hr (130° bath). The resin was collected on a coarse glass frit, washed with methanol (10 ml), resuspended in 1.0 *M* potassium acetate in 2-methoxyethanol (150 ml), and heated gently at reflux for 10 hr; this cycle was repeated again. After the third reflux period, the resin was collected, washed with 2-methoxyethanol, water, and methanol (two 100-ml portions each), and vacuum dried to furnish the hydroxymethyl-resin (22.2 g). The infrared spectrum showed a strong hydroxyl band at 2.8 μ, new bands at 7.3, 8.5, and 12.3 μ, and the absence of carbonyl absorption.

Boc-L-valyloxymethyl-resin. The hydroxymethyl-resin (5.00 g, 6.8 mmol OH) was allowed to swell in dichloromethane, filtered to remove excess solvent, and shaken for 99 hr at room temperature with a solution of N^{α}-*tert*-butyloxycarbonyl-L-valine (1.48 g, 6.8 mmol) and *N,N'*-carbonyldiimidazole (1.10 g, 6.8 mmol) in a mixture of dichloromethane (50 ml), chloroform (10 ml), and dry dimethylformamide (3 ml). The resin was collected, washed with four 50-ml portions of dichloromethane, and shaken for 21 hr with a solution of acetic anhydride (20 ml, 0.21 mol) and pyridine (40 ml, 0.5 mol) in benzene (40 ml). The resin was collected, washed with four 100-ml portions of dichloromethane and three 10-ml portions of methanol, and vacuum dried. The resulting Boc-L-valyloxymethyl-resin (4.89 g) exhibited infrared bands at 2.93 (w, secondary urethane NH), 5.73 (s, ester carbonyl), and 5.80 μ (s, urethane carbonyl). The absence of hydroxyl absorption at 2.7–2.9 μ indicated that the excess hydroxymethyl sites had been completely acetylated. Four 10-mg resin samples were suspended in 12 *M* hydrochloric acid (2.0 ml), acetic acid (1.0 ml), and phenol (1.0 ml) and hydrolyzed in evacuated, sealed tubes for 24 hr at 110°. By quadruplicate amino acid analysis, 384 ± 4 μmol of valine was present per gram of Boc-L-valyloxymethyl-resin.

Deca(L-lysyl)-L-valine. A. Using Boc-Lys(2,4-Cl_2Z). The Boc-L-valyloxymethyl-resin (0.851 g, 328 μmol Val) was placed in a 20-ml reaction vessel and converted into N^{α}-Boc-deca[N^{ϵ}-(2,4-Cl_2Z)-L-lysyl]-L-valyloxymethyl-resin by the stepwise addition of ten protected lysine residues. Each residue was added manually by the following five-step cycle: (a) deprotection for 60 min with 50% TFA–CH_2Cl_2, (b) neutralization with 0.50 *M* ethyldiisopropylamine in CH_2Cl_2, (c) coupling for 60 min with a CH_2Cl_2 solution 0.12 *M* in N^{α}-*tert*-butyloxycarbonyl-N^{ϵ}-(2,4-dichlorobenzyloxycarbonyl)-L-lysine (1.00 mmol) and 0.12 *M* in *N,N'*-dicyclohexylcarbodiimide (1.00 mmol), (d) neutralization as in step b, and (e) coupling as in step c. Two residues were added per day; the synthesis was stopped at night only after step c or e.

One cycle of the synthesis required the resin to be washed 41 times with 10-ml washes over 5.5 hr as follows: (a) washing three times for 1 min with CH_2Cl_2, 1 min with 50% CF_3COOH–CH_2Cl_2, 60 min with 50% CF_3COOH–CH_2Cl_2, three times for 1 min with CH_2Cl_2, 1 min with 1.3 *M* 2-propanol in CH_2Cl_2 (to react with any strong electrophiles present before addition of the amine), and six times for 1 min with CH_2Cl_2; (b) washing three times for 1 min with 0.5 *M* ethyldiisopropylamine in CH_2Cl_2 and six times for 1 min with CH_2Cl_2; (c) shaking for 5 min with 0.25 *M* Boc-Lys(2,4-Cl_2Z) in CH_2Cl_2 (4.0 ml) and CH_2Cl_2 (0.5 ml), adding to this 0.26 *M* DCC in CH_2Cl_2 (3.8 ml) and CH_2Cl_2 (0.5 ml) and shaking for 60 min, draining, and washing three times for 1 min with CH_2Cl_2; (d) repetition of step b; and (e) repetition of step c.

After ten cycles, part of the dried resin (50 mg) was treated with anhydrous HF (2.7 ml) and dry anisole (0.3 ml) for 60 min at 0°. The HF was evaporated under water aspiration and most of the anisole was evaporated under high vacuum. The resin was collected on a glass frit, washed with three 3-ml portions of ether to remove anisole and its by-products, and washed with five 2-ml portions of CF_3COOH to remove the crude peptide. The CF_3COOH solution was evaporated to dryness under water aspiration; the residue was slurried with water (5.0 ml) and microfiltered through a Millipore filter. Part of this solution (0.30 ml) was diluted with buffered 0.2 *M* sodium chloride (4.20 ml) and adjusted to pH 5.5 with 12 *M* hydrochloric acid.

Part of this solution (1.00 ml) was chromatographed on an 0.9 × 50 cm column of carboxymethylcellulose preequilibrated with buffered 0.2 *M* sodium chloride. The column was eluted with an aqueous sodium chloride gradient delivered by a Beckman Accu-Flo pump at 65 ml/hr. The constant-volume gradient was formed by placing buffered 0.2 *M* sodium chloride (250 ml) in a stoppered vessel and continuously replacing this well-stirred solution by buffered 1.0 *M* sodium chloride from a second vessel. The column eluate was continuously reacted with ninhydrin and monitored spectrophotometrically at 570 nm to provide curve B of Figure 2.

In a semipreparative chromatographic run, 20% of the column eluate was utilized to monitor the separation and 80% was delivered to a fraction collector. The fraction corresponding to the midpoint of the Lys_{10}Val peak was evaporated to dryness, hydrolyzed under vacuum with 6 *M* aqueous HCl (110°, 24 hr), and analyzed; the observed Lys:Val ratio was 10.1:1.00.

B. Using Boc-Lys(Z). The synthesis of decalysylvaline was repeated using the same resin, solutions, equipment, and procedures described above, except that N^{α}-*tert*-butyloxycarbonyl-N^{ϵ}-benzyloxycarbonyl-L-lysine was the lysine reagent. Chromatographic analysis of a sample of the crude peptide mixture furnished curve A of Figure 2. In a second chromatographic run the branched-peptide peaks appearing after the peak due to Lys_{10}Val were enhanced for more accurate integration by analyzing a fivefold larger sample of the peptide mixture.

Crystal and Molecular Structure of the Salt (1-Methylnicotinamide)(+) Aden-9-ylacetate(−) Dihydrate. A Model for the Intramolecular Interactions of Oxidized Nicotinamide Adenine Dinucleotide

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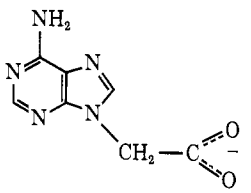
Abstract: The X-ray crystal structure of the salt 1-methylnicotinamide(+) aden-9-ylacetate(−) dihydrate has been determined. The aromatic rings form layered walls in which ribbons of coplanar adenine rings alternate with ribbons of coplanar nicotinamide rings. Short contacts between the stacked parallel rings suggest that the intermolecular forces between the stacked rings are partially charge transfer in character. There are no hydrogen bonds between the nicotinamide and the adenine residues. The significance of these observations in terms of the structure and biological function of NAD^+ is discussed.

