220 MHz PROTON NUCLEAR MAGNETIC RESONANCE STUDY OF THE GEOMETRIC DISPOSITION OF THE BASE PAIRS IN THE OXIDIZED AND REDUCED PYRIDINE NUCLEOTIDES*

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SUMMARY

Differential shielding of the symmetrically located C_2H and C_6H of the pyridine moiety by adenine indicates that the geometric disposition between the pyridine base and the D-ribofuranose ring is syn in α-DPN, β-DPN, β-DPNH, TPNH and the acetylpyridine analog of β-DPNH and anti in the acetylpyridine analogs of β-DPN and TPN. An anti-syn overlap, i.e. adenine is anti and pyridine is syn, is proposed for the stacked conformation of α-DPN, β-DPN, TPN, β-DPNH and the acetylpyridine analog of β-DPNH. An anti-anti overlap is proposed for the stacked conformation in the acetylpyridine analogs of β-DPN and TPN.

Several recent reports from this laboratory on the low and high frequency nuclear magnetic resonance (NMR) spectra of pyridine coenzymes have enabled us to unravel the subtle and finer features of their molecular geometry (1-6). The data in the current communication give insight regarding the relative geometry between the pyridine ring and the ribose as well as the geometric disposition between the base pairs in the oxidized and reduced pyridine nucleotides. Examination of the data (Table I and Fig. 1) indicates that the pyridine protons appear considerably upfield from those of mononucleotides, suggesting that in all the dinucleotides tested, there is strong intramolecular interaction between their base pairs and that the base pairs are stacked in parallel planes. In the cases of α-DPN, β-DPN, TPN, β-DPNH, (AcPy)DPNH (the acetylpyridine analog of β-DPNH) and TPNH, the C_2H has under-

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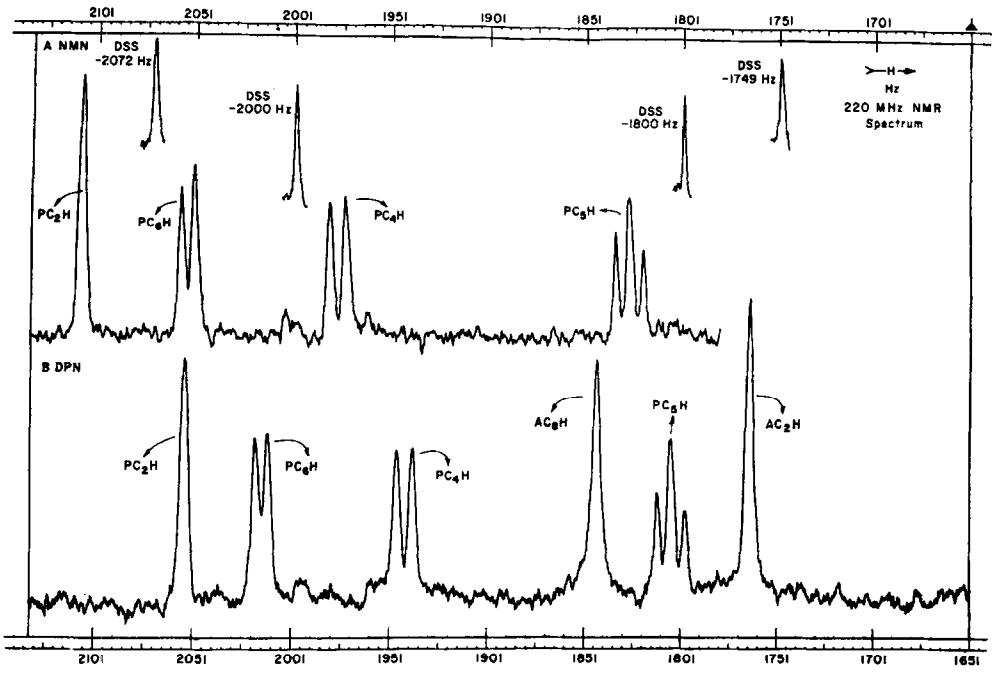

Fig. 1. 220 MHz NMR spectra of β-NMN (top) and β-DPN (bottom) taken at a sweepwidth of 500 Hz using sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) as an internal standard.

