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NAD(P)$^+$-NAD(P)H Model. 43. Formation of 1,4-Dihydronicotinamide in the Reaction of Pyridinium Salt and Glyceraldehyde

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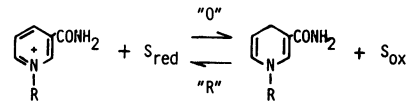
It has been found that *N*-substituted 1,4-dihydronicotinamides are produced by the reaction of *N*-substituted 3-carbamoylpyridinium salts with glyceraldehyde and its analogous compounds. A mechanism of the reaction is suggested.

The reduced and oxidized forms of nicotinamide adenine dinucleotide act as redox reagents in biological reactions. The reactions have been accepted with great interest because of a variety of excellent features promised by enzymes and have prompted organic chemists to design model reactions. As one of typical model compounds for NAD$^+$ and NADH, 1-benzyl-3-carbamoylpyridinium salt (BNA$^+$) and 1-benzyl-1,4-dihydronicotinamide (BNAH) have widely been used.



NAD$^+$: R = RPPA NADH : R = RPPA

BNA$^+$: R = CH_2Ph BNAH : R = CH_2Ph

(RPPA represents the dinucleotide residue of NAD$^+$)

As to the reaction of "R" direction many kinds of model reactions have been achieved and valuable insights have been accumulated.[1] However, its reverse reaction, that is, the reduction of pyridinium salt to 1,4-dihydropyridine[2] as a model reaction of "O" direction has not so widely been investigated despite the redox reactions between NAD$^+$ and NADH are reversible in biological systems. This makes it difficult to establish a cyclic reaction system where an NADH model is catalytically used.[3]

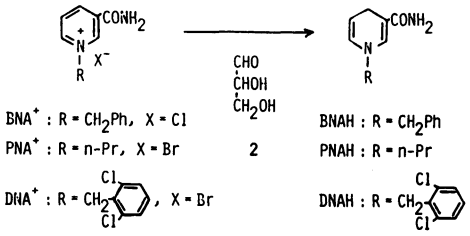
For the preparation of BNAH from BNA$^+$ sodium dithionite is an effective and exclusive reductant from the view points of the yield and selectivity toward 1,4-dihydropyridine derivative over its 1,6-isomer. On the other hand, the reducion with sodium borohydride produces large amounts of 1,6-dihydropyridine and tetrahydropyridine derivatives as well as the desired product. The use of other reductants such as sodium cyanoborohydride[4] and formic acid[5] the 1,4-selectivity does not afford good results, neither.

As a model for alcohol dehydrogenases, reduction of pyridinium salt by alkoxides[6] and alcohol[7] has been reported, although the results are not satisfactory. Deazaflavin[8] and 3-hydroxymethylacridinium iodide[9] that have partial structures similar to NAD$^+$ can be reduced by alcohol easily and various excellent reactions including cyclic reaction systems are reported. However, these systems are hardly be regarded as suitable models for NAD$^+$. From this standpoint it is desired to establish more efficient system for "O" directed reaction using, especially, organic compounds as reductants.

It is well known that glyceraldehyde 3-phosphate dehydrogenase (GAPDH) catalyzes the reduction of NAD$^+$ by glyceraldehyde 3-phosphate (**1**) coupled with oxidative phosphorylation of **1**.[10] The model reaction of this enzyme has not been reported yet. Previously it was reported that glyceraldehyde (**2**) reacts with NAD$^+$ to form a product which has a UV-absorption at 340 nm, similarly to NADH. The product, however, had no ability as the reduced coenzyme.[11] This product was thought to be an adduct of NAD$^+$ and **2** containing a 1,4-dihydropyridine moiety. When we tried the reaction of the same type using BNA$^+$ and **2**, reduced product, BNAH, was obtained in addition to such an adduct.[12] We will describe herein the details of the reaction as well as the reaction mechanism.

Results and Discussion

A borate-buffered solution of BNA$^+$ and **2** was refluxed in the presence of chloroform. The color of the aqueous layer turned yellow. At the same time reduced product with a UV-absorption at 355 nm was extracted to the organic layer. The product was ascertained to be BNAH by comparison of its NMR spectrum with that of the authentic sample. It was also confirmed that the extracted product was almost pure *N*-substituted 1,4-dihydronicotinamide contaminated by less than 3% of its 1,6-isomer. No other product was detected. The results are summarized in Table 1.



BNA$^+$: R = CH_2Ph, X = Cl BNAH : R = CH_2Ph

PNA$^+$: R = n-Pr, X = Br PNAH : R = n-Pr

DNA$^+$: R = CH_2(Cl, Cl), X = Br DNAH : R = CH_2(Cl, Cl)

The reduction must be carried out under alkaline conditions. Though the rate of the reaction increased at elevated pH's, the yield of BNAH was not improved appreciably. The yield was also independent of the presence or absence of air and light.