The pyridine nucleotides, NAD^+ and $NADP^+$, are required by various biochemical processes in all known forms of life. These coenzymes function as electron carriers in metabolic processes through the reversible reduction of their nicotinamide moiety. Thus a molecule of NAD^+ that had been oxidized in one

metabolic process will be used as an oxidizing agent in another such process.

Spectroscopic,[1-3] nmr,[4-6] and chemical[7] data indicate that in aqueous solutions NAD^+ maintains a folded conformation such that the adenine and the nicotinamide rings are stacked. However, the detailed geometry of its molecular configuration is unknown. The determination of this detailed geometry could shed light on the as of now unknown biological role of the adenine moiety of the pyridine nucleotides. Such a determination could best be done using the techniques of X-ray crystal structure analysis. Unfortunately, exhaustive efforts in several laboratories have failed to produce crystals of naturally occurring pyridine nucleotides that are suitable for X-ray analysis. Likewise, solutions containing an N1-quaternized nicotinamide salt together with a neutral adenine compound have precipitated as crystals of the individual solutes rather than as cocrystals containing both components of the mixture.

The observations that salts often crystallize more readily than neutral compounds and that N1-quaternized nicotinamide derivatives are cations suggested that a salt consisting of such a cation together with an anion incorporating an adenine residue would form a model for adenine–nicotinamide interactions that could be analyzed by single-crystal X-ray diffraction techniques. Accordingly, it was found that a salt containing the aden-9-ylacetate anion (**1**) and the 1-methylnicotinamide cation (**2**) in one to one stoichiometric



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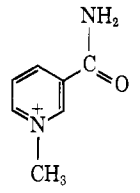

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ratio crystallized in a manner suitable for X-ray diffraction analysis.

The present report describes the X-ray analysis of the above crystal. In this structure the adenine and the nicotinamide rings associate by the formation of parallel stacks in which they appear to interact, in part, through charge-transfer interactions. The adenine and the nicotinamide rings do not form mutual hydrogen bonds even though the crystal structure is extensively hydrogen bonded. The biological significance of these observations is discussed.

Experimental Section

Sodium Aden-9-ylacetate. A suspension of sodium adenide was prepared in accordance with the method of Carraway, Huang, and Scott[8] by suspending 25 g (0.19 mol) of adenine and 5 g (0.21 mol) of NaH (57% dispersed in oil) in 300 ml of dimethylformamide and then stirring the suspension for 1 hr at room temperature. Methyl bromoacetate (38 g, 0.25 mol) was added dropwise to the suspension, causing it to clear. The solution was stirred overnight at room temperature, evaporated to 25% of its initial volume, and then cooled in an ice bath. The resultant white precipitate (methyl aden-9-ylacetate) was recrystallized from hot 66% aqueous ethanol, suspended in 100 ml of 1 *M* NaOH, and refluxed for 90 min. The solution was cooled and 1 l. of ethanol was added. The resultant white precipitate was dried at 110° to yield 6.4 g (16% yield) of sodium aden-9-ylacetate.

The uv spectrum of the product in aqueous solution very closely resembles that of 9-ethyladenine. Its ir spectrum (in KBr) has peaks at 1384, 1399, 1620, and 1650 cm^{-1}, which is indicative of the presence of a carboxyl group.[9]

Crystalline Salt 1-Methylnicotinamide(+) Aden-9-ylacetate(−) Dihydrate. A column of Amberlite IR120 cation exchange resin in the acid form was converted to the 1-methylnicotinamide(+) form by passing through it a sixfold molar excess of 0.5 *M* 1-methylnicotinamide iodide (that had been synthesized by the method of Martin and Hull[10]). A 0.12 *M* solution of sodium aden-9-ylacetate was passed through the resulting column to yield a solution of the salt 1-methylnicotinamide(+) aden-9-ylacetate(−). This was demonstrated by the identity of the uv spectrum of the column effluent with that of an aqueous solution containing a 1:1 stoichiometric ratio of 1-methylnicotinamide chloride and sodium aden-9-ylacetate. Needle-shaped colorless crystals of 1-methylnicotinamide(+) aden-9-ylacetate(−) dihydrate were grown at room temperature by diffusing ethanol into the column effluent. A small crystal (0.1 × 0.1 × 0.8 mm) was glued to a glass fiber along its long (*a*) axis. Preliminary oscillation, Weissenberg, and precession photographs indicated that the crystal had monoclinic lattice symmetry. Systematic absences of the *h*0*l* reflections for *l* odd and of the 0*k*0 reflections for *k* odd disclosed the space group to be $P2_1/c$.

All subsequent X-ray measurements were made with a Picker FACS-1 diffractometer employing a pyrolytic graphite monochrometer and Cu radiation (λ 1.5418 Å). The unit cell parameters, as determined by the least-squares analysis of the angular positions of 12 independent reflections, are $a = 7.496 \pm 0.003$, $b = 11.322 \pm 0.003$, $c = 19.794 \pm 0.004$ Å, and $\beta = 94.42 \pm 0.03°$. The unit cell volume is 1675.9 $Å^3$. The buoyant density of the crystals, as determined by flotation in an *n*-hexane–$CHCl_3$ mixture, was found to be 1.445 g/cm^3. This is in good agreement with the density of 1.448 g/cm^3 calculated for the asymmetric unit of the unit cell containing the species 1-methylnicotinamide(+) aden-9-ylacetate(−) dihydrate ($C_{14}N_7O_6H_{19}$, mol wt 365.35).

X-Ray diffraction peak counts were measured using the θ–2θ scan mode, a scan rate of 1°/min, a scan range of 0.85°, and a take-off angle of 2.5°. Stationary background counts of 20 sec duration each were taken at both limits of each scan. A total of 2659 unique reflections were measured to the limit $2\theta = 125°$. All computer calculations were performed using an IBM 360-75 computer.

The (0,6,0), the (0,0,16), and the (4,0,−4) reflections were monitored every 50 reflections during the data collection. An analysis of the intensities of these standard reflections revealed no apparent abnormality in the data collection process.