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gone a much larger upfield shift than C_6H; on the other hand, in the cases of (AcPy)DPN and (AcPy)TPN (respectively the acetylpyridine analogs of β-DPN and TPN), the C_6H has undergone a much larger upfield shift than C_2H. This difference in the upfield shifts between the C_2H and C_6H could arise from the interaction of the adenine moiety. The folding interaction of adenine in α-DPN, β-DPN, TPN, β-DPNH and (AcPy)DPNH is such that it shields the pyridine C_2H more than C_6H and that there is restricted rotation around the pyridine ribose glycosidic linkage. If the pyridine ring is able to rotate freely, a rotation of 180° around the glycosidic bond would enable the C_2H and C_6H protons of the pyridine ring to exchange their positions with respect to the plane of the juxtaposed adenine ring because the C_2H and C_6H are located symmetrical to the glycosidic bond and then the adenine ring would shield the C_2H and C_6H to the same extent, i.e. the C_2H and C_6H would experience the same average shielding

by the adenine ring. The adenine ring could shield the pyridine C_2H more than C_6H only if the adenine moiety interacts with that part of the pyridine which contains the amide side chain, i.e. the folding interaction between the base pairs in β-DPN, α-DPN, TPN, β-DPNH, (AcPy)DPNH and TPNH is such that the adenine interacts with that part of pyridine which contains the side chain at C_3. Examination of the molecular models clearly reveals that such interactions could prevail only if the pyridine ring assumes a syn conformation with respect to the D-ribofuranose ring. On the other hand, Table I shows that in the cases of (AcPy)DPN and (AcPy)TPN the pyridine C_6H has undergone a much larger upfield shift compared to C_2H. This suggests that the geometry of the folding interaction in (AcPy)DPN and (AcPy)TPN is such that, on the average, the adenine spends considerable amount of time on that part of the acetylpyridine ring which does not contain the $-COCH_3$ side chain. In agreement with these arguments one observes relatively very little shielding of the $-CH_3$ group of (AcPy)DPN and (AcPy)TPN by the adenine ring current magnetic anisotropy and significant shielding of the $-CH_3$ group of (AcPy)DPNH. Again, examination of molecular models reveals that such interactions between the base pairs could prevail only if the acetylpyridine ring assumes an anti conformation with respect to the D-ribofuranose ring in (AcPy)DPN and (AcPy)TPN and a syn conformation in (AcPy)DPNH.

The above conclusions have been further verified by studying the effect of ionization of the secondary phosphate in β-NMN, α-NMN and (AcPy)MN. Table II contains the chemical shift data on β-NMN, α-NMN and (AcPy)MN at pH values 8.0 and 4.5. The pK for the ionization of the secondary phosphate in the mononucleotides would lie between 6 and 7. The pK values for the protonation of the nicotinamide ring are much lower. The data in Table II show that in β-NMN at pH 8.0, the chemical shifts of C_2H and C_6H are shifted downfield by the phosphate which is present as a dianion at that pH. The specific deshielding of the C_2H of the pyridine ring by the phosphate dianion would agree with a syn conformation for β-NMN. It is not surprising that in the case of

TABLE I. The chemical shifts of the nucleotides and the upfield shifts in the dinucleotides from the corresponding mononucleotides.[a]

