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Conformation of Pyridine Dinucleotides in Solution*

John Jacobus

ABSTRACT: Evidence which has been previously presented for a helical conformation of pyridine dinucleotides is reinterpreted. Possible alternate conformations are discussed and it is concluded that the experimental data cannot be interpreted to define unambiguously the molecular geometry of pyridine dinucleotides.

Investigations directed toward the elucidation of the conformation of pyridine dinucleotides have yielded experimental data which has been rationalized on the basis of a helical model (Sarma *et al.*, 1968a,b, 1970; Sarma and Kaplan, 1969a,b, 1970a,b). A reinterpretation of the experimental data indicates that although a helical model may be correct, more prosaic alternatives are plausible, and the choice between a number of these alternate interpretations of the experimental data does not lead to a unique molecular geometry of dinucleotides in solution, *i.e.*, the data can be interpreted without consideration of any particular model of the molecules in question and need not be a consequence of the helicity of these molecules.

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Definitions

Nuclei or groups of nuclei in molecules can be classified on the basis of symmetry (Mislow and Raban, 1967). The ramifications of such classification as applied to nuclear magnetic resonance spectroscopy, as well as to chemical phenomena in general (Mislow, 1966), have been enunciated (Mislow and Raban, 1967). In order to facilitate the ensuing discussion, these classifications will be defined and employed. Numerous examples have been presented elsewhere (Mislow and Raban, 1967) and will not be reiterated.

Nuclei or groups of nuclei in molecules that can be interconverted by a rotational symmetry operation (Mislow, 1966) are defined as *equivalent*. Equivalent nuclei or groups reside in equivalent environments; such nuclei or groups are isochronous (chemical shift equivalent) in nuclear magnetic resonance spectroscopy.

Nuclei or groups of nuclei in molecules that can be interconverted by a reflectional, but not by a rotational, symmetry

TABLE 1: Nuclear Magnetic Resonance Consequences of Symmetry Based Classifications.

Type of Nuclei or Group	Solvent	
	Achiral	Chiral
Equivalent	Isochronous	Isochronous
Enantiotopic	Isochronous	Anisochronous
Diastereotopic	Anisochronous	Anisochronous

operation are defined as *enantiotopic*. Enantiotopic nuclei or groups reside in enantiomeric environments; such nuclei or groups are isochronous in achiral circumstances, *e.g.*, achiral solvents, but are, in principle, anisochronous (chemical shift nonequivalent) in chiral circumstances, *e.g.*, chiral solvents. Such protons are also chemically distinct in chiral circumstances, *e.g.*, in enzymatic processes.

Nuclei or groups of nuclei in molecules that have the same connectivity (are joined to the same atom in the same way) but that cannot be interconverted by any symmetry operation are defined as *diastereotopic*. Diastereotopic nuclei or groups reside in diastereomeric environments; such nuclei or groups are always, in principle, anisochronous. Such protons are, in principle, chemically distinct in all circumstances, chiral or achiral.

The nuclear magnetic resonance consequences (Mislow and Raban, 1966) of symmetry based nuclei or group classification are summarized in Table I.

The term *diastereomer* shall be employed, as advocated by Mislow (1966), to include all stereoisomers which are not enantiomers.

Discussion

The Sarma–Kaplan helical model for pyridine nucleotides is, in essence, based on the following experimental observations. (1) Nuclear magnetic resonance chemical shift differences in the adenine and nicotinamide residue protons as a function of pH (Sarma *et al.*, 1968a,b). (2) Differential shielding of the C-2 and C-6 nicotinamide protons, stated to be "symmetrically" disposed with reference to the *N*-glycoside bond (Sarma and Kaplan, 1969a). (3) An AB quartet in the nuclear magnetic resonance spectrum of some reduced pyridine nucleotides for the two C-4 nicotinamide protons (Sarma and Kaplan, 1969b, 1970a). (4) The appearance of four *N*-methyl resonances in the nuclear magnetic resonance spectrum of MENAD[1] (Sarma *et al.*, 1970).

