Correction. In the article "A Structure of Pyridine Nucleotides in Solution," by Oppenheimer, N. J., Arnold, L. J. & Kaplan, N. O., which appeared in the December 1971 issue of Proc. Nat. Acad. Sci. USA 68, 3200-3205, the column heads for Table 1, p. 3202, should read:

\[ \text{PC₄H₄} \quad \text{PC₄H₄B} \quad \text{J₅₄A} \quad \text{J₅₄B} \]

Correction. In the article "Effect of Dibutyryladenosine 3':5'-Cyclic Monophosphate on Growth and Differentiation in Caulobacter crescentus," by Shapiro, L., Agabian-Keshishian, N., Hirsch, A. & Rosen, O. M., which appeared in the May 1972 issue of Proc. Nat. Acad. Sci. USA 69, 1225-1229, the bacteria—Escherichia coli B—shown in Table 3, p. 1228, should read: Escherichia coli Crookes.

Correction. In the article "Insulin Receptors in Human Circulating Cells and Fibroblasts," by Gavin, J. R., III, Roth, J., Jen, P. & Freychet, P., which appeared in the March 1972 issue of Proc. Nat. Acad. Sci. USA 69, 747-751, the fifth line from the bottom of the right-hand column on p. 747 should read "Tris buffer [25 mM Tris-10 mM dextrose-1 mM EDTA-1.4 mM sodium acetate-5.0 mM KCl-120 mM NaCl-2.4 mM MgSO₄-1% bovine serum albumin (pH 7.4)]."

A Structure of Pyridine Nucleotides in Solution
(pyridine coenzymes/epimerization/cyanide adducts/220 MHz NMR/ring conformation)

NORMAN J. OPPENHEIMER, LYLE J. ARNOLD, AND NATHAN O. KAPLAN

Department of Chemistry, University of California at San Diego, La Jolla, Calif. 92037

Contributed by Nathan O. Kaplan, October 12, 1971

ABSTRACT Re-examination of the structure of pyridine coenzymes in solution by use of the 220-MHz high-frequency nuclear magnetic resonance spectrometer indicates that there is primarily one folded structure that is in rapid equilibrium with an open form. Reduced DPN+ and reduced analogs of DPN+ exist predominantly with the B side of the dihydropyridine ring folded against the adenine moiety. (The oxidized coenzymes appear to exist in the same folded structure.) Furthermore, the ribose protons undergo very little conformational change upon reduction of the pyridine ring; this observation strongly suggests a considerable similarity between the folded forms of the oxidized and reduced coenzymes. A model of the folded structure is presented.

From early experiments with the 60-MHz nuclear magnetic resonance (NMR) instrument, it was suggested that many folded forms of the pyridine coenzymes might exist in solution. More recent studies from our laboratory on these coenzymes with the high-frequency 220-MHz NMR instrument suggested the possibility that two folded forms of DPN+ as well as TPNH (P + M helices), exist in solution. These two folded structures were thought to be in slow equilibrium with each other through an intermediate open form. Careful re-examination of the spectra, together with new data, indicate that such an interpretation is not correct and that probably only one distinct folded conformation of the coenzyme, which is in rapid exchange with an open structure, exists in solution. Here, we give evidence that we feel with some certainty describes the structure of the folded form of the coenzyme. In addition, we discuss the reasons why we believe it was erroneously concluded that two helical forms existed rather than one.

MATERIALS AND METHODS

TPN+, DPN+, DPNH, (AcPy)DPN+, (AcPy)DPNH, and α-DPN+ (P-L Biochemicals) were used without further purification. β-NMN+ was prepared with purified snake-venom diesterase, and deaminio DPN+ was prepared by deamination of DPN+. (1). NMNH and α-DPNH were prepared by chemical reduction with dithionite (2), as were the compounds nonspecifically labeled with deuterium α-DPNDαβ, β-DPNDαβ, β-NMNDαβ, and deaminio DPNDαβ by dithionite reduction in D2O. DPND and (AcPy)DPND labeled in the A position were synthesized by incubation of 50 μmol of the oxidized nucleotide with 5 μmol of [U-3H]ethanol (Mallinckrodt, 99.8% D) and horse liver or yeast alcohol dehydrogenase (P-L Biochemicals) in 3 ml of 0.05 M (NH4)2CO3, titrated to pH 10.5 with NH4OH. The extent of the reaction was followed spectrophotometrically and, after more than 90% of the oxidized form was converted to the reduced form, the reaction was quenched by the addition of 10–12 drops of 2 M Ba(SCN)2. The solution was then centrifuged and any precipitate was discarded. The volume was increased to 5 ml with acetone; once again the solution was centrifuged and the precipitate was discarded. The addition of two volumes of acetone (10 ml) and cooling in an ice bath produced a flocculent, pale-yellow, barium nucleotide precipitate. This precipitate was collected by centrifugation and washed with a small amount of acetone to remove any remaining Ba(SCN)2. The barium precipitate was then dissolved in water and converted to the sodium salt by the addition of sodium sulfate until no additional barium sulfate precipitate was formed. The white barium sulfate precipitate was removed by centrifugation and the remaining liquid was lyophilized to give an 80% yield of the respective A-labeled nucleotides. Spectrophotometric analysis showed the nucleotides to be more than 98% reduced.