NUCLEOTIDES	The chemical shifts of the pyridine protons						
	C_2H Hz	C_6H Hz	C_5H Hz	A	C_4^b Hz	B	CH_3 Hz
α-NMN	2052.0	2004.0	1810.0		1971.0		
α-DPN	1997.2	1959.0	1778.0		1934.1		
Upfield shift	54.8	45.0	32.0		36.9		
β-NMN	2112.0	2052.0	1828.5		1978.5		
β-DPN	2055.5	2016.5	1806.3		1942.3		
Upfield shift	56.5	35.5	22.2		36.2		
β-NMN	2112.0	2052.0	1828.5		1978.5		
β-TPN	2042.0	2000.0	1795.0		1932.0		
Upfield shift	70.0	52.0	33.5		46.5		
(AcPy)MN	2093.5	2097.0	1846.5		1997.5		614.8[c]
(AcPy)DPN	2066.5	2028.0	1810.5		1960.5		607.0
Upfield shift	27.0	69.0	36.0		37.0		7.8
(AcPy)MN	2093.5	2097.0	1846.5		1997.5		614.8[c]
(AcPy)TPN	2053.5	2012.0	1801.5		1953.3		607.0
Upfield shift	40.0	85.0	45.0		44.2		7.8
β-NMNH	1555.0	1072.0	1084.0	642.7		642.7	
β-DPNH	1510.5	1051.5	1041	560.5		581.9	
Upfield shift	45.5	20.5	43	82.2		60.8	
β-NMNH	1555.0	1072.0	1084.0	642.7		642.7	
β-TPNH	1516.0	1044.6	1084.3	575.6		601.4	
Upfield shift	39.0	27.4	0	67.1		41.3	
(AcPy)MNH	1647.0	1094.0[d]	1134.3	628.0		628.0	487.0
(AcPy)DPNH	1595.0	1066.8	1070	548.7		548.7	457.0
Upfield shift	52.0	27.2	64	79.3		79.3	30.0

a The NMR spectra were obtained on a high resolution varian HRSC-IX superconducting solenoid 220 MHz NMR system. The measurements were made on 0.1 M solutions in D_2O. The oxidized coenzymes were examined at 23°, pH 7.0. The reduced coenzymes were examined at various temperatures at pH 8.5 to unravel and assign the dihydropyridine C_6H and C_5H. The data given above were obtained at 5°, unless otherwise stated. The chemical shifts of both oxidized and reduced coenzymes are independent of concentration (10) and hence could be interpreted in terms of intramolecular interactions.

b The geminal C_4 hydrogens of the dihydropyridines in β-DPNH and TPNH give an AB quartet in the 220 MHz NMR system indicating that they reside in different electronic and geometric environments and hence their shifts are expressed separately (3,4).

c Not observable in D_2O. d Data at 23°.

TABLE II. Effect of ionization of secondary phosphate on the chemical shifts of pyridine mononucleotides.[a]

NUCLEOTIDES	pH	The chemical shifts of the pyridine protons			
		C_2H Hz	C_6H Hz	C_4H Hz	C_5H Hz
β-NMN	4.5	2084.5	2041.4	1978.5	1828.5
β-NMN	8.0	2112.0	2052.0	1978.5	1828.5
Downfield shift		27.5	10.6	0	0
α-NMN	4.5	2048.5	2001.0	1969.5	1807.0
α-NMN	8.0	2052.0	2004.0	1971.0	1810.0
Downfield shift		3.5	3.0	1.5	3.0
(AcPy)MN	4.5	2095.5	2060.0	2003.5	1842.0
(AcPy)MN	8.5	2093.5	2097.0	1997.5	1846.5
Downfield shift		-2.0	37.0	-6.0	4.5

a The NMR spectra were obtained on a high resolution varian HRSC-IX superconducting solenoid 220 MHz NMR system, using 2,2-dimethyl-2-silapentane-5-sulfonate as an internal standard.

α-NMN, we do not observe any specific deshielding of the base protons (Table II). This is because the 5'phosphate and the base reside on opposite sides of the ribose ring. The ionization of the secondary phosphate deshields C_6H more than C_2H in (AcPy)MN (Table II). This would suggest that the geometric relationship between the ribose and the acetylpyridine ring is anti in (AcPy)MN. In Fig. 2 we have illustrated the syn conformation for β-NMN and β-NMNH and the anti conformation for (AcPy)MN.