Structure Determination and Refinement. The intensities, *I*, were corrected for Lorentz and polarization factors. Their standard deviations, $\sigma(I)$, were calculated according to the method of Stout and Jensen[11] with an instrumental instability factor of 0.02. A total of 1033 reflections had $I < 2.33\sigma(I)$ and hence were considered to be unobserved (the remainder are observed at the 98% confidence level). No absorption corrections were made due to the small size of the crystal. The normalized structure factors were calculated using the method of polynomial regression to determine $\langle I \rangle$ as a function of $\sin\theta/\lambda$.[12]

The structure was solved by the reiterative application of the Sayre equation[13] using a modified version of the program REL.[14] The positions of all 26 nonhydrogen atoms in the structure appeared

(1) N. J. Leonard and R. A. Laursen, *Biochemistry*, **4**, 365 (1965).
(2) J. R. Barrio, J. A. Secrist, III, and N. J. Leonard, *Proc. Nat. Acad. Sci. U. S.*, **69**, 2039 (1972).
(3) J. A. Secrist, III, and N. J. Leonard, *J. Amer. Chem. Soc.*, **94**, 1702 (1972).
(4) R. H. Sarma, V. Ross, and N. O. Kaplan, *Biochemistry*, **7**, 3052 (1968).
(5) R. H. Sarma, M. Moore, and N. O. Kaplan, *Biochemistry*, **9**, 549 (1970).
(6) J. Jacobus, *Biochemistry*, **10**, 161 (1971).
(7) N. J. Oppenheimer, L. J. Arnold, and N. O. Kaplan, *Proc. Nat. Acad. Sci. U. S.*, **68**, 3200 (1971).
(8) K. L. Carraway, P. C. Huang, and T. G. Scott in "Synthetic Procedures in Nucleic Acid Chemistry," Vol. 1, W. W. Zorbach and R. S. Tipson, Ed., Wiley, New York, N. Y., 1968, p 3.
(9) L. J. Bellamy, "The Infra-red Spectra of Complex Molecules," Wiley, New York, N. Y., 1958.
(10) R. B. Martin and J. G. Hull, *J. Biol. Chem.*, **239**, 1237 (1964).
(11) G. H. Stout and L. H. Jensen, "X-ray Structure Determination," Macmillan, New York, N. Y., 1968.
(12) D. Voet, *J. Amer. Chem. Soc.*, **94**, 8213 (1972).
(13) D. Sayre, *Acta Crystallogr.*, **5**, 60 (1952).
(14) R. E. Long, *Diss. Abstr.*, **26**, 3651 (1966).

as the highest peaks in the E map based on the 174 (phased) reflections with $|E| > 1.5$. The structure was refined by the method of full matrix least squares. The quantity minimized in this process was $\Sigma w(|F_o| - |F_c|)^2$ where $w = 1/\sigma^2(I) = 1/\sigma^2(F_o)$. The atomic scattering factors for nonhydrogen atoms were taken from Cromer and Waber.[15] The atomic scattering factors for hydrogen atoms were those of Stewart, Davidson, and Simpson.[16] Three cycles of refinement of the scale factor, the atomic positions, and the isotropic temperature factors reduced the discrepancy index, $R(= \Sigma||F_o| - |F_c||/\Sigma|F_o|)$, from its initial value of 0.393 to the unacceptably high value of 0.239. An examination of those reflections with the greatest values of Δ $(=||F_o| - |F_c||)$ revealed that for them $|F_o|$ was always less than $|F_c|$. Many of these deviant reflections occurred in small groups of no more than six reflections that had been measured sequentially during the data collection process. Hence it appears that there was some intermittent instrumental instability during the data collection process that systematically caused the intensitites of some of the reflections to be seriously underestimated. Therefore in the succeeding refinement process, reflections were omitted from a given cycle of refinement if, for their calculated deviation, $\Delta/\sigma(F_o) > 5$ immediately preceding that refinement cycle. Likewise, those reflections with $\Delta > 5$ were omitted from the difference Fourier maps.

Two additional cycles of isotropic refinement reduced the discrepancy index to $R = 0.173$. This was followed by two cycles of refinement of the scale factor, the atomic positions, and the anisotropic temperature factors. This refinement reduced the discrepancy index to $R = 0.083$. (At this point, of the 108 reflections with $\Delta/\sigma(F_o) > 5$, only 29 reflections had $|F_o| > |F_c|$. Of these, 28 were in the range $10 > \Delta/\sigma(F_o) > 5$.) The difference Fourier map at this stage of refinement disclosed the positions of all but three of the expected hydrogen atoms. A difference Fourier map based on a structure factor calculation that included these known hydrogen atomic positions revealed the positions of the three missing hydrogen atoms. The atomic positions and the isotropic temperature factors of the hydrogen atoms were then refined for two cycles. This was followed by two cycles of refinement of all the atomic positions, the anisotropic temperature factors for nonhydrogen atoms and the isotropic temperature factors for hydrogen atoms. This reduced the discrepancy index to its final value of $R = 0.056$ (based on the 1575 observed reflections with $\Delta/\sigma(F_o) > 5$).[17] The final parameter shifts were all less than the estimated standard deviations of their respective parameters. The height of the highest peak in the difference Fourier map based on the final parameters was 0.20 e/Å^3.

The final discrepancy index based on all 1626 observed reflections is $R = 0.103$. Of the 51 reflections with $\Delta/\sigma(F_o) > 5$, only one has $|F_o| > |F_c|$. This was the (0,2,1) reflection for which $\Delta/\sigma(F_o)$ is 6.66. The reduction of the number of deviant reflections as the refinement of the structure progressed and the elimination of those with $|F_o| < |F_c|$ indicate the validity of the data filtration technique used.

Results

The atomic numbering scheme used in this communication is presented in Figure 1. Table I contains the final fractional coordinates and thermal parameters for all atoms in the asymmetric unit together with their standard deviations as estimated from the variance–covariance matrix of the final cycle of least-squares refinement. The thermal parameters for all atoms are in their expected ranges with the possible exception of those atoms that comprise the water molecules and the nicotinamide methyl group. However, it is often observed that such groups have greater freedom of motion than the other groups in a crystal.

The Molecular Structure. Figure 1 presents the covalent bond distances and bond angles found in the crystal structure. The adenine and the pyridinium ring bond distances and angles are in excellent agreement with the corresponding quantities reported in previously determined adenine[18] and pyridinium[19–22]

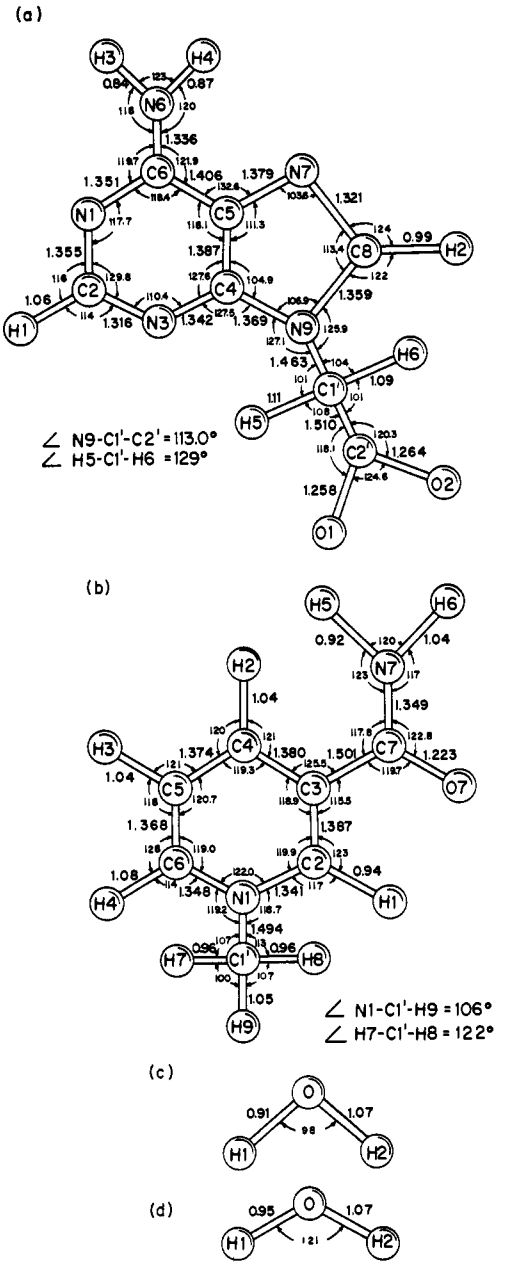

Figure 1. The atom numbering scheme and the covalent bond distances (in Å) and angles (in deg) for (a) the aden-9-ylacetate anion, (b) the 1-methylnicotinamide cation, (c) water molecule 1, and (d) water molecule 2. The bond lengths are uncorrected for thermal motion. The average standard deviations of the various quantities, as determined from the variance–covariance matrix of the final cycle of least-squares refinement, are 0.005 Å and 0.4° for the distances and the angles, respectively, that involve only nonhydrogen atoms. For distances and angles involving hydrogen atoms these quantities are 0.05 Å and 3°, respectively.