DPND and the other reduced analogs labeled on the B-side were produced by coupling the reduction of the oxidized coenzyme to dithiopentrol through pig heart diaphorase (3). Typically, 28 μmol of the oxidized coenzyme was reacted with 65 μmol of dithiopentrol in the presence of 5 μmol of lipamide, and enough pig heart diaphorase (Sigma lot 73B-1560) to complete the reaction in about 15 min, when 5 ml of 0.05 M NH4HCO3 in D2O was used. Again, the extent of the reaction was followed spectrophotometrically and the reduced nucleotides were purified by barium precipitation, as described above, except that after the initial addition of barium, a small amount of NH4OH was added to bring the pH to about 8. Cyanide adducts were formed by titration of the acid salt of the oxidized coenzyme in D2O with 1 M KCN in D2O to pD 8.6.

The proton magnetic resonance (PMR) spectra were recorded on a Varian HR-220 Nuclear Magnetic Resonance Spectrometer with field sweep. Decoupling was done with a Wavetek 131A voltage-controlled generator. Computer averaging was done on a Fabritek Computer Model 1074. The
spectra were routinely run at a high spinning rate (>100 revolutions per sec), with a Teflon plug inserted to prevent vortexing, at 20–21°C (as measured with an ethylene glycol standard). The samples were lyophilized twice in D₂O to remove exchangeable protons and were run as 200- to 250-μl samples at 40–50 mM concentration. The pD measured was the pH read on a Corning model 12 pH Meter + 0.4. The residual HDO absorption was routinely tuned to less than 1.0 Hz. Sodium 2,2-dimethyl-2-silapentane-5-sulfonate (B. Merck Ag Germany) was used as the internal standard and the samples were run in D₂O (Mallinkrodt 99.9%).

**STRUCTURE OF REDUCED PYRIDINE**

Patel (4) and Sarma and Kaplan (5, 6) have interpreted their data as being consistent with a model in which DPND exists in two folded forms, termed the $P$ and $M$ helices. Such a conclusion was derived from the following observations:

i. In DPND specifically labeled with deuterium on the pyridine $C_4$ A-side, it was reported that the pyridine $C_4$ B-side proton give two resonances 14-Hz apart. This evidence was interpreted as being “consistent only with a slow exchange between the $P$ and $M$ helices.” (6).

ii. The pyridine $C_4$ protons of DPND were found to be an asymmetric A-B quartet. It was thought that, “the observed asymmetric A-B quartet results from the overlap of the two expected A-B quartets from the two unequally populated, nonequivalent helical forms of DPND” (6).

iii. It was found that the $C_4$ protons in (AcPy)DPNH appeared as a broad singlet. This was consequently interpreted as probably being due to the fast exchange between two helical forms (6).

**Specific Labeling.** We have reinvestigated the PMR spectra of specifically labeled DPND and some of its analogs and have observed that all of the pyridine $C_4$ protons of the A-side and B-side labels appear as singlets. Lines c, d, and e of Fig. 1 show the specific labels for DPND and of Figs. 2 and 3 the specific labels for (AcPy)DPND. These labeling experiments do not agree with earlier studies (6). It was found, however, that DPND specifically labeled with deuterium on the A-side by the use of the same materials as in the original investigation gave the lower spectrum of Fig. 4. The partially observed A-B quartet indicates that this “specifically” labeled sample is obviously contaminated by a substantial quantity of unlabeled DPND. It was also found that upon oxidation of this material to DPN⁺ with lipo-dehydrogenase, a B-specific enzyme, the pyridine $C_4$ position was contaminated with 30% hydrogen. This indicates that the original “specifically” labeled DPND₄H₈ contained about 30% DPND. Furthermore, with a slight amount of detuning, i.e., recording of the spectrum at lower than optimal resolution, we have generated the upper spectrum of Fig. 4, which is essentially identical to that found earlier (6). The origin of the contamination that produced the unlabeled DPND is obscure, since both yeast and horse liver
enzymes have subsequently given us “pure” labels (as shown in Fig. 1).

