DELINEATION OF THE INTIMATE DETAILS OF THE BACKBONE CONFORMATION OF PYRIDINE NUCLEOTIDE COENZYMES IN AQUEOUS SOLUTION

Kurethara S. Bose and Ramaswamy H. Sarma
Department of Chemistry
State University of New York at Albany
Albany, New York 12222

Received August 13, 1975

SUMMARY: The noise free 300 MHz ^{1}H NMR spectra of β-DPN$^+$, recorded in the Fourier mode at 12°and 68°Chave been completely analysed by extensive computer simulation. It is shown, whether the coenzyme exists as an equilibrium mixture of folded $\rightleftarrows$ extended forms (12°C) or in overwhelminghly extended forms (68°C), the backbone of both the nicotinamide and adenine fragments preferentially exist in ^{2}E-gg-g'g' conformation. This orientation is significantly different from those reported in the solid state for the extended species in contact with the enzyme where ^{2}E-tg-g'g' and ^{3}E-tg-g'g' orientations have been observed. It is suggested that specific interactions of the backbone with the various amino acid residues in the enzyme induces conformational aberrations in the backbone. Intimate details of the backbone conformation of the extended forms of AcPy-DPN$^+$ and β-TPN$^+$ are also presented.

INTRODUCTION

There have been a number of studies on the aqueous solution conformation of pyridine nucleotides using such diverse tools as ^{1}H NMR spectroscopy, fluorescence transfer, circular dichrosim and temperature jump and a complete list of references upto 1972 is given in a series of three articles and a review paper on this subject from this laboratory (1-4). Recently there have been extensive ^{13}C NMR (5-7) and theoretical studies (8). Despite these intensive studies, one does not know the intimate details of the orientation of the backbone in aqueous solution for this important class of coenzymes. In this paper we present these details, obtained by completely analysing their complex 300 MHz ^{1}H NMR spectra. The detailed backbone conformation so derived is then compared to that reported from X-ray studies for the molecule in contact with the enzyme.

EXPERIMENTAL METHODS

The pyridine coenzymes were purchased from P. L. Biochemicals and the samples were lyophilized three times from 99.8% D_2O. The 0.05M solutions were prepared in '100'% D_2O with pD 7.5.^{1}H NMR spectra at 300 MHz were recorded in Fourier Transform mode at 12°and 68°C for β-DPN$^+$ and at 68° for AcPy-DPN$^+$ and TPN$^+$ using a Varian 300 MHz NMR System. The spectra were analyzed using a UNIVAC 1108 computer using LAOCN III. The lineshapes of the spectra were computer simulated from the derived data using a program developed in this laboratory. The experimentally observed and computer simulated spectra are given in Fig. 1. In view of the complexity of the spectra, the agreement between the observed and calculated spectra should be considered as an excellent one. The derived data are presented in Table 1.

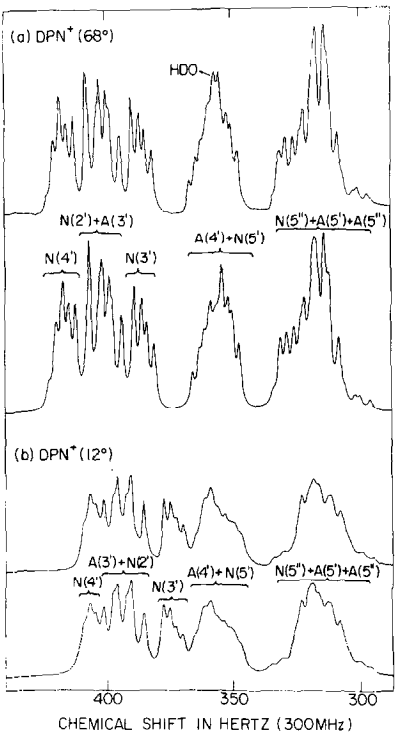
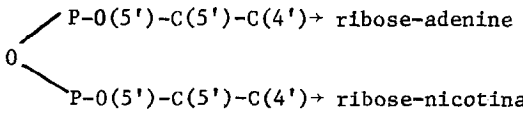


Fig. 1. Top of 1a shows the observed 300 MHz ^{1}HNMR spectrum of β-DPN$^+$, 0.05 M, pD 7.5 at 68°. The corresponding computer simulated spectrum is shown at the bottom of Fig. 1(a). Only the complex region from the backbone are shown. The designations A and N stand respectively for the adenine and nicotinamide fragments of the molecule. Fig 1(b) show respectively the observed (top) and computer simulated spectra of β-DPN$^+$ at 12°.

