Use of Fast Fourier Transform ^1H Nuclear Magnetic Resonance Spectroscopy to Determine the Helical Sense of Pyridine Dinucleotides

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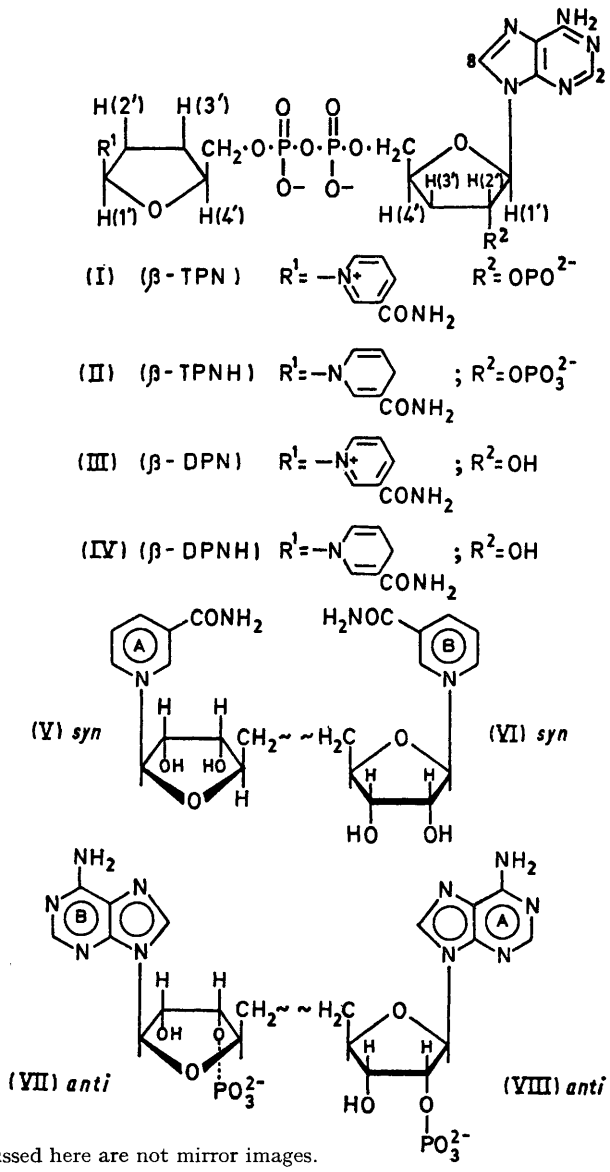
Summary Fast Fourier transform techniques have been used to determine the ^1H chemical shifts of pyridine coenzymes in the concentration range 0·4—0·001M and the data have been used to determine the helical sense of the dinucleotides.

MOLECULAR models show that the most probable† helical arrangements which the dinucleotides (I)—(IV) can assume are as follows. (a) The backbone of the dinucleotide makes a turn of a right-handed helix so that the B side (VII) of adenine interacts with the A side of pyridine (V). We designate this conformation as (*P*)-*B-anti-A-syn*, (*P*) describing the chirality, the first letter B followed by *anti* designating the side of adenine facing the pyridine and the conformation of adenine with respect to its glycosidic linkage, the second letter A followed by *syn* designating the side of pyridine facing the adenine and the conformation of the pyridine with respect to its glycosidic linkage. An alternate conformation (*P*)-*B-anti-B-syn* can be generated by torsional variation of the backbone. (b) The backbone of the dinucleotide makes a turn of a left-handed helix so that the A side of adenine (VIII) stacks over the B side of pyridine (VII). This conformation is designated as (*M'*)-*A-anti-B-syn*.‡ An alternate conformation (*M'*)-*A-anti-A-syn* can also be created by torsional variation of the backbone.

In the (*M'*)-helical system, the B side of adenine resides outside the helix and the entire B surface and the nearby environment are free from substituents from the D-ribose fragment (VII). Hence, if a dimer is formed between two (*M'*) helices one would expect the two B surfaces of the two molecules involved to stack in parallel planes and cause considerable ring-current upfield shifts of the adenine 2-H, 8-H, and 1′-H and relatively small upfield shifts of adenine 2′-H and 3′-H. In the (*P*)-helical system, the A side of adenine lies outside the helix and the neighbourhood of this side is highly crowded from substituents originating from the D-ribose (VIII). This is particularly so for β-TPN (I) and β-TPNH (II) where the bulky 2′-phosphate group will hinder a free close overlap between two A sides, should a dimer form between the two (*P*) helices. The ring-current upfield shifts caused by such stacking interactions between



† Jardetzky and Wade-Jardetzky[1] have proposed 64 possibilities.

‡ (*M'*) is used rather than (*M*) because the two helical forms discussed here are not mirror images.

two (*P*) helices will be considerably smaller compared to those involving two (*M'*) helices.

