PREPARATION AND PROPERTIES OF 3-CYANO PYRIDINE AD$^+$, A NEW ANALOGUE OF NAD$^+$

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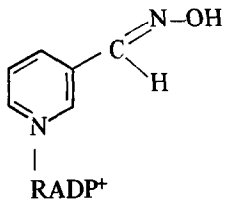
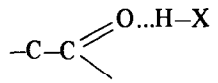
From a consideration of NAD$^+$ and its known alcohol dehydrogenase-active analogues, it is seen that only those compounds having a C=O or C=S group in the 3-substituent and suspected on thermodynamic grounds of being active in redox systems catalysed by dehydrogenases have been tested, with the exception of *1*

1



which is active with LADH and YADH** [1]. It therefore appeared interesting to us to determine the possible activity of the analogue of NAD$^+$ having a nitrile function in place of the amide group. The nitrile group participates in hydrogen bonding by means of the nitrogen lone pair of electrons [2], but the geometry of the hydrogen bonded system C–C≡N...H–X is different from the system



It is thus evident that replacing the amide group of NAD$^+$ by a cyano group will allow determination of the bonding site of NAD$^+$. The exchange method (transglycosidation) used by Kaplan et al. [3], which is catalysed by DPNases extracted from animal tissue, cannot be used for the preparation of 3-cyano Py AD$^+$. The latter is not surprising in view of the fact that 3-cyano pyridine is a weak base ($pK_a \simeq 1.45$). It seems that the lower pK_a limit for exchange by DPNase extracts of beef spleen and pig brain is of the order of 3.1 [3, 4].

The facile preparation of thio-NAD$^+$ proved useful for this preparation, since the thio-NAD$^+$ could be converted to 3-cyano Py AD$^+$ by silver or mercury (II) ions [5]. Experiments with mercury (II) chloride in the presence of primary amine [6] were unsuccessful with thio-NAD$^+$ as substrate; on the other hand, thio-NADH was converted to 3-cyano Py ADH under these conditions, but a mixture of products resulting from secondary reactions was obtained. However, the use of silver nitrate [7] gave 3-cyano Py AD$^+$ in 70 to 80% yields from thio-NAD$^+$.

3-cyano Py AD$^+$ has an absorption maximum in the U.V. at λ_{max} = 259 nm (ϵ = 17,100). In an aqueous molar solution of potassium cyanide, a new absorption band appears at 322 nm (ϵ = 5,000) and the absorption at 260 nm decreases (ϵ = 16,200). In the presence of ethanol and YADH we notice two absorption bands: λ_{max} = 324 nm (ϵ = 3,900) and λ_{max} = 260 nm (ϵ = 15,000).

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** *Abbreviations:*
3-cyano Py AD$^+$: 3-cyano pyridine adenine dinucleotide;
thio NAD$^+$: thio nicotinamide adenine dinucleotide;
LADH: the alcohol dehydrogenase extract of horse liver;
YADH: the alcohol dehydrogenase extract of yeast.

These spectroscopic results are compatible with a 1,4-dihydropyridine [8]. The 3-cyano Py AD^+, like all 3-cyano pyridinium salts, is unstable in alkaline media [9].

The position of the redox equilibrium of 3-cyano Py AD^+, determined by the oxidation of ethanol in the presence of YADH, was $K = 10^{-8}$ mole l^{-1}. We have determined the Michaelis constants of 3-cyano Py AD^+ with LADH and with YADH. These are listed in table 1.

Table 1

	NAD^+	3-Cyano Py AD^+
YADH	1.5×10^{-4} mole l^{-1} (2.8×10^{-4} at pH 7.8 [10])	2.8×10^{-4} mole l^{-1}
LADH	7.5×10^{-5} mole l^{-1} (7×10^{-6} [11])	1.4×10^{-4} mole l^{-1}

0.1 pyrophosphate buffer (pH 7.5)
EtOH 0.2 M for YADH
0.02 M for LADH

The equilibrium constant for the redox equilibrium is higher than that of 3-acetyl Py AD^+ (2.8×10^{-9} mole l^{-1}) [13], in agreement with the strongly electron withdrawing properties of the nitrile group.

The Michaelis constant of 3-cyano Py AD^+ with YADH is close to that of NAD^+. From this we concluded that either the contribution of the hydrogen bond of the group at position C-3 to the stability of the enzyme-coenzyme complex is weak or that the enzymatic group taking part in this bond does not have a rigid geometry. It can be estimated that the separation between the lone pair of the carbonyl C–C=O and the lone pair of the nitrile C–C≡N in NAD^+ and its analogue, when the Pyridinium nuclei are placed in the same position, is of the order of 1 Å.

The Michaelis constant for NAD^+ with LADH, as determined in this work, was about ten times greater than reported values (II), and is thought to be a result of the commercial enzyme preparation used; however the ratio K_m, NAD/K_m 3-CN Py AD is about the same for LADH and YADH. The Michaelis constant for 3-acetyl Py AD^+ is comparable to that of NAD^+ with both enzymes [13]. Thus it seems that for the two enzymes studied, there is no absolute steric requirement for the 3-substituent on the coenzyme Pyridinium ring.

Experimental

The thio-NAD^+ was prepared by the transglycosidation method [14] and freed from NAD^+ by chromatography on Dowex-1.

The thio-NAD^+ (200 mg), dissolved in acetate buffer 0.2 M (6 ml) pH 4.5, was treated with a 0.1 N silver nitrate solution in 1:1 methanol-water (6 ml). After a few minutes, 1 N HCl was added and the black precipitate was centrifuged. Desalting of the clear solution on Sephadex G-10 and chromatography on Dowex-1 $HCOO^-$ yielded 3-CN Py AD^+ (eluted from Dowex using 0.3 N formic acid). Lyophilisation of eluate from Dowex gave a white solid which was shown to be pure by chromatography on a DEAE column.

The treatment of this product with NAD^+ nucleotidase (pig brain) liberated 3-cyano Pyridine as detected by thin layer chromatography.

The 3-cyano Py AD^+ is not very stable. Within a month at 4°C in solution at pH 6, a slow displacement of the λ_{max} of the dihydro species from 324 nm to 330 nm was observed. After the action of the nucleotidase, in addition to 3-cyano Pyridine, nicotinamide was also detected.

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