These data have been interpreted in terms of a helical model, *viz.*, if a helical model is *a priori* assumed, and if it is further assumed that decreasing the pH (increasing the acidity) of the medium results in an unfolding of the helical molecule, the chemical shift data *can* be interpreted as indicative of unfolding. With the same *a priori* assumption, differential shielding of the C-2 and C-6 nicotinamide protons *can* be interpreted as consistent with the assumed structure if a particular conformation of the nicotinamide residue relative to the "juxtaposed" adenine ring is chosen. Similarly, with the assumed model the α and β C-4 nicotinamide protons of NADH would reside in different environments, would be chemically and magnetically nonequivalent, and would thus exhibit an AB spectrum. Further, if a particular conformation of the nicotinamide residue relative to the "juxtaposed" adenine ring is chosen, and if both the s-cis- and s-trans-torsional diastereomers about the nicotinamide–amide linkage exist, four *N*-methyl resonances should be observed for MENAD.

The rationalizations enunciated in this summarized work, although appealing in light of the research objective, *viz.*, the elucidation of the conformation of nucleotides, must be considered speculative. Alternate interpretations of these data, which do not require a helical model, are possible and plausible, yet have not been considered thus far.

Oxidized Nucleotides

If one considers conformations of nicotinamide dinucleotides in which *both* the P and M helices exist, it is immediately obvious that *the stereochemical relationship of the two helices is that of diastereomers, and not enantiomers*. In general, the nuclear magnetic resonance chemical shifts of corresponding nuclei in diastereomers are different, *i.e.*, the resonance lines are anisochronous, and "resonance doubling" is observed (Raban and Mislow, 1967; Jacobus *et al.*, 1968; Jacobus and Raban, 1969; Jacobus and Jones, 1970). Thus, if two helices (P and M) are present, it would be predicted that single resonances for any nuclei in the molecule should not be observed, *i.e., all resonances should be doubled.* Inspection of the published nuclear magnetic resonance spectra (Sarma *et al.*, 1968a, Figure 5, p 3056; 1968b, Figure 1, p 4360; Sarma and Kaplan, 1969a, Figure 1, p 781) indicate that *this is not the case*, *e.g.*, the C-2 nicotinamide proton of β-DPN is a singlet, the C-2 nicotinamide proton of α-DPN is a singlet, and the C-2 nicotinamide proton of β-NMN is a singlet, as are all other resonances in the absence of hydrogen spin–spin coupling.

These observations, uncommented upon by the original authors, can be rationalized by *any* of the following models. (1) A helical model, consisting of both P and M helices, in which the nonequivalent diastereotopic hydrogen resonances from the P and M helices are vanishingly small, *i.e.*, the signals are accidentally coincident. (2) A single static helix, either P or M, which is not interconverting with other helical or linear species. (3) A "helical" model in which an infinitely rapid equilibrium, on the nuclear magnetic resonance time scale, exists between the P and M helices and all torsional diastereomers and in which only an average chemical shift for the various nonequivalent diastereotopic proton resonances is observed. (4) A nonhelical model constrained to a single torsional diastereomer about some bond or bonds linking the nicotinamide and adenine residues. (5) A nonhelical model in which all molecular torsional modes are of low energy. Thus, there are at least five models which are consistent with the original data, ranging from a helical model to a nonhelical model.

Attempted rationalization of any of these models on the basis of pH-dependent chemical shifts is hazardous. Protonation of the molecule at any site will produce torsional variations which will be transmitted throughout the entire molecule. Even subtle torsional variations will result in torsional diastereomers, which *cannot* be expected to exhibit the same chemical shifts a the original molecule. Thus, it is dubious that such data can be relied upon to define molecular geometry.

[1] Abbreviation used is: MENAD, *N*-methyl-*N*-ethylnicotinamide–adenine dinucleotide.