Fast Fourier transform techniques enabled us to obtain high quality ^{1}H n.m.r. spectra of β-TPN, β-DPN (III), β-TPNH and β-DPNH (IV) in the concentration range 0·001—0·4M. Contrary to Jardetzky and Wade-Jardetzky's report,[1] the chemical shifts of pyridine coenzymes show a concentration dependence (Figure 1). Significant concentration-dependent perturbations of adenine resonances suggest the formation of dimers in which intermolecular stacking occurs between juxtaposed adenine fragments. The pair β-TPN and β-DPN shows very dramatic difference in their concentration profiles and for the first time, we have been able to observe experimentally shifts to lower fields originating from stacking interactions (Figure 1). If both β-DPN and β-TPN existed as (*M'*)-helices one would not expect them to show any differences in their concentration profiles because in the (*M'*)-helices, as described earlier, the B surface of adenine (VII) (whose environment is free from substituents from ribose) is involved in stacking interactions in dimer formation. We have constructed the most probable stacking arrangement between the adenine fragments of two (*P*)-*B-anti-A-syn* molecules of β-TPN (Figure 2), and experimental and theoretical data for this model are in the Table. Given the uncertainty involved in the measurements of *z* and *p* (Table) and the assumptions involved in the calculations,[2-4] the agreement of the theoretically predicted direction and magnitude of the shifts to the corresponding observed ones is good. Such an agreement enables us to conclude that a single molecule of β-TPN exists in the (*P*)-helical form and dimerization involve two (*P*)-helices. The data do not indicate whether the molecular geometry of β-TPN is (*P*)-*B-anti-A-syn* or (*P*)-*B-anti-B-syn*. In the case of β-TPNH similar treatment of concentration data leads to the conclusion that the molecule may exist as (*P*)-*B-anti-B-anti* or (*P*)-*B-anti-A-anti*. In view of the present findings

TABLE

The horizontal (z) *and in plane* (p) *axis for the protons in* β-TPN *dimers shown in Figure* 2.

Protons	*p*/Å[a]	*z*/Å[a]	Observed Δδ/Hz[b]	Ring current zone[c] Adenine[d]	Benzene[e]	— Benzene[f]
A-2-H	3·0	3·6	−16·0	s	−20 Hz	−8·1 Hz
A-8-H	5·0	3·6	0·0	n	n	n
A-1'-H	4·8	3·3	0·0	n	n	n
A-2'-H	5·1	2·5	+7·5	d	+6·2 Hz	+2·2 Hz
A-3'-H	7·5	3·4	+5·0	d	d	+1 Hz

[a] Measured values for *p* and *z* are accurate only to ± 0·5 Å. [b] − refers to upfield shifts, + refers to downfield shifts. [c] s = shielding, n = neutral, d = deshielding. [d] From ref. 2. [e] From ref. 3. [f] From ref. 4.

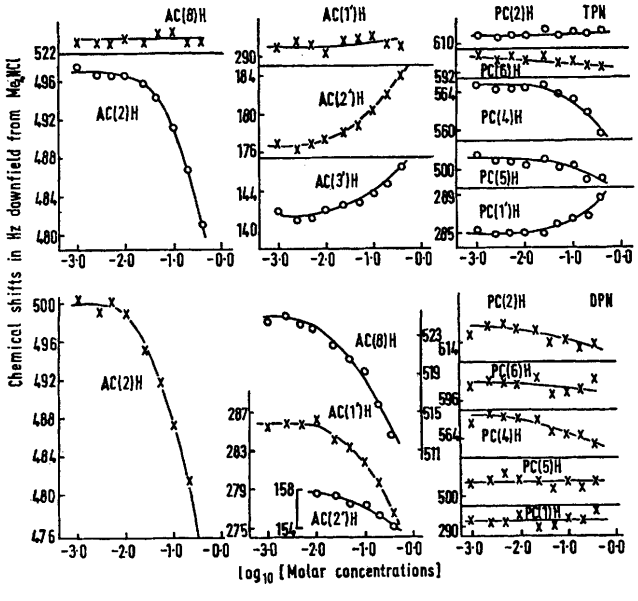


FIGURE 1. *Concentration dependence of the chemical shifts of* β-TPN (*top*) *and* β-DPN (*bottom*). (*A* = *adenine*, *P* = pyridine). *Chemical shifts are expressed in Hz* (100 MHz) *downfield from* $Me_4N^+Cl^-$. *The previous assignment*[1] *of* AC(1')H *and* PC(1')H of β-TPN *was found to be erroneous.*

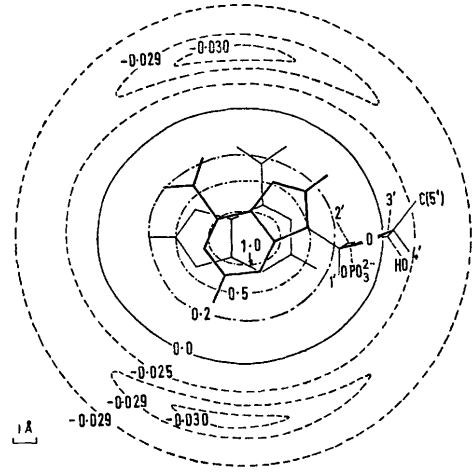


FIGURE 2. *Geometric orientation between the two adenine fragments in the dimer of the* (P)-B-anti-A-syn *conformation of* β-TPN. *Isoshielding lines* (z = 3·4 Å) *are from ref.* 2. *Ribose in the diagram appears puckered, but no puckering is intended other than to show the various atoms of the ribose.*

one must conclude that the concept of a slow exchange between the (*P*) and (*M'*)-helices originally proposed by Patel[5] and later adopted by Sarma and Kaplan[6] is not true at least for β-TPNH and β-TPN.[7]

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[7] Data given by N. Oppenheimer, L. Arnold, and N. O. Kaplan (*Proc. Natl. Acad. Sci. U.S.A.*, 1971, **68**, 3200) demonstrate that the evidence suggested for a slow exchange was due to an erroneous interpretation which resulted from the inherent poor homogeneity of the first 220 MHz n.m.r. spectrometer.