RESULTS AND DISCUSSION

Conformation of the Pyrophosphate Backbone: The backbone in the dinucleotide coenzymes contains the framework:



The energy minimum conformations about C(4')-C(5') and C(5')-O(5') are illustrated in I through VI. We have discussed elsewhere in considerable detail (9-12) how to translate the observed values of $J_{H(4')-P(5')}$, Σ and Σ' (Table 1) into conformer populations about the C(4')-C(5') and C(5')-O(5') bonds. The results of such a calculation are given in Table 2. The data reveal that the C(4')-C(5') and C(5')-O(5') bonds of both the adenine and nicotinamide parts of the coenzyme are flexible but show considerable preference for the gg (I) and g'g' (IV) orientations at the temperatures studied. The data further reveal that elevation of temperature and the

Table 1. Chemical Shifts and Coupling Constants of β-DPN, AcPy-DPN and β-TPN; 0.05M Solutions at pD 7.5. Data from 300MHz Spectra

	β-DPN (12°)		β-DPN (68°)		AcPy-DPN (68°)		β-TPN (68°)	
	A[a]	N	A	N	A	N	A	N
Chemical Shifts, (Hz)[c]								
H(1')	842.9	865.8	865.2	884.6	865.3	895.2	875.3	888.3
H(2')	472.0	389.9	469.1	406.5	471.2	408.8	776.6	400.3
H(3')	396.7	374.1	398.3	385.3	399.7	389.6	421.0	380.7
H(4')	358.8	406.6	356.5	417.3	355.0[b]	423.6	351.0[b]	406.8
H(5')	323.1	355.2	321.1	355.5	322.6	358.0[b]	328.2	431.9
H(5'')	308.1	316.5	309.4	322.2	311.6	324.7	298.6	313.6
H(8)	1561.1		1567.3		1568.4		1568.3	
H(2)	1460.4		1508.4		1507.2		1512.4	
H(2)		1840.9		1853.3		1857.7		1845.5
H(4)		1688.7		1714.6		1734.0		1711.5
H(5)		1497.7		1523.4		1530.2		1522.4
H(6)		1786.3		1809.0		1825.7		1799.4
Coupling Constants, J(Hz)								
H(1')-(2')	5.8	5.1	5.5	5.4	5.6	4.9	5.3	4.8
H(2')-(3')	5.2	5.2	5.3	5.2	5.3	5.2	5.2	5.2
H(2')-P(2')	-	-	-	-	-	-	5.65	-
H(3')-(4')	3.6	2.7	3.8	3.0	3.8	2.9	5.1	2.9
H(4')-(5')	3.2	2.4	2.8	2.8	3.1	2.1	2.7	2.4
H(4')-(5'')	3.4	2.2	4.0	2.5	3.8	2.7	5.3	2.7
$\Sigma=J_{4'-5'} + J_{4'-5''}$	6.6	4.6	6.8	5.3	6.9	4.8	8.0	5.1
H(4')-P(5')	~2.1	2.5	~1.8	2.1	-	2.4	-	2.4
H(5')-(5'')	-11.8	-12.0	-12.2	-11.8	-11.9	-11.6	-11.5	-12.0
H(5')-P(5')	4.6	4.1	5.0	4.5	5.2	-	5.0	4.3
H(5'')-P(5')	5.1	5.0	6.0	5.4	5.8	5.6	5.3	5.6
$\Sigma'=J_{H5'-P5'} + J_{5''-P5'}$	9.7	9.1	11.0	9.9	11.0	-	10.3	9.9

[a] A and N denote adenine-ribose and nicotinamide-ribose respectively.

[b] As these signals are overlaped by HDO peak the chemical shifts are approximate. The positions given are the ones used in computer simulation.

[c] Chemical shifts are given in hertz (300 MHz System) from tetramethyl ammonium chloride.

consequent increase in the population of the open form (13,14) has only a relatively minor influence on the time average backbone conformation of β-DPN$^+$. The close similarity in the values of gg and g'g' populations (Table 2) of the nicotinamide-ribose part of β-DPN$^+$, AcPy-DPN$^+$ and β-TPN$^+$ indicates the essential similarity of the backbone conformation of the nicotinamide-ribose portion in all these coenzymes.

Table 2. *Conformational Details of β-DPN, AcPy-DPN and β-TPN in the Solution State and DPN-Enzyme Complexes in the Solid State*