(15) D. T. Cromer and J. T. Waber, *Acta Crystallogr.*, **18**, 104 (1965).
(16) R. F. Stewart, E. R. Davidson, and W. T. Simpson, *J. Chem. Phys.*, **42**, 3175 (1965).
(17) The observed and calculated structure factors for the structure will appear following these pages in the microfilm edition of this volume of the journal. Single copies may be obtained from the Business Operations Office, Books and Journals Division, American Chemical Society, 1155 Sixteenth St., N.W., Washington, D. C. 20036, by referring to code number JACS-73-3763. Remit check or money order for $3.00 for photocopy or $2.00 for microfiche.
(18) D. Voet and A. Rich, *Progr. Nucl. Acid Res. Mol. Biol.*, **10**, 183 (1970).
(19) Y. Takeuchi and R. Pepinsky, *Z. Kristallogr.*, **109**, 29 (1957).
(20) C. Rérat, *Acta Crystallogr.*, **15**, 427 (1962).
(21) L. Dix-Edixhoven and C. H. Stam, *Recl. Trav. Chim. Pays-Bas*, **88**, 577 (1969).
(22) A. Cammerman, L. H. Jensen, and A. T. Balaban, *Acta Crystallogr., Sect. B*, **25**, 2623 (1969).

Table I. The Final Atomic Coordinates and Thermal Parameters[a]

Atom[b]	x	y	z	β_{11} or B	β_{22}	β_{33}	β_{12}	β_{13}	β_{23}
AN1	0.2154 (6)	0.7894 (3)	−0.0789 (2)	0.0218 (12)	0.0059 (3)	0.0022 (1)	−0.0003 (5)	0.0013 (3)	0.0006 (1)
AC2	0.1560 (8)	0.8703 (4)	−0.0355 (2)	0.0234 (17)	0.0056 (4)	0.0029 (1)	−0.0007 (6)	0.0006 (4)	0.0006 (2)
AN3	0.1683 (6)	0.8711 (3)	0.0312 (2)	0.0228 (13)	0.0058 (3)	0.0024 (1)	−0.0003 (5)	0.0011 (3)	0.0001 (1)
AC4	0.2517 (7)	0.7734 (3)	0.0555 (2)	0.0206 (14)	0.0054 (4)	0.0019 (1)	−0.0011 (6)	0.0010 (3)	0.0001 (2)
AC5	0.3213 (7)	0.6822 (3)	0.0188 (2)	0.0179 (14)	0.0050 (3)	0.0016 (1)	−0.0015 (5)	0.0003 (3)	−0.0001 (2)
AC6	0.3013 (7)	0.6933 (3)	−0.0521 (2)	0.0162 (14)	0.0056 (4)	0.0020 (1)	−0.0024 (5)	0.0003 (3)	0.0000 (2)
AN7	0.3973 (5)	0.5961 (3)	0.0611 (2)	0.0214 (12)	0.0062 (3)	0.0018 (1)	0.0004 (5)	0.0008 (3)	0.0002 (1)
AC8	0.3772 (8)	0.6378 (4)	0.1224 (2)	0.0245 (16)	0.0065 (4)	0.0018 (1)	−0.0012 (6)	0.0010 (4)	0.0003 (2)
AN9	0.2896 (6)	0.7429 (3)	0.1220 (1)	0.0236 (13)	0.0057 (3)	0.0018 (1)	−0.0012 (5)	0.0015 (3)	−0.0004 (1)
AN6	0.3683 (7)	0.6137 (4)	−0.0930 (2)	0.0283 (14)	0.0072 (4)	0.0016 (1)	0.0004 (6)	0.0020 (3)	0.0001 (2)
AC1′	0.2558 (9)	0.8133 (4)	0.1816 (2)	0.0287 (19)	00.073 (5)	0.0020 (1)	−0.0032 (8)	0.0019 (4)	−0.0011 (2)
AC2′	0.4109 (8)	0.8922 (4)	0.2049 (2)	0.0231 (15)	0.0057 (4)	0.0021 (1)	0.0018 (6)	0.0011 (4)	0.0001 (2)
AO1	0.3962 (5)	0.9438 (3)	0.2605 (1)	0.0272 (12)	0.0110 (4)	0.0020 (1)	−0.0020 (5)	0.0012 (2)	−0.0019 (1)
AO2	0.5434 (5)	0.9024 (2)	0.1696 (1)	0.0258 (11)	0.0072 (3)	0.0030 (1)	−0.0018 (4)	0.0028 (3)	−0.0010 (1)
NN1	0.2091 (6)	0.3018 (3)	0.1128 (2)	0.0220 (13)	0.0064 (3)	0.0024 (1)	−0.0011 (5)	0.0017 (3)	0.0003 (2)
NC2	0.1634 (8)	0.3421 (4)	0.0502 (2)	0.0151 (13)	0.0052 (4)	0.0026 (1)	−0.0012 (5)	0.0004 (3)	0.0001 (2)
NC3	0.2112 (7)	0.2791 (3)	−0.0057 (2)	0.0176 (14)	0.0050 (4)	0.0024 (1)	−0.0004 (5)	0.0007 (3)	0.0002 (2)
NC4	0.3042 (7)	0.1746 (4)	0.0044 (2)	0.0154 (14)	0.0059 (4)	0.0029 (1)	0.0001 (6)	0.0009 (3)	−0.0001 (2)
NC5	0.3470 (8)	0.1357 (4)	0.0694 (3)	0.0246 (18)	0.0054 (4)	0.0037 (2)	0.0002 (6)	0.0022 (4)	0.0008 (2)
NC6	0.3023 (8)	0.2009 (4)	0.1238 (2)	0.0231 (16)	0.0067 (4)	0.0028 (1)	0.0005 (6)	0.0012 (4)	0.0011 (2)
NC7	0.1488 (7)	0.3304 (4)	−0.0734 (2)	0.0166 (14)	0.0061 (4)	0.0024 (1)	−0.0018 (6)	0.0010 (3)	0.0001 (2)
NN7	0.1976 (7)	0.2741 (4)	−0.1291 (2)	0.0275 (15)	0.0076 (4)	0.0023 (1)	0.0026 (6)	0.0018 (3)	−0.0003 (2)
NO7	0.0556 (5)	0.4192 (2)	−0.0761 (1)	0.0255 (11)	0.0068 (3)	0.0028 (1)	0.0038 (4)	0.0017 (3)	0.0004 (1)
NC1′	0.1539 (12)	0.3709 (5)	0.1721 (3)	0.0358 (23)	0.0083 (6)	0.0023 (1)	0.0010 (9)	0.0023 (5)	−0.0004 (2)
W1O	0.7610 (7)	0.0917 (3)	0.2054 (2)	0.0383 (16)	0.0104 (4)	0.0026 (1)	−0.0076 (6)	−0.0005 (3)	0.0010 (2)
W2O	0.1133 (8)	0.1044 (3)	0.2472 (2)	0.0302 (15)	0.0093 (4)	0.0030 (1)	−0.0001 (6)	−0.0024 (3)	0.0001 (2)
AH1	0.080 (6)	0.942 (3)	−0.058 (2)	3.4 (0.9)					
AH2	0.419 (6)	0.597 (3)	0.165 (2)	2.9 (1.0)					
AH3	0.338 (5)	0.622 (3)	−0.135 (2)	2.5 (0.8)					
AH4	0.416 (8)	0.549 (4)	−0.076 (2)	6.6 (1.5)					
AH5	0.144 (8)	0.870 (4)	0.161 (2)	6.2 (1.6)					
AH6	0.259 (6)	0.749 (3)	0.223 (2)	4.2 (1.0)					
NH1	0.104 (6)	0.415 (3)	0.047 (2)	3.8 (1.1)					
NH2	0.349 (6)	0.128 (3)	−0.036 (2)	3.6 (0.9)					
NH3	0.427 (6)	0.061 (3)	0.079 (2)	4.5 (1.0)					
NH4	0.335 (6)	0.180 (3)	0.177 (2)	3.9 (1.0)					
NH5	0.278 (8)	0.213 (4)	−0.127 (2)	6.2 (1.5)					
NH6	0.142 (7)	0.305 (4)	−0.175 (2)	5.5 (1.3)					
NH7	0.090 (9)	0.318 (5)	0.199 (3)	8.0 (1.7)					
NH8	0.107 (7)	0.447 (4)	0.159 (2)	6.2 (1.5)					
NH9	0.271 (12)	0.385 (6)	0.204 (3)	13.6 (2.8)					
W1H1	0.752 (7)	0.135 (4)	0.167 (2)	5.6 (1.2)					
W1H2	0.666 (9)	0.025 (5)	0.190 (2)	7.9 (1.7)					
W2H1	0.205 (12)	0.049 (6)	0.258 (3)	12.6 (2.9)					
W2H2	−0.016 (14)	0.077 (8)	0.226 (4)	15.3 (4.0)					