One would expect the C_6H of dihydronicotinamides to appear at much lower fields than C_5H because the C_6 is directly bonded to an electronegative nitrogen. Thus the C_6H of N-benzyl-1,4-dihydronicotinamide (8) appears 220 Hz* downfield from C_5H. However, the data in Table I show that in the cases of reduced mono- and dinucleotides, the C_6H appears either at a higher field than C_5H or at a slightly lower field than C_5H! This is probably related to the fact that the dihydropyridine is held in a syn conformation. (Fig. 2). In a syn conformation, the C_6H is located on the same side as the -OH groups

*The original data converted to 220 MHz NMR systems.

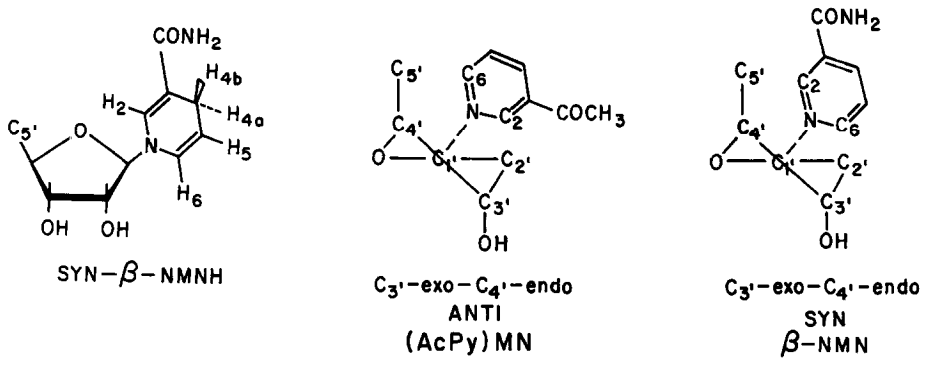

Fig. 2. The syn conformation of β-NMN and β-NMNH and the anti conformation of (AcPy)MN. The ribose conformations are taken from Sarma and Kaplan (6).

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of the ribose and resides juxtaposed to them. Then the magnetic anisotropic shielding of the -OH groups can prevail on C_6H and this in turn could offset the deshielding effect by the electronegative ring nitrogen. Comparison of the chemical shifts of C_2H and C_6H of α- and β-DPN in Table I shows that the D-ribofuranose shields the protons of the base which reside in space on the same side as the hydroxyl groups like the α-isomer. Even though β-NMN exist in syn conformation, the C_6H appears at fields lower than C_5H because the anisotropic shielding of the C_6H by the neighboring -OH groups of D-ribofuranose is not sufficient to offset the strong deshielding effect from the contiguous positively charged ring nitrogen which is part of an aromatic conjugated system.

Figs. 3A and 3B respectively show the anti-syn and the anti-anti overlap between the base pairs in α-DPN, β-DPN and TPN. From the discussion presented above, one could easily discard the anti-anti overlap. In addition, it could be seen very clearly that the geometry in Fig. 3B would cause the adenine to shield the C_6H of nicotinamide more than C_2H, contrary to the data in Table I. The upfield shift data in Table I indicate that α-DPN, β-DPN and TPN do not have exactly identical stacked conformations; but the anti-syn overlap in Fig. 3A would describe the general features of the stacked conformation in all the three cases and that their conformations are remarkably similar. It