These findings indicate that our earlier DPND preparations were most likely contaminated by DPNH. Hence, it appears that the combination of the impure DPND, plus improper tuning, gave the spurious double-peak spectrum for the C\textsubscript{4} proton in DPND, which in turn led to the incorrect interpretation that there were two helical conformations in slow exchange. As a result, it is our strong belief that caution with respect to resolution should be exercised with the 220-MHz instrument in order to prevent similar artifacts as the one shown in Fig. 4.

Fast Exchange. Our specific labeling studies indicate that DPNH, (AcPy)DPNH, TPNH, deaminodPNH, and \(\alpha\)-DPNH exist in fast exchange between their various conformations with respect to the NMR time scale, since the resonances appear as single peaks that result from the rapid averaging of all conformations.

Asymmetric A-B Quartet. It is clearly observable from Fig. 1 that the A-B quartet of the pyridine C\textsubscript{4} protons of DPNH is not symmetrical, but that the downfield A-proton is higher than the upfield B-proton. This apparent asymmetry can be accounted for by puckering of the dihydropyridine ring, which causes the upfield B-proton to be more highly split by the pyridine C\textsubscript{4} proton than the downfield A-proton, and by an \(\alpha\)-DPNH contaminant formed by epimerization (this will be discussed below). Consequently, an asymmetric A-B quartet in DPNH is not necessarily consistent with “two unequally populated, nonequivalent helical forms of DPNH” (6).

Nicotinamide Ring Puckering. As can be seen in Figs. 1, 2, and 3 and Table 1, a difference exists between the spin–spin coupling constants of the pyridine C\textsubscript{4} A and B protons to the pyridine C\textsubscript{4} proton, not only in \(\beta\)-DPNH but also in TPNH, and in the other reduced analogs as well. This difference in coupling constants is consistent with a puckered dihydropyridine ring, in which the B-proton moves equatorial and the A-proton moves axial. This preferential puckering (see Fig. 5), which is nonexistent in the respective mononucleotides and for the dinucleotides at high temperature or in CD\textsubscript{3}OD, is brought about by the folding of the dihydronicotinamide ring against the adenine ring. This observation shows that when DPNH and its analogs are folded, they exist predominantly in a conformation or conformations with the pyridine C\textsubscript{4} B-proton towards the adenine ring and pyridine C\textsubscript{4} A-proton away from the adenine ring. This observation would also indicate that these reduced nucleotides do not exist as a pair of \(P\) and \(M\) helices, since nearly equal populations of \(P\) and \(M\) helices would tend to average out any puckering interactions.

**Chemical-Shift Differences of Pyridine C\textsubscript{4} Protons.** With regard to the above model, one would, \(a\ priori\), expect a considerable chemical-shift difference between the A and B pyridine C\textsubscript{4} protons, due to differential anisotropic shielding by the adenine ring. However, since the dihydronicotinamide ring is puckered, the B-proton experiences a large diamagnetic deshielding with respect to the A-proton, due to the adjacent C-C single-bonds and the ring double-bonds (see Fig. 5) (7). This effect is directly opposed to that produced by the adjacent adenine ring. As a result, the chemical shift one observes between the A- and B-protons is the small difference between these large opposing effects. This observation explains why some dinucleotides such as (AcPy)DPNH can have nearly magnetically equivalent pyridine C\textsubscript{4} protons, and yet be folded as mentioned above.

\((\text{AcPy})\text{DPNH, an Unresolved A-B Quartet.}\)** As can be seen in Figs. 2 and 3 and Table 1, specific labeling experiments and spectra taken at low temperature clearly show that the pyridine C\textsubscript{4} protons of (AcPy)DPNH do not appear as a singlet, but as an unresolved A-B quartet. At lower temperature, it is possible to resolve the side peaks of the A-B quartet; at 7°C, the A-B quartet as shown in Fig. 3 is consistent with the superimposed stick diagram calculated by the use of a value of 11.5 Hz for the chemical-shift difference obtained from specific labeling experiments and a geminal, spin–spin coupling constant of 19 Hz measured from DPNH. As indicated by Figs. 1, 2, and 3, deuteration shifts the resonance about 3-Hz upfield due to the slight shielding of the remaining proton by deuterium.