[a] The positional parameters are expressed as fractions of unit cell edges. Anisotropic temperature parameters are expressed as $T = \exp[-(h^2\beta_{11} + k^2\beta_{22} + l^2\beta_{33} + 2hk\beta_{12} + 2hl\beta_{13} + 2kl\beta_{23})]$. Isotropic temperature factors for hydrogen atoms are of the form $\exp(-B \sin^2\theta/\lambda^2)$. Standard deviations, as determined from the variance–covariance matrix of the final cycle of least-squares refinement, are given in parentheses and refer to the least significant digits of their corresponding parameters. [b] The prefix A refers to the aden-9-ylacetate anion, N refers to the 1-methylnicotinamide cation, W1 refers to water molecule 1 and W2 refers to water molecule 2. The atomic numbering scheme is the same as that given in Figure 1.

containing structures, respectively. The bond parameters for the amide group and the carboxyl group are also within their expected ranges. Only the bond distances and angles involving hydrogen atoms on the nicotinamide methyl group and on the two water molecules are significantly distorted from their expected values. However, this is probably an artifact of the refinement process because such groups are often found to be somewhat disordered.

The molecular configuration of neighboring adenine and nicotinamide residues is illustrated in Figure 2.[23] Both of these residues are highly planar, as is shown in Table II. In the nicotinamide ion the amide group is nearly coplanar with the pyridinium ring. The dihedral angle between the least-squares planes through the nonhydrogen atoms of these two groups is 5.7°. The orientation of the amide group relative to the ring is with the C7–N7 bond nearly parallel to the C2–C3 bond. This is opposite to what was found in the structure of nicotinamide,[24] in which the C7–O7 bond was parallel to the C2–C3 bond. However, it is in agreement with the amide group orientation found in the structures 1-benzyl-1,4-dihydronicotinimide[25] and 1-(*n*-propyl)-1,4-dihydronicotinamide.[26] Thus it appears that the orientation of the amide group relative to the coplanar pyridinium ring is largely controlled by intermolecular forces within the crystal.

Intermolecular Interactions. The aromatic rings in the crystal structure form layered walls in which ribbons of coplanar adenine rings alternate with ribbons of coplanar nicotinamide rings. This is illustrated in

(23) C. K. Johnson, "ORTEP: A FORTRAN Thermal Ellipsoid Plot Program for Crystal Structure Illustrations," ORNL-3794, Oak Ridge National Laboratory, Oak Ridge, Tenn., 1965.

(24) J. D. Wright and G. S. D. King, *Acta Crystallogr.*, 7, 283 (1954).

(25) I. L. Karle, *Acta Crystallogr.*, **14**, 497 (1961).

(26) H. Koyama, *Z. Kristallogr.*, **118**, 51 (1963).

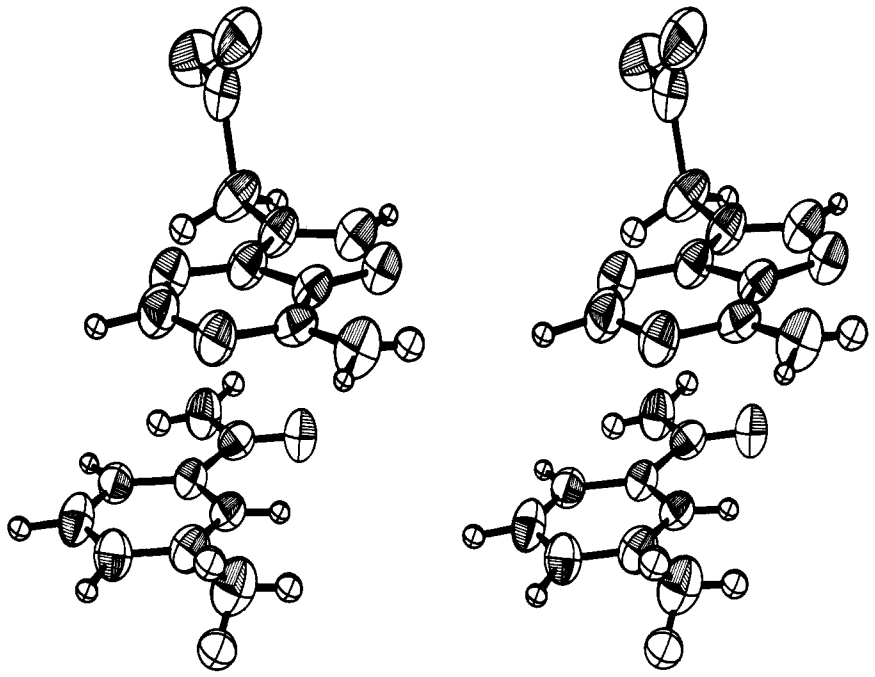

Figure 2. A stereoscopic view of the asymmetric unit of the crystal structure. The nonhydrogen atoms are depicted as thermal ellipsoids of a size such that the vibrating atoms have a 50% probability of being found within them. The hydrogen atoms are represented as spheres of a size such that the atoms have a 25% probability of being found within them. The water molecules have been omitted for the sake of clarity.