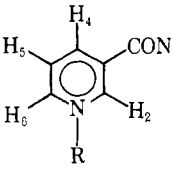
Rationalizations of the geometric disposition of the base pairs in oxidized and reduced pyridine nucleotides (Sarma and Kaplan, 1969a) on the basis of C-2 and C-6 nicotinamide chemical shifts have been advanced. The statement: "If the pyridine ring is able to rotate freely, a rotation of 180° around the glycosidic bond would enable the C-2 H and C-6 H protons of the pyridine ring to exchange their positions with respect to the plane of the juxtaposed adenine ring because the C-2 H and C-6 H are located symmetrical (*sic*) to the glycosidic bond and then the adenine ring would shield the C-2 H and C-6 H to the same extent . . ." (*loc. cit.*, p 781) is untenable; any torsional mode about the nicotinamide–ribose bond will result in conformational changes in the entire molecule, such changes altering *all* intramolecular distances, which in turn will alter *all* shielding and deshielding parameters. The rationalizations provided, in which specific intramolecular interactions are chosen in support of a particular model, a helix, in which the conformation of the base pairs is defined, must be dismissed as fanciful. The observed chemical shift differences of various resonance signals in the compared molecules (mono- to dinucleotides and di- to trinucleotides) are most succinctly rationalized as being intimately connected with some *undefined* conformational change in the total structure of the molecule as the backbone is altered, *i.e.*, such shifts may or may not be associated to molecular helicity.

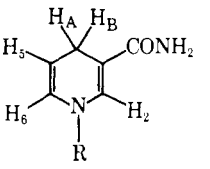
Interesting, though uncommented upon, chemical shifts are those of β-TPN relative to β-DPN in which the nicotinamide C-2 and C-6 protons are shifted upfield 13.5 and 16.5 Hz, respectively, the TPN resonances appearing at higher field than the corresponding DPN resonances, whereas in β-TPNH relative to β-DPNH the C-2 proton is shifted downfield 5.5 Hz and the C-6 proton is shifted 6.9 Hz upfield (Table II). Of more interest is the shift of the C-4 proton of β-TPN relative to β-DPN, the β-TPN resonance 10.3 Hz upfield from that of β-DPN, indicating that, at least in an assumed helical structure, the intramolecular ring interactions in TPN are "stronger" than those in DPN, in harmony with the related C-2 and C-6 proton shifts. However, the two C-4 protons of β-TPNH relative to β-DPNH are both shifted downfield, indicative of "weaker" interactions in TPNH than in DPNH, and the shifts in opposite directions of the corresponding C-2 and C-6 proton resonances defy plausible interpretation. Any of these shifts could be rationalized with subtle conformational changes, not necessarily associated with helicity, provided a proper model is selected. We defer such rationalization as fanciful speculation.

The quadrupling of the *N*-methyl resonance of MENAD has been cited as evidence that specific torsional diastereomers about the amide bond of two helical conformations exist (Sarma *et al.*, 1970). Although helical conformations can be employed to rationalize the multiple *N*-methyl resonances in MENAD, they are not *necessary*. *Any* molecular restraint, slow on the nuclear magnetic resonance time scale, will serve to quadruple the *N*-methyl resonance if both the s-cis- and s-trans- (s refers to the "single" carboxyl-nitrogen amide bond) diastereomers are present (Jacobus and Jones, 1970). Thus, any of the following models will suffice to rationalize the multiplicity of the *N*-methyl resonance; the list is not intended to be exhaustive. (1) A nonhelical species, consisting of s-*cis*- and s-*trans*-amide diastereomers, with slow, on the nuclear magnetic resonance time scale, rotation about *any* bond linking the adenine and nicotinamide residues. (2) A hydrogen-bonded species (coplanar), consisting of s-*cis*- and s-*trans*-amide diastereomers, with slow, on the nuclear magnetic resonance time scale, rotation about any bond linking the adenine and nicotinamide residues. (3) A single helical species, either P or M, consisting of s-*cis*- and s-*trans*-amide diastereomers, constrained to two torsional diastereomers about the nicotinamide C-1′ bond. (4) Two helical species (P and M), consisting of s-*cis*- and s-*trans*-amide diastereomers, constrained to a single torsional diastereomer about the nicotinamide C-1′ bond. (5) Two helical species (P and M), consisting of only a single s-*cis*- or s-*trans*-amide diastereomer, constrained to two torsional diastereomers about the nicotinamide–C-1′ bond.

In light of the discussion concerning the interpretation of a single nicotinamide C-2 proton in NAD (*vide supra*), we defer choice as to the most attractive of these alternatives.

TABLE II: Chemical Shifts of Pyridine Nucleotides.[a]





Compound	C-2 H	C-6 H	C-5 H	A	C-4 H	B
β-NMN	2112.0	2052.0	1828.5		1978.5	
β-DPN	2055.5	2016.5	1806.3		1942.3	
β-TPN	2042.0	2000.0	1795.0		1932.0	
β-NMNH	1555.0	1072.0	1084.0	642.7		642.7
β-DPNH	1510.5	1051.5	1041	560.5		581.9
β-TPNH	1516.0	1044.6	1084.3	575.6		601.4

[a] Data from Sarma and Kaplan (1969a); chemical shifts relative to 2,2-dimethyl-2-silapentane-5-sulfonate at 220 MHz.