**Epimerization.** The pyridine C\textsubscript{4} protons occur as an asymmetric A-B quartet with regard to both their height and area (6). While the asymmetry in height could be explained by the difference in the spin–spin coupling constant to the pyridine C\textsubscript{4} proton, this effect alone cannot explain the difference in area, which can be as great as 20%. This difference in area can be explained on the basis of a slow epimerization of the pure \(\beta\)-DPNH to about a 10:1 mixture of \(\beta\)-DPNH with the enzymatically inactive \(\alpha\)-DPNH form, and the fortuitous appearance

### Table 1. Chemical-shift difference of the PC\textsubscript{4}H\textsubscript{2} at 20°C

<table>
<thead>
<tr>
<th></th>
<th>PC\textsubscript{4}H\textsubscript{8}</th>
<th>PC\textsubscript{4}H\textsubscript{4}</th>
<th>J\textsubscript{5-4A}</th>
<th>J\textsubscript{1-7A}</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\beta)-DPNH</td>
<td>599.2</td>
<td>576.8</td>
<td>2.5</td>
<td>3.6</td>
</tr>
<tr>
<td>TPNH</td>
<td>612</td>
<td>588</td>
<td>2.5</td>
<td>3.8</td>
</tr>
<tr>
<td>(AcPy)DPNH</td>
<td>557.8</td>
<td>548</td>
<td>2.4</td>
<td>3.8</td>
</tr>
<tr>
<td>DeaminodPNH</td>
<td>652.2*</td>
<td>531.2*</td>
<td>2.2</td>
<td>3.9</td>
</tr>
<tr>
<td>(\alpha)-DPNH†</td>
<td>600</td>
<td>590.5</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>(\beta)-NMMH</td>
<td>671.5</td>
<td>671.5</td>
<td>3.4†</td>
<td>3.4†</td>
</tr>
</tbody>
</table>

The concentration was 40–50 mM, pD 8.5. The chemical-shift differences are \(\pm 0.1\) Hz, while the absolute chemical-shift is \(\pm 1.0\) Hz. Measured directly from the PC\textsubscript{4}H in the specifically labeled coenzyme by spin decoupling the PC\textsubscript{4}H.

* 7°C.
† Arbitrary assignment of A and B protons.
‡ Measured directly from the PC\textsubscript{4}H.

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**Fig. 5.** Model for \(\beta\)-DPNH. This illustrates the puckering that takes place when the dihydronicotinamide ring folds against the adenine ring. It also shows the opposing effects controlling the chemical differences of the A and B protons.
ance of the α pyridine C₄ protons as a singlet under the central
downfield peak of the β-pyridine C₄ A-B quartet (Fig. 1).

Mild heating causes extensive epimerization of α-DPNH to
ezymatically active β-DPNH (8). We have found that heating
of pure α-DPNH or β-DPNH at 100°C in an inert atmosphere
for a few minutes yields an equilibrium mixture of
10:1 β-DPNH to α-DPNH. Thus, enzymatically prepared
β-DPNH quickly acquires different trace amounts of α-DPNH
as a contaminant (Fig. 6). It can be seen from the 220-MHz
NMR spectra of α-DPNH (Fig. 7) that the pyridine C₄ protons
come as a broad, unresolved A-B quartet (as determined
by nonspecific labeling) centered at 597 Hz. (See Table 1).
At 21°C, the downfield central peak of the β pyridine C₄
A-proton comes at about 597 Hz, right on top of the two-proton peak of the α-DPNH. Thus, an α-DPNH contaminant
of as much as 10% can give a 20% enhancement in the area
under the β pyridine C₄ A-proton. We have not observed the
epimerization of oxidized α-DPN⁺ and β-DPN⁺ with heating.