Table II. Deviations, in Ångstroms, of Nonhydrogen Atoms from the Least-Squares Plane through the Indicated Atoms

Adenine atom	Deviation[b]	Nicotinamide atom	Deviation[c]
N1	−0.002	N1	−0.005
C2	−0.005	C2	−0.005
N3	0.003	C3	0.006
C4	−0.005	C4	0.002
C5	0.001	C5	−0.011
C6	0.012	C6	0.013
N7	−0.014	C7	−0.034[a]
C8	0.009	N7	0.020[a]
N9	0.002	O7	−0.129[a]
N6	0.068[a]	C1′	−0.032[a]
C1′	0.092[a]		
rms deviation	0.007	rms deviation	0.008
		Amide atom	Deviation[d]
		C3	−0.001
		C7	0.004
		N7	−0.001
		O7	−0.002
		rms deviation	0.003

[a] Atoms not included in the least-squares fit. [b] The equation of the least-squares planar of the adenine ring is $6.5817x + 5.4177y - 1.1562z = 5.7878$. [c] The equation of the least-squares plane of the pyridinium ring is $6.4776x + 5.6959y - 1.0640z = 2.9581$. [d] The equation of the least squares plane of the amide group is $6.1848x + 6.3944y - 0.9390z = 3.0975$.

Figures 3[23] and 4.[23] Within each ribbon, neighboring coplanar rings are related by a center of symmetry located in the plane of the rings. Within the adenine ribbon, pairs of neighboring adenine molecules associate through the formation of two hydrogen bonds connecting atoms N6 and N7 on one adenine molecule with atoms N7 and N6, respectively, on the other. This mode of adenine–adenine base pairing has often been observed in crystal structures containing adenine derivatives[18] and is also found in the structure of double helical polyadenylic acid.[27]

Neighboring ribbons of coplanar rings form stacks of parallel molecules as is illustrated in Figure 3. The dihedral angle between the least-squares plane of an adenine ring and that of an adjacent nicotinamide ring is 1.6°. The stacked rings associate more closely than can be accounted for by normal stacking interactions. The root mean square perpendicular deviations of the atoms of the adenine rings on alternate sides of a nicotinamide ring are 3.261 and 3.317 Å, respectively, from the least-squares plane of the nicotinamide ring. Such interplanar distances are significantly less than the accepted distance for normal stacking interactions of 3.4 Å.[28,29] Those contacts between atoms in neighboring rings that are less than 3.35 Å are presented in Table III. It can be seen that some of them are significantly less than the accepted minimal van der Waals interatomic contacts (3.4 Å for C–C, 3.2 Å for C–N, and 3.1 Å for C–O).[28] It should be noted that the deviation of the amide group from coplanarity with its attached pyridinium ring is largely due to a rotation about the nicotinamide C6–C7 bond in a direction such that nicotinamide atom O7 appears to be pulled in toward the adenine imidazole ring on which it is stacked.

The water molecules and the carboxyl oxygen atoms form polar walls parallel to and separating the symmetry related walls of stacked aromatic rings. This structural feature is maintained by a complex system of hydrogen bonds as can be seen in Figure 4. This

(27) A. Rich, D. R. Davies, F. H. C. Crick, and J. D. Watson, *J. Mol. Biol.*, **3**, 71 (1961).
(28) L. Pauling, "The Nature of the Chemical Bond," Cornell University Press, Ithaca, N. Y., 1960.
(29) C. E. Bugg, J. M. Thomas, M. Sundaralingham, and S. T. Rao, *Biopolymers*, **10**, 175 (1971).

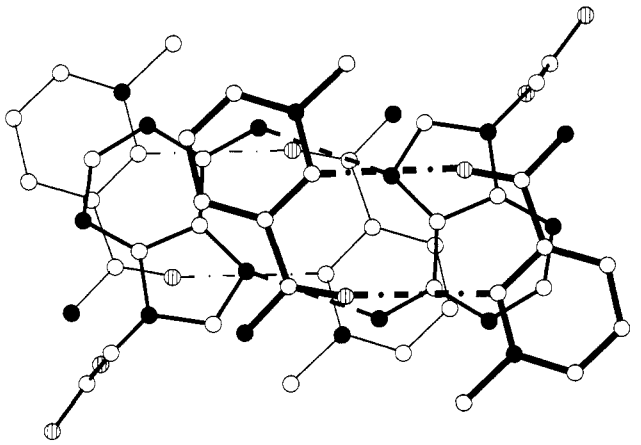

Figure 3. A projection of three consecutive layers of the stacked walls of aromatic rings onto the plane of the nicotinamide ring. This illustrates the stacking relationships in the structure. Hydrogen bonds are represented as dashed lines and C–H···O hydrogen-bond-like interactions are represented as dot-dashed lines. Carbon atoms are depicted as open circles, nitrogen atoms are filled circles and oxygen atoms are shaded circles. Hydrogen atoms have been omitted for the sake of clarity.

system includes all the possible hydrogen bond donor groups in the structure except for the adenine N–H group that is involved in the adenine–adenine pairing. The acceptor atoms for these hydrogen bonds include both water oxygens, both carboxyl oxygens, and adenine N1. Hydrogen bond length data are given in Table III.

Nicotinamide atom O7 is the only oxygen atom in the structure that does not participate in normal hydrogen bonding. However, as can be seen from Table III, its distances from atoms C2a and H1a of an adjacent coplanar nicotinamide residue are closer than can be accounted for by normal van der Waals interactions.[28] The NC7–NO7–NC2a and the NC7–NH1a–NC2a angles are 168.1 and 161°, respectively. The nicotinamide C2–H1 group is expected to be particularly acidic due to the stabilizing influence of its neighboring quaternary amine and amide groups. Hence the C2–H1 group and the amide oxygen atom appear to be participating in a typical C–H···O hydrogen-bond-like interaction.[12] As can be seen from Figures 3 and

Table III. Intermolecular Contacts[a]

	Bond length, Å		Bond length, Å
1. Ring Stacking Contacts Less Than 3.35 Å for Nonhydrogen Atoms			
NC2–AN1a	3.294		
NC3–AN3a	3.319		
NC4–AC5b	3.304		
NC5–AC6b	3.310		
NC5–AN3c	3.344		
NC6–AN6b	3.333		
NC7–AC4a	3.268		
NN7–AC8b	3.332		
NO7–AC4a	3.220		
NO7–AC5a	3.329		
NO7–AN9a	3.245		
NO7–AN6	3.252		
2. Hydrogen Bonding Contacts			
AN6–AN7b	2.993	AH4–AN7b	2.17
AN6–AO1d	2.995	AH3–AO1d	2.28
NN7–AO2b	2.941	NH5–AO2b	2.09
NN7–W2Oe	2.838	NH6–W2Oe	1.85
W1O–AN1b	2.860	W1H1–AN1b	1.97
W1O–AO2c	2.753	W1H2–AO2c	1.70
W1O–AO1c	2.791	W2H1–AO1c	1.86
W2O–W1Of	2.709	W2H2–W1Of	1.70
3. C–H···O Hydrogen-Bond-like Contacts			
NO7–NC2a	3.223	NO7–NH1a	2.33

[a] The bond lengths are uncorrected for thermal motions. Standard deviations for the various bond types are given in the legend of Figure 1. The prefix A refers to the aden-9-ylacetate anion, the prefix N refers to the 1-methylnicotinamide cation, and W1 and W2 refer to water molecules 1 and 2, respectively. Lower case letters accompanying the atom numbers refer to atoms related to those in Table I by the following symmetry operations: (a) $-x$, $1-y$, $-z$; (b) $1-x$, $1-y$, $-z$; (c) x, $-1+y$, z; (d) x, $^3/_2-y$, $-^1/_2+z$; (e) x, $^1/_2-y$, $-^1/_2+z$; (f) $-1+x$, y, z.

