*, **221**, 823 (1956).
(32) A. M. Stein, J. K. Lee, C. D. Anderson, and B. M. Anderson, *Biochemistry*, **2**, 1015 (1963).
(33) The chemical shift anisotropy mechanism, $(T_1^{CSA})^{-1}$, is usually zero since the magnitude of the chemical shift range for ^1H's is quite small. The scalar coupling mechanism, $(T_1^{SC})^{-1}$, can be discounted because of the small value of γ_N for ^{14}N compared to γ_H. For further details see ref 27, p 21.
(34) T. C. Farrar and E. D. Becker, "Pulse and Fourier Transform NMR Introduction to Theory and Applications", Academic Press, New York, N.Y., 1971, p 46.
(35) P. L. Johnson, C. L. Maier, and I. C. Paul, *J. Am. Chem. Soc.*, **95**, 5370 (1973).
(36) P. L. Johnson, J. K. Frank, and I. C. Paul, *J. Am. Chem. Soc.*, **95**, 5377 (1973).
(37) J. K. Lrank, N. N. Thayer, and I. C. Paul, *J. Am. Chem. Soc.*, **95**, 5386 (1973).
(38) Reference 34, p 57.
(39) G. A. Jeffrey, *Acc. Chem. Res.*, **2**, 344 (1969).
(40) R. E. Schirmer, J. P. Davis, and J. H. Noggle, *J. Am. Chem. Soc.*, **94**, 2561 (1972).
(41) A. P. Zens, P. J. Fogle, R. B. Dunlap, T. A. Bryson, P. D. Ellis, and R. R. Fisher, unpublished results.
(42) Similar trends have been independently observed by Kaplan and coworkers, personal communication.
(43) W. D. Hamill, Jr., R. J. Pugmire, and D. M. Grant, *J. Am. Chem. Soc.*, **96**, 2885 (1974).
(44) T. J. Williams, R. M. Riddle, P. D. Ellis, R. R. Fisher, T. A. Bryson, and R. B. Dunlap, unpublished results.
(45) T. J. Williams, A. P. Zens, P. D. Ellis, R. R. Fisher, T. A. Bryson, and R. B. Dunlap, unpublished results.
(46) This argument is just a generalization of that put forth in ref 4.
(47) D. G. Cross and H. F. Fisher, *Biochemistry*, **8**, 1147 (1969).
(48) G. Weber, *Nature (London)*, **180**, 1409 (1957).
(49) S. F. Velick in "Light and Life", W. D. McElroy and B. Glass, Ed., Johns Hopkins Press, Baltimore, Md., 1961.
(50) P. Walter and N. O. Kaplan, *J. Biol. Chem.*, **238**, 2323 (1963).
(51) C. Sandorfy, "Electronic Spectra and Quantum Chemistry", Prentice-Hall, Englewood Cliffs, N.J., 1964, p 4.

Synthesis and Biological Activity of *enantio*-[5-Valine]malformin, a Palindrome Peptide

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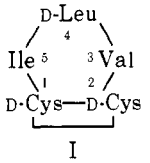
Abstract: The cyclic pentapeptide disulfide *enantio*-[5-valine]malformin (II) was prepared by stepwise synthesis in solution. The ring was closed by the azide method and the dithiol was oxidized to the disulfide. The product is the enantiomer of a palindrome peptide, [5-valine]malformin; it has one-tenth of the biological activity of natural malformin in causing curvatures on corn roots, inhibiting adventitious root formation, and stimulating the growth of etiolated bean cuttings. It induces malformations on bean seedlings which are identical with those induced by the parent compound.

Malformins, a family of metabolic products of *Aspergillus niger*, induce malformations on higher plants,[1] inhibit adventitious root formation,[2] have antibacterial[3] and cytotoxic properties, and under some conditions stimulate plant growth.[4] The initially proposed[5] structure for malformin A_1, a principal member of the family hereafter referred to as malformin, was recently revised.[6,7] The revised sequence, I, was confirmed by synthesis.[7]



I

The examination of structure-activity relationships in malformins, including a study of their different stable conformations,[8] necessitated additional efforts toward the synthesis of malformin analogs. This work, however, was hampered by the limited availability of one of the constituent amino acids, D-cystine. Therefore, we decided to prepare and study the enantio form of a malformin.

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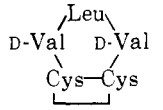
The decision to synthesize *enantio*-[5-valine]malformin[9] was based on the following considerations. In some biologically active members of the malformin family, isoleucine is replaced by valine.[10] Although the sequence of these constituents has not been determined, it seemed to be likely that there are no additional structural differences between malformin and other members of the family, or if there are differences, they do not affect the biological activity in any major way. Therefore, we expected that the enantio analog of [5-valine]malformin (II) would be a suitable model for a



II

study of the conformation of malformins and also hoped that the synthetic material would be biologically active. This hope was supported by the antibacterial properties of retro-enantio analogs of gramicidin S[11] and of enniatins.[12] The topochemical[13] similarity between a microbial peptide and its enantiomer is further enhanced if the direction of the chain is reversed.[14] Such retro enantiomers are closely