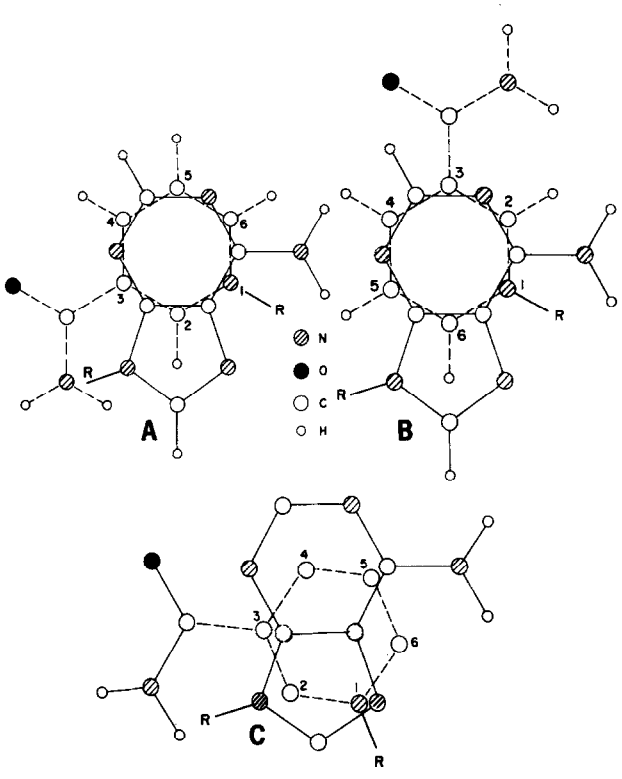

Fig. 3A. The anti-syn overlap between the base pairs in α-DPN, β-DPN and TPN. This overlapping structure is drawn from skeletal molecular models in which the adenine moiety is held in anti and the nicotinamide in syn conformations. The solid lines represent adenine and the broken lines the pyridine which lies below adenine. The letter R stands for the ribose adjacent to the base pairs. Schweizer et al. (7) have shown that in 5'AMP the adenine is held in the anti conformation. Bender and Grisolia (9) have concluded from rotatory dispersion and circular dichroism data that the adenine in pyridine nucleotides is in the anti conformation. Elsewhere we have presented evidence (1) that the six-membered ring of adenine is involved in the folding interaction in oxidized coenzymes. The overlap in 3A is such that the six-membered ring of adenine would shield all the pyridine protons; in addition pyridine C_2H would undergo additional shielding by the imidazole portion of adenine. 3B shows the anti-anti overlap between the base pairs. 3C shows the anti-syn structure of the stacked conformation in β-DPNH.

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is obvious that such similar dispositions between the base pairs in α- and β-DPN cannot have resulted if both have identical backbone conformations and this difference in the backbone conformation may account for the enzymatic inactivity of α-anomers. In the cases of the analogs (AcPy)DPN and (AcPy)TPN, the anti-anti overlap in Fig. 3B would, in general, describe the structure of the stacked conformation--only such overlap could explain the upfield shifts ob-

served in Table I. This difference in the molecular geometrics of β-DPN and (AcPy)DPN as well as TPN and (AcPy)TPN can have very important consequences with regard to steric course of reductions by dehydrogenases. Thus a syn arrangement of the pyridine ring is β-DPN and TPN and an anti arrangement of the same in (AcPy)DPN and (AcPy)TPN could change the sides of the pyridine ring to which the hydride is added during enzymatic reduction of the oxidized coenzymes and their acetylpyridine analogs by dehydrogenases and substrates.

The anti-syn overlap shown in Fig. 3C very closely describes the structure of the stacked conformations in β-DPNH and the general features of the stacked conformations of (AcPy)DPNH. The overlap is such that, on the average, the C_4 protons would spend considerable time in the strong shielding zone of the adenine like the center of the pyrimidine portion and hence would undergo the maximum shielding. Also, the interaction is such that the C_5H and C_2H would undergo pronounced shielding, the C_6H being shielded the least. The lack of shielding of C_5H in TPNH suggests that the stacked conformation of TPNH is different from that of β-DPNH and (AcPy)DPNH because of the bulky monoester phosphate. It is to be noted that the relative geometry between the pyridine and the ribose is anti in (AcPy)DPN and syn in (AcPy)DPNH. This is in keeping with our observation that during reduction of oxidized coenzymes, the ribose adjacent to the pyridine changes its conformation from C_3'-exo-C_4'-endo to C_2'-endo-C_3'-exo (3,4 and 6) and the new conformation of the ribose, in turn, determine its relative geometry with the pyridine base.

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