Misassignment of PC₃ - PC₄ Protons. In 60-MHz NMR
studies of the pyridine nucleotides, it was observed that upon
reduction of DPN⁺ to DPNH, little change occurred in the
region that corresponds to 1300 Hz at 220 MHz. Studies (9,
10) on ribose and deoxyribose bases have indicated that ab-
sorptions in this region were C₄ proton, thus, the two
overlapping doubles in DPNH were also assigned as the
adenine and pyridine C₄ ribose protons (11). With the 220-
MHz NMR spectrometer, it was possible to resolve these
peaks clearly; the sharp downfield doublet was assigned to
the adenine C₄ proton and the broad upfield doublet was
assigned to the pyridine C₄ proton (12). Furthermore, it was
found that a doublet of triplets and a sharp doublet resided
under the residual-water peak at normal temperature. At
elevated temperatures, these peaks separated from each other
and from the water peak and were assigned as the pyridine C₃
and pyridine C₄ proton, respectively (6).

It is quite evident that there are problems with these as-
signments. The model compounds N-methyl-1,4-dihydropy-
rinamide (13) and 1-(2,6-dichlorobenzyl)-1,4-dihydropy-
rinamide (14), clearly show in their 100-MHz PMR spectra
that not only is the pyridine C₄ proton found about
1.5-ppm downfield from the pyridine C₃ proton, but also there
are extensive long-range spin–spin interactions with the
pyridine C₃ and C₄ protons. Conversion of these results to
220-MHz chemical shifts shows that the pyridine C₄ proton
would come as a broad doublet at 1340 Hz in the N-methyl-
and at 1380 Hz in the 1-(2,6-dichlorobenzyl)-1,4-dihydropy-
rinamide. The pyridine proton absorptions in these model
compounds all come within 0.1 ppm of their corresponding
pyridine absorptions in unfolded DPNH if the broad doublet
in DPNH assigned as the “pyridine C₄’ ribose” proton is
assigned as the pyridine C₃ proton. The broadening of the
“pyridine C₄’ ribose” proton was accounted for by invoking
nitrogen quadrapole-coupling between the pyridine nitrogen
and the C₄’ ribose proton, based on the apparent observation
of increased broadening at high temperature (12). As can be
seen from Figs. 6 and 8, the “pyridine C₄’ ribose” proton of
DPNH does not appreciably broaden with elevated tempera-
ture, suggesting that the broadening is indeed due to unre-
solved spin–spin coupling instead of quadrapolar or kinetic
relaxation. This unresolved, long-range spin–spin coupling
would explain why the protons of the dihydropyridine ring
appear considerably broader than do the protons of the ade-
nine ring.

Furthermore, PMR temperature studies of DPNH (see
Fig. 9 of ref. 6) clearly show that the pyridine C₃ proton,
which is supposedly spin coupled to the sharp doublet of the

![Fig. 6. Adenine C₄ region of β-DPNH computer-averaged 16 times, 10% α-DPNH contamination resulting from incubation at 100°C for 5 min.](image)

![Fig. 7. 20 mM α-DPNH, pH 8.2, at 220 MHz.](image)
isomers

no

with

and

74°C,

where

can

conclusively,

5.5

DPN+

5.5

"pyridine

C₆"

proton,

is able to move through this doublet

with no evidence whatsoever of any second-order distortions

that would characterize such a coupled system where the

crystal-shift differences are of the same order of magnitude

as that of their coupling constant, as is the case of the pyridine

C₄ protons.

Thus, there is a strong evidence for the assignment of the

broad doublet at 1315 Hz as the pyridine C₄ proton, and not as

the pyridine "C₁' ribose". To demonstrate this assignment

conclusively, spin-decoupling experiments were undertaken

at 74°C, where the pyridine C₄ proton is clearly visible.

The results can be seen in Fig. 8; the broad doublet that has shifted

to 1330 Hz decouples the pyridine C₄ proton and vice versa,

which proves that it is indeed the pyridine C₄ proton. Further,

it was observed that the sharp doublet at 1050 Hz could be
decoupled by irradiation of a partially hidden triplet at 1025 Hz,

and thereby assigning these two protons as the pyridine C₁' and

C₄' ribose protons, respectively.