Reduced Nucleotides

Examination of *the two C-4 nicotinamide protons in reduced nucleotides*, in light of the chiral moieties in the backbone of the molecule, immediately reveals that they *are diastereotopic*. Rotation by 180° about the nicotinamide–ribose bond (not a symmetry operation since the original molecule is not restored) does *not* interconvert the two protons in relation to the whole molecule; *the protons are not equivalent*. Reflection of the total molecule does not interconvert the two protons in relation to the whole molecule, such reflection simultaneously converts *d*-ribose into *l*-ribose; *the protons are not enantiotopic*. The spacial relationship of the two protons in relation to the rest of the molecule can never become equivalent; *the protons are diastereotopic and*, whether observably or not, *chemically and magnetically nonequivalent*, in principle, *under all conditions*. The observed magnetic nonequivalence or accidental coincidence of these protons (Sarma and Kaplan, 1969b, 1970a) is not a function of any specific conformational disposition of the molecule, and no molecular process conceivable for this molecule can serve to make the protons equivalent.

Statements to the effect that the observation of two C-4 protons in reduced nucleotides make the following conclusions possible. (1) There is no rotation about the dihydropyridine–ribose glycosidic linkage at rates exceeding about

10^2 sec^{-1}. (2) The reduced pyridine nucleotides exist in a folded conformation. (3) The existence of two types of dehydrogenases one stereospecific to the A side of the ring and the other stereospecific to the B side, is not surprising in view of the nonequivalence of the electronic and geometrical environments of these two protons, are untenable (Sarma and Kaplan, 1969b). The nonequivalence is not a function of rotation or folding, and the nonequivalence of the two protons is demonstrable even with achiral reagents (Sarma and Kaplan, 1970a). The two C-4 protons are intrinsically diastereotopic and should not be expected to react at identical rates or exhibit identical nuclear magnetic resonance chemical shifts in any conformation, helical or nonhelical. Failure to observe nuclear magnetic resonance nonequivalence cannot be construed as evidence for chemical or magnetic equivalence; the chemical shift difference is simply too small to be observed, *i.e.*, the resonance frequencies are accidentally coincident, not equivalent.

The observation of two resonance signals for the single nicotinamide C-4 proton of (*R*)-DPND has been presented as evidence that a rapid exchange between the P and M helices is not occurring. Such resonance doubling can occur if *any* conformational change in the molecule is slow on the nuclear magnetic resonance time scale. Thus, the statement: "The observation of the peaks B and B′ (Figure II) from (*R*)-DPND is consistent only with a slow exchange between the P and M helices" (Sarma and Kaplan, 1970a, p 545) is untenable; any slow torsional process in a linear molecular array will produce the same result.

Summary

The conformation of pyridine nucleotides in solution, on the basis of the presently available experimental evidence, cannot be unambiguously assigned. Failure to observe expected resonance doublings of various nicotinamide and purine proton resonance signals in a number of nucleotide derivatives is most consistent with a model with complete torsional freedom over the entire molecule or a model possessing a singular molecular restraint. The observation of four *N*-methyl resonance signals for MENAD is consistent with any model possessing two molecular restraints. *Attempts to apply any particular model of a pyridine coenzyme to rationalize coenzyme–enzyme–substrate interactions or to rationalize the mechanisms of enzymatic processes must, at present, be considered extremely hazardous.*

Added in Proof

Recent X-ray structure determination of the lactate dehydrogenase–NAD enzyme–coenzyme complex indicates that the NAD moiety is an essentially linear array in the complex (Adams *et al.*, 1970). Although the geometry of NAD associated with an enzyme cannot be construed to reflect the geometry of NAD in solution, the X-ray structure serves to illustrate an important concept; even if the structure of NAD and NADH could be unequivocally designated in solution, in the course of the approach of the coenzyme to an enzyme and subsequent binding thereto, it cannot be expected that the solution conformation of the coenzyme would remain unaltered.

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