Conformation about:	Solution State				β-DPN-Enzyme Complexes in the Solid State	
	β-DPN (12°)	β-DPN (68°)	AcPy-DPN (68°)	β-TPN (68°)	Binary[a]	Ternary[a]
C(4')-C(5') bond A	gg (64%)	gg (62%)	gg (61%)	gg (50%)	tg (100%)	tg (100%)
C(4')-C(5') bond N	gg (84%)	gg (77%)	gg (82%)	gg (79%)	tg (100%)	tg (100%)
C(5')-O(5') bond A	g'g' (79%)	g'g' (72%)	g'g' (72%)	g'g' (77%)	g'g' (100%)	g'g' (100%)
C(5')-O(5') bond N	g'g' (83%)	g'g' (78%)	-	g'g' (78%)	g'g' (100%)	g'g' (100%)
Ribofuronose ring[b] A	^2E (66%)	^2E (64%)	^2E (64%)	^2E (50%)	^3E (100%)	^3E (100%)
Ribofuronose ring[b] N	^2E (75%)	^2E (72%)	^2E (73%)	^2E (73%)	^2E (100%)	^3E (100%)
Amplitude of pucker, τ_m, of adenine-ribose[c]	38°	37°	38°	42°	-	-

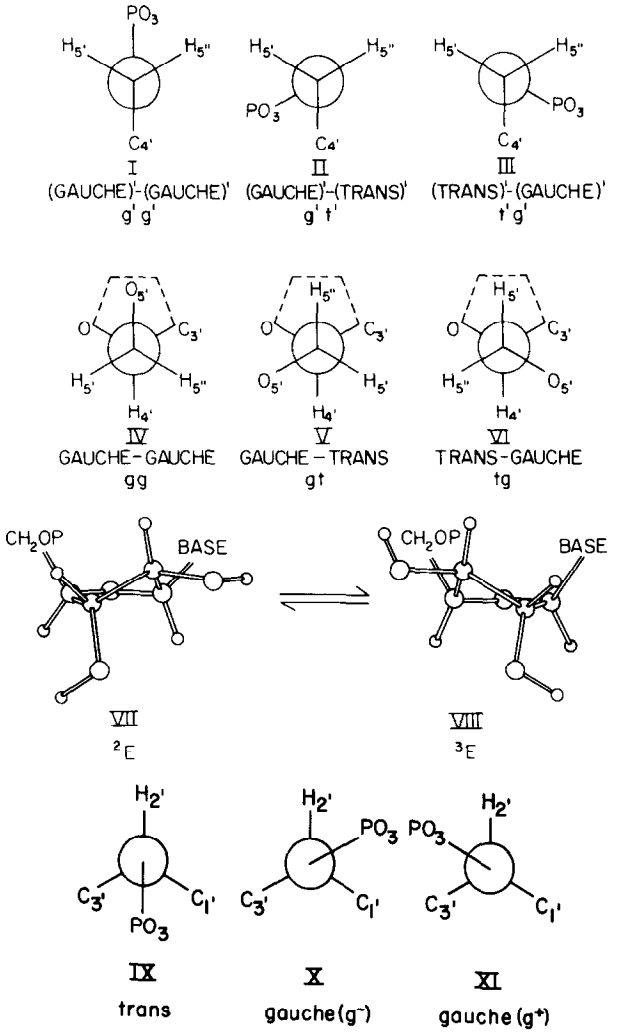
[a] Data from reference (23).

[b] Computed from $J_{3'-4'}$ using Altona-Sundaralingam approach. (16)

[c] Computed from reference (21).

The adenine-ribose gg and g'g' populations of β-DPN^+ and its acetyl derivative are almost identical at 68°; but the gg population of β-TPN^+ shows a significant relative reduction. This decrease is related to the presence of the additional 2'-phosphate group present in β-TPN^+.

Conformation of the D-Ribose Rings. In aqueous solution the D-ribose moiety of the nucleosides and nucleotides can be qualitatively described as a two state equilibrium $^2E \rightleftharpoons {}^3E$ (VII and VIII) although one is essentially dealing with a flexible ring system of continuously changing conformations (3). The ^2E populations of the ribose rings computed from the vicinal $J_{3'-4'}$ coupling constants are given in Table 2. For these calculations the $J_{3'-4'}$ coupling constants have been used rather than the $J_{1'-2'}$ because of the electronegativity difference in the base substituents at C(1'), nicotinamide-ribose having a positively charged nitrogen and adenine-ribose a neutral nitrogen at C(1'). Except in the case of the adenine-ribose part of β-TPN^+, the observed ^2E populations indicate a preference for the ^2E conformation on the part of the coenzyme ribofuranose rings. In the case of β-TPN^+, the ^2E:^3E populations is about 50:50. The lower value of the ^2E population should be attributed to the additional 2'-phosphate group present in the adenine-ribose ring. Temperature has



no significant effect on the relative population of ^{2}E and ^{3}E conformers (Table 2). The constancy in the sum $J_{1'2'} + J_{3'4'}$ in the case of the adenine-ribose of β-DPN^{+} at 12°and 68°C probably indicates that amplitude of pucker, τ_m, remains almost the same at both temperatures (15,16). But the nicotinamide-ribose shows an increase of 0.6 Hz in the above sum when the temperature increases from 12°to 68°C. This may indicate an increase in τ_m, as $J_{1'-2'} + J_{3'-4'}$ is expected to increase with an increase in τ_m (15,16). However, any such increase in τ_m should be very small because the magnitude of $J_{2'-3'}$ remains the same at both the temperatures (15,16).