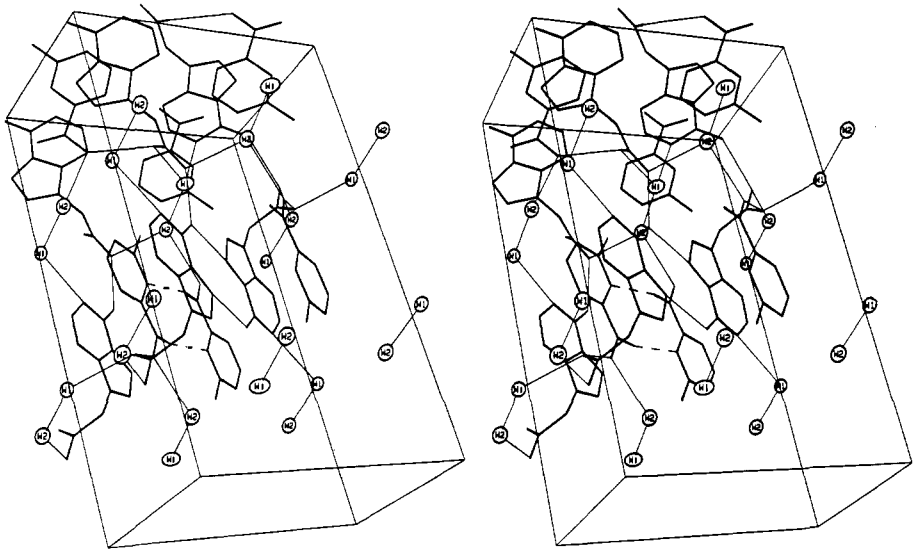

Figure 4. A stereoscopic view of a portion of the crystal structure illustrating the packing relationships among its molecules. Covalent bonds are shown as thick lines, unit cell outlines and hydrogen bonds are shown as thin lines and C–H···O hydrogen-bond-like interactions are shown as dashed lines. Water oxygen atoms are represented as thermal ellipsoids such that the vibrating atoms have a 25% probability of being found within them. Hydrogen atoms have been omitted for the sake of clarity.

4, these interactions occur in pairs so that adjacent coplanar nicotinamide groups form a sort of cyclic dimer about a center of symmetry.

Discussion

The most noteworthy features of the crystal structure are the anomalously close face-to-face contacts between neighboring adenine and nicotinamide rings. This suggests that a charge-transfer bond has formed between the adenine and the nicotinamide rings. However, as can be seen in Figure 3 and from Table III, the amount of overlap between neighboring rings is far from the maximal amount that is expected for strong charge-transfer bonds.[30] In addition, the majority of the anomalously close stacking contacts involves interactions between polar groups, such as the nicotinamide amide group, and polarizable groups, such as the adenine imidazole ring. Such intermolecular configurations are typical of those in which the stacking contacts are of normal length.[29] This is indicative that the forces underlying the stacking interactions in the present structure consist mainly of the more prosaic electrostatic and London dispersion types.[31]

It is known that NAD^+ maintains a folded conformation in aqueous solutions.[1-7] Model building studies indicate that the nicotinamide and the adenine rings in NAD^+ can easily assume either of the stacking conformations illustrated in Figure 3 (although it is not suggested here that either of these stacking conformations are necessarily the dominant ones in NAD^+). Charge-transfer bands have been observed in the ultraviolet spectrum of aqueous solutions containing derivatives of N1-quaternized nicotinamide together with those of adenine.[32,33] However, a charge-transfer band in the spectrum of NAD^+ has not been reported. Nevertheless, it is conceivable that such a band exists but is masked by the hypochromism observed on cleaving the pyrophosphate bond in NAD^+.[1] Thus, it is possible that intramolecular charge-transfer bonding is partially responsible for maintaining the conformation of NAD^+.

In cocrystals containing adenine and uracil residues or cytosine and guanine residues, hydrogen bonds are invariably formed between unlike bases.[18] Infrared,[34-36] nmr,[37-39] and chromatographic binding[40] studies of these bases in both aqueous and nonaqueous solutions have shown that there is strong affinity for hydrogen bonding between the members of Watson–Crick base pairs, but that the strength of such hydrogen bonds between the members of non-Watson–Crick base pairs is less, often by many orders of magnitude. This phenomenon, which has been termed "electronic complementarity,"[34,35] is important in maintaining the structural integrity of nucleic acids and in assuring the fidelity of information transfer in the various processes of protein synthesis.

The absence of hydrogen bonds between the adenine and the nicotinamide residues in the present structure suggests that adenine and nicotinamide rings are not electronically complementary. This would rationalize why mixed crystals containing the 1-methylnicotinamide cation and neutral adenine derivatives have not been isolated. However, the reason why NAD^+ has a stacked conformation rather than an internally hydrogen bonded one in aqueous solutions is more attributable to hydrophobic forces, and perhaps to charge-transfer forces, than to any lack of electronic complementarity between its adenine and nicotinamide components. Thus, even molecules with electronically complementary components, such as FAD,[41,42] maintain a stacked conformation in aqueous solutions.[43] Nevertheless, the lack of electronic complementarity between its components may be an important determinant of the conformation of NAD^+ in nonpolar environments such as might be found on the surface of an enzyme.

The problem motivating the present study is to determine the function of the adenine moieties of NAD^+ and $NADP^+$. These coenzymes are employed in a great variety of metabolic processes in all known forms of life. Yet in all of these myriads of organisms the chemical structures of the pyridine nucleotides are invariable. This suggests that the adenine component of these coenzymes has a function that is indispensable to the basic metabolic apparatus of all life forms. This is because evolutionary processes tend to modify the nonessential components of molecules that take part in biological processes. Because of the large through-the-bonds distance of the adenine ring from the nicotinamide ring in NAD^+, it can be safely assumed that the adenine component of NAD^+ does not directly affect the reduction potential of this coenzyme. It has been observed that the adenine component of NAD^+ is required for the binding of the coenzyme to various enzymes.[44] In fact, the function of the adenine moiety of the NAD^+ cofactor of the dogfish enzyme lactate dehydrogenase would appear to be only that of a binding handle. In this enzyme the adenosine moiety of NAD^+ probably acts as an allosteric effector required for the binding of the NMN^+ moiety to the enzyme.[44] Yet the NAD^+ molecule is observed to bind to the enzyme in an extended conformation.[45,46] Hence there could be no direct interactions of any significance between the adenine and nicotinamide components of this NAD^+. However, the role of a binding handle that has no other function is a passive one and therefore such a molecular handle would be subject to changes in its structure through the processes of evolution. In-