The correct assignment of the pyridine C₄ and pyridine C₁' ribose protons has great significance in formulating a structure

for the reduced pyridine coenzymes. It can be seen from Table

2 that the conformation of the pyridine ribose has undergone

little change upon reduction, i.e., the configuration remains

essentially the same as that of the oxidized pyridine ribose,

contrary to previous reports. With use of the correct assign-

ment, the pyridine C₁' ribose proton is observed to shift 1.4-
ppm upward upon reduction, much more in line with the effects

that would have been expected when the magnetic and

chemical environment of that proton is changed from adjacent

to the positively charged nitrogen of the aromatic pyridine

ring to adjacent to an uncharged, nonaromatic dihydropyridine

ring of DPNH. Thus, the nonequivalence of chemical

shifts observed in the ¹⁹F NMR spectra of the pyrophosphate

backbone of DPN⁺, as compared to their chemical equiva-

lence in DPNH (16), would appear more likely to be the

result of interaction between the ¹⁹F and the positively

charged pyridine ring than due to changes in the pyrophos-

phate configuration. Finally, without the proper assignments

of the pyridine C₂ and C₄ protons, it has been impossible

previously to reach any conclusions about the orientation of the
dihydropyridine moiety with respect to the ribose ring (17).

**Oxidized Nucleotides**

**Cyanide Adducts.** DPN⁺ and the oxidized analogs cannot

be studied directly as can DPNH, since they do not possess a

pyridine C₄ methylene group. DPN⁺ and the oxidized analogs
do give clues to their folded conformation when their cyanide

adducts are studied. First, cyanide can add to either side of

the pyridine C₁ carbon to produce two isomers of the cyanide

adduct, as can be seen in Fig. 9. These nonequivalent isomeric

adducts have different PMR spectra in aqueous solution, so

that the observed spectra consist of the superposition of their

**Table 2. Ribose coupling constants**

<table>
<thead>
<tr>
<th></th>
<th>A₂₁-₄</th>
<th>A₂₂-₄</th>
<th>A₂₃-₄</th>
<th>P₂₁-₄</th>
<th>P₂₂-₄</th>
<th>P₂₃-₄</th>
</tr>
</thead>
<tbody>
<tr>
<td>DPN⁺</td>
<td>5.5</td>
<td>5.0</td>
<td>4.5</td>
<td>5.4</td>
<td>6.1</td>
<td>2.3</td>
</tr>
<tr>
<td>DPNH</td>
<td>5.5</td>
<td>5.5</td>
<td>3.8</td>
<td>7.4</td>
<td>4.0</td>
<td>*</td>
</tr>
<tr>
<td>NMN⁺</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>5.8</td>
<td>5.2</td>
<td>2.0</td>
</tr>
<tr>
<td>NMNH</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>7.1</td>
<td>5.4</td>
<td>2.1</td>
</tr>
<tr>
<td>AMP</td>
<td>5.5</td>
<td>5.8</td>
<td>5.9</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>(AcPy)DPN⁺</td>
<td>6.0</td>
<td>5.0</td>
<td>*</td>
<td>5.2</td>
<td>4.6</td>
<td>2.7</td>
</tr>
<tr>
<td>(AcPy)DPNH</td>
<td>5.5</td>
<td>4.5</td>
<td>4.5</td>
<td>6.2</td>
<td>*</td>
<td>*</td>
</tr>
</tbody>
</table>

Values are ±0.2 Hz, pD is about 8.5 and concentration is
about 40–50 mM at a temperature of 20°C. These coupling
constants are all consistent with a C₁'-exo-C₁' endo configuration
of the ribose ring (10).

* These coupling constants were obscured by other absorptions.
Pyridine adenine ring orientation as dinucleotide adducts. Studies of the DPNH and the reduced analogs exist predominantly with the B-side of the dihydropyridine ring folded against the adenine ring; furthermore, this folded interaction puckers the dihydropyridine ring in such a manner that the B-proton moves equatorial and the A-proton moves axial. From cyanide-adduct studies, (AcPy)DPN⁺, and probably the other oxidized analogs, fold with primarily one side of the pyridine ring against the adenine ring. Interestingly, both chemical oxidation by ferricyanide and reduction by hydrosulfite show a marked preference for the removal and addition of the pyridine C₆ A-proton (21). This observation suggests that the specific folded conformation of the oxidized dinucleotides, as indicated by the cyanide adducts, maintains the same pyridine–adenine ring orientation as in the reduced dinucleotides. This hypothesis is further supported by the fact that the ribose protons of the oxidized dinucleotides undergo very little conformational change upon reduction, indicating that no major conformational changes have taken place.

We have preliminary results that indicate that the structures for the oxidized and reduced coenzymes are in fact identical; a full discussion of these findings will be presented in a later publication.

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