Conformation of the 2'-Phosphate Group. From the known three bond $J_{P-O-C-H}$ coupling constants of approximately 21 Hz and 3Hz in the trans and gauche orientations (17-19) respectively the conformational populations of the three energetically favorable staggered conformations (IX, X, XI) about the C(2')-O(2') bond can be approximately

computed. The observed value of 6.7 Hz for $J_{H(2')-P(2')}$ for β-TPN^+ can be translated into ≃ 20% t (IX) and ≃ 80% g+/g- (X, XI) conformations. For reasons outlined elsewhere (20) one may assume that the trans (IX) conformation does not contribute toward the time average orientation of the 2' phosphate group. If this were the case the observed $^3J_{PH}$ values (Table 1) gives the approximate angle of orientation of the 2' phosphate group with respect to the H(2') as about ± 41° (g^+ and g^-, XI, X). An unambigous distinction between g^+ and g^- orientations is not possible. However, a comparison of the H(3') and H(1') chemical shifts in β-TPN^+ and β-DPN^+ (Table 1) shows that the 2' phosphate group in β-TPN^+ deshields H(3') more than H(1'). According to the effects of phosphate groups on the chemical shifts of neighboring protons (21), this observation may indicate a preference for g^+ orientation for the phosphate group in the present case.

Comparision of the Coenzyme Conformations on the Enzyme Surface and Solution: The preferred orientation of the backbone of β-DPN^+ on the enzyme surface as derived from the solid state X-ray crystallographic data are given in Table 2. The complexity of the electron density pattern in the X-ray diffraction studies have prevented a unique assignment of the coenzyme conformation on the enzyme surface and the available data are the best fit (22,23). The X-ray diffraction data of β-DPN^+ bound to cytoplasmic malate dehydrogenase in a binary complex (23) and dogfish M_4 lactate dehydrogenase in the ternary complex (22) with pyruvate indicate that the coenzyme exists in an extended conformation on the enzyme surface as the free coenzyme in aqueous solution at a higher temperature as 68°C. However the C(4')-C(5') bonds of the enzyme bound coenzyme are in the tg (VI) conformation, where as the preferred conformation of these two C(4')-C(5') bonds in the free β-DPN^+ in the extended form is gg (IV). A 5Å resolution X-ray structural analysis of β-DPN^+ dogfish lactacte dehydrogenase binary complex indicates a gg orientation; but the conformational assignments are rather arbitrary and hence less reliable. The observation that at both 12°C(folded ⇄ extended) and 68°C (overwhelmingly extended), for the free coenzyme, gg is the preferred conformation argues against the possibility that the existence of β-DPN^+ in the extended form is responsible for the unusual orientation of its C(4')-C(5') bonds while on contact with the enzyme. A comparison of the present NMR data with the X-ray results, thus, indicates a twist in the backbone conformation about the C(4')-C(5') bonds which preserves the solution g'g' conformational preference on the enzyme surface, but changes the solution gg preference to a tg conformational orientation about C(4')-C(5') bonds. This twist is probably a consequence of the extensive hydrogen bonding between the pyrophosphate group of β-DPN^+ and the protein residues suggested to occur in the DPN-enzyme complex (22,23). The X-ray structure of β-DPN^+ bound to cytoplasmic malate dehydrogenase (23) indicates a preferred ^3E conformation for the adenine-ribose and ^2E conformation for the nicotinamide ribose while the respective conformations are 3-endo in M_4

lactate dehydrogenase ternary complex (22). As has already been mentioned these derived ribose conformation are the best fit and are not unique assignments. The present NMR data indicate an appreciable preference by both the ribose rings of β-DPN^+ at 12° and 68°C for the ^2E conformation. As the conformational energy difference between the ^2E and ^3E forms are low the conformational preference of the ribose rings of the coenzyme molecule on the enzyme surface may be dictated by the various interactive forces between the sugar rings and the amino acids.

In Table 2 also given are the intimate details of the backbone conformation of AcPy-DPN^+ and β-TPN^+ at 68°C, a temperature at which these coenzymes are known to exist overwhelmingly in extended conformation. These data are provided with the hope that in the near future X-ray determinations of their conformation in contact with the enzyme will become available and a comparison of the type attempted in the present paper can be undertaken at that time.

ACKNOWLEDGEMENT

This research was supported by grants from National Cancer Institute of NIH (CA12462) and National Science Foundation (BO28015-001). One of the authors (K.S.B.) thanks Mr. Che-Hung Lee for helping with the computor simulation of the spectra.

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