(30) R. S. Mulliken, *J. Amer. Chem. Soc.*, **74**, 811 (1952).
(31) H. DeVoe and I. Tinoco, *J. Mol. Biol.*, **4**, 500 (1962).
(32) G. Cilento and S. Schreier, *Arch. Biochem. Biophys.*, **107**, 102 (1964).
(33) B. M. Anderson and M. L. Reynolds, *Arch. Biochem. Biophys.*, **114**, 299 (1966).
(34) Y. Kyogoku, R. C. Lord, and A. Rich, *Science*, **154**, 518 (1966).
(35) Y. Kyogoku, R. C. Lord, and A. Rich, *Proc. Nat. Acad. Sci. U. S.*, **57**, 250 (1967).
(36) J. Pitha, R. N. Jones, and P. Pithova, *Can. J. Chem.*, **44**, 1045 (1966).
(37) M. Raszka and N. O. Kaplan, *Proc. Nat. Acad. Sci. U. S.*, **69**, 2025 (1972).
(38) L. Katz and S. Penman, *J. Mol. Biol.*, **15**, 220 (1966).
(39) R. R. Shoup, H. T. Miles, and E. D. Becker, *Biochem. Biophys. Res. Commun.*, **23**, 194 (1966).
(40) H. Tuppy and E. Küchler, *Biochim. Biophys. Acta*, **80**, 669 (1964).
(41) Y. Kyogoku and B. S. Yu, *Bull. Chem. Soc. Jap.*, **42**, 1387 (1969).
(42) D. Voet and A. Rich, *Proc. Nat. Acad. Sci. U. S.*, **68**, 1151 (1971).
(43) M. Kainosho and Y. Kyogoku, *Biochemistry*, **11**, 741 (1972).
(44) A. McPherson, Jr., *J. Mol. Biol.*, **51**, 39 (1970).
(45) M. J. Adams, A. McPherson, Jr., M. G. Rossman, R. W. Schevitz, and A. J. Wonacott, *J. Mol. Biol.*, **51**, 31 (1970).
(46) M. G. Rossman, M. J. Adams, M. Buehner, G. C. Ford, M. L. Hackert, P. J. Lentz, Jr., A. McPherson, Jr., R. W. Shevitz, and I. E. Smiley, *Cold Spring Harbor Symp. Quant. Biol.*, **36**, 179 (1971).

deed, it would appear that the specificity of adenine as a binding handle would be rather limited due to the large number of metabolically indispensable small molecules that contain an adenine moiety. Therefore, it is likely that the function of the adenine component in NAD^+, at least in one indispensable metabolic process, is something more than that of a passive binding handle.

At present this hypothetical function of the adenine component of NAD^+ is unknown. However, the present study suggests that it might involve the adsorption of an NAD^+ molecule in a stacked configuration to its binding site on an enzyme surface. During the reduction of this coenzyme molecule the function of its adenine moiety might be to act as a conduit for the transfer of an electron pair from the enzyme surface or from the substrate to the nicotinamide ring. The formation of a charge-transfer complex between the adenine and the nicotinamide rings of NAD^+ would facilitate such an electron pair transfer because it is expected that adenine would be the donor group[47] and nicotinamide would be the acceptor group[48] in such an intramolecular charge-transfer complex.

It is also possible that the conformation of NAD^+ bound to some enzymes is significantly different from that of its reduction product, NADH, bound to the same enzyme. For example, if the NAD^+ formed the postulated internally stacked complex, the NADH might assume an internally hydrogen bonded conformation. Alternatively, the NADH might be in an extended configuration such as that it assumes when bound to the dogfish enzyme lactate dehydrogenase.[46] Structural studies on NADH model compounds are presently being carried out so that conformational differences between NAD^+ and NADH can be more accurately assessed.

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(47) B. Pullman and A. Pullman, *Proc. Nat. Acad. Sci. U. S.*, **44**, 1197 (1958).

(48) B. Pullman and A. Pullman, *Proc. Nat. Acad. Sci. U. S.*, **45**, 136 (1959).

Determination of Pyrimidine Nucleoside Syn, Anti Conformational Preference in Solution by Proton and Carbon-13 Nuclear Magnetic Resonance[1]

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Abstract: The glycosidic conformation of 11 pyrimidine nucleosides and one quinazoline nucleoside in solution has been investigated by ^1H and ^{13}C nmr spectroscopy. Proton chemical shift data as well as vicinal furanose coupling constants indicate that most of these nucleosides are preferentially anti. Bulky groups such as methyl at position 6 or a 5,6-fused benzene ring shift the torsional angle into the syn range. Measurements of the vicinal $^3J_{C_2-H_{1'}}$ about the glycosidic bond in cytidine and 6-methylcytidine confirm the conclusions based upon chemical shift data. Although the torsional angle may be altered somewhat, the relative proportion of syn and anti conformers appears to be about the same in DMSO as in water. Examination of 2′,3′-*O*-isopropylidene derivatives indicates that significant changes in the furanose conformation are less a determinant of glycosidic conformation than steric interaction between substituents on the base and ribose moieties.

An understanding of the conformational details of oligonucleotides and polynucleotides will undoubtedly be attained at least in part from thorough investigation of the conformational properties of the individual monomers. In pursuit of this goal, numerous investigations on nucleoside and nucleotide crystals and their solutions have been carried out. The solid state furanose conformation in nucleosides, nucleotides, and polynucleotides has been shown to be quite similar.[2] Nmr[3] studies of uridylic acid monomers[4] and polymers[4b] have been interpreted as indicating comparable furanose conformations in solution as well, at least for this particular example.

One important facet of nucleoside conformation is the relative position of the base and sugar moieties about the glycosidic bond, governed by the torsional angle, χ.[2a,5] X-Ray data,[2a,5,6] potential energy calculations,[7] and nmr studies[8] have shown that χ is dependent upon furanose ring puckering and that pyrimidine nucleosides generally exist in the anti

(1) Presented in part at the 15th Biophysical Society Meeting, New Orleans, La., Feb 1971, Abstracts No. WPM-M3; "Conformation of Biological Molecules and Polymers" (Proceedings of the Fifth Jerusalem Symposium), Israel Academy of Science and Humanities, Jerusalem, 1972, in press.

(2) (a) M. Sundaralingam, *Biopolymers*, 7, 821 (1969); (b) S. Arnott and D. W. L. Hukins, *Nature (London)*, **224**, 886 (1969).

(3) Abbreviations: nmr, nuclear magnetic resonance; pmr, proton magnetic resonance; cmr, carbon-13 nuclear magnetic resonance; CD, circular dichroism.

(4) (a) T. Schleich, B. J. Blackburn, R. D. Lapper, and I. C. P. Smith, *Biochemistry*, **11**, 137 (1972); (b) G. P. Kreishman and S. I. Chan, *Biopolymers*, **10**, 159 (1971).

(5) J. Donohue and K. N. Trueblood, *J. Mol. Biol.*, **2**, 363 (1960).

(6) A. E. V. Haschemeyer and A. Rich, *ibid.*, **27**, 369 (1967).

(7) A. V. Lakshminarayanan and V. Sasisekharan, *Biopolymers*, **8**, 475 (1969).

(8) J. H. Prestegard and S. I. Chan, *J. Amer. Chem. Soc.*, **91**, 2843 (1969).