Limitation of structure for the reductant was examined. Sugars such as D-ribose and D-glucose, simple aldehydes such as formaldehyde, acetaldehyde, and glyoxal exerted no ability for the reaction of BNA$^+$. Since α-ketols are known to be good reducing agents for flavins,[13] they might be promising candidates for the

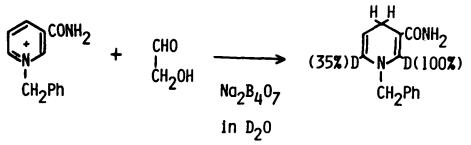
Table 1. Reduction of pyridinium salts[a]

Pyridinium salt[b]	α-Ketol[b]	pH	Reaction time/h	Yield of 1,4-dihydronicotinamide/%[c]
BNA+[d]	**3**	9.3— 9.5	18	22
BNA+	**2**	8.5— 8.7	30	22
BNA+	**2**	9.3— 9.5	18	21 (15)
BNA+	**2**	9.9—10.0	18	17 (11)
BNA+	**2**	11.4—11.5	10	8
PNA+	**2**	9.3— 9.5	18	(7)
BNA+	**3**	9.3— 9.5	18	21 (17)
DNA+	**3**	9.3— 9.5	3	(19)
BNA+	**4**	9.3— 9.5	18	22 (17)
DNA+	**5**	9.3— 9.5	18	(14)
BNA+	**6**	9.3— 9.5	14	25
BNA+	**7**[e]	9.1— 9.2	3 d	(4)

a) In 0.05 M $Na_2B_4O_7$ in the presence of chloroform at its reflux temperature. b) 0.01 M. c) Yields based on pyridinium salt were determined by the intensity of an absorbance at 355 nm (ε=6100 for BNAH). Values in parentheses indicate the yields measured on an NMR spectrometer using *p*-nitrotoluene as an internal standard. d) Reaction under nitrogen in the dark. e) 0.05 M.

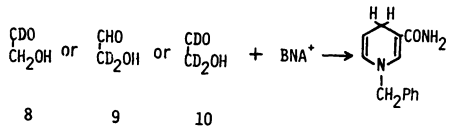
present reduction. As shown in Table 1, glycolaldehyde (**3**), dihydroxyacetone (**4**), 2-hydroxypropanal (**5**), acetol (**6**), and L-ascorbic acid (**7**) were effective. On the other hand, acetoin, methyl lactate, lactamide, and lactic acid were not.

In order to confirm the origin of the hydrogen on the 4-position of produced BNAH, several reactions were undertaken using deuterated materials. First of all the reaction of BNA+ with **3** was carried out in D_2O and isotope contents in BNAH were found to be 100% on the 2-position, 35% on the 6-position, and 0% on the 4-position, respectively. Since the hydrogens on the 2- and 6-positions of BNA+ are replaced by protons from the solvent appreciably under the same conditions as that for the reaction described above except for the absence of **3**, there is no doubt that deuteriums in the BNAH produced were introduced before the reduction and the hydrogens on the 4-position came from somewhere else other than water.



This result excludes the mechanism which, as suggested for the reduction of flavin,[14] involves an electron-transfer process from the enediol derived from the corresponding α-ketol to the model. The isomerization of initially formed 1,6-isomer to the 1,4-derivative[15] is also implausible.

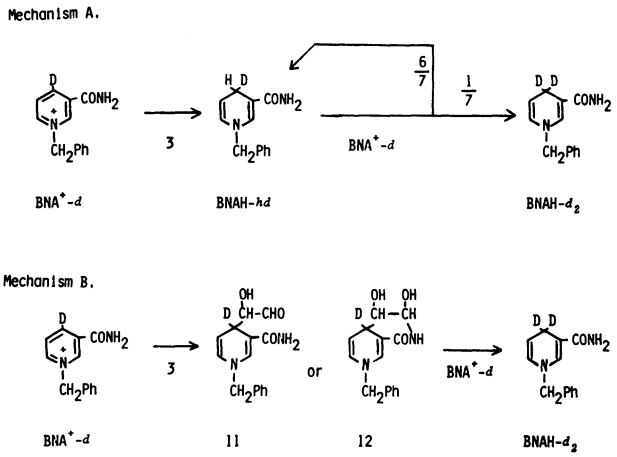
Secondly, deuterated glycolaldehydes were employed. Three types of mono- or dideuterated compounds (**8**, **9**, and **10**) were synthesized and subjected to the reaction with BNA+. In any case, however, produced BNAH contained no deuterium so far as in the limitation of NMR spectroscopy.



8 9 10

A suggested mechanism for the reduction of NAD+ catalyzed by GAPDH involves a Cannizzaro-type direct hydride transfer from the carbonyl carbon of **1** to the 4-position of NAD+, which is supported by the experiment using tritium labelled on the carbonyl carbon of **1**.[16] On the other hand, in the present system neither direct hydrogen transfer nor introduction of a proton from water was observed and the oxidized product from **3** could not be detected in spite of our best efforts.

When BNA+ whose 4-position was deuterated (BNA+-*d*) was employed for the reaction with **3**, both hydrogens on the 4-position of BNAH were deuterated.



Scheme 1.

In order to account for the result that the origin of the deuterium is not other than that on the 4-position of BNA+-*d*, following two possibilities can be considered. One is a mechanism which involves a fast transhydrogenation between BNAH and BNA+ and the other is the one which involves a formation of an adduct containing a 1,4-dihydropyridine moiety and the adduct is the true reductant (Scheme 1). Mechanism A shown in Scheme 1 indicates that by transhydrogenation, which is a well known reaction,[17] initially produced BNAH-*hd* reduces BNA+-*d* to afford BNAH-*hd* and BNAH-d_2 with the ratio of 6 to 1 because of the isotope effect of 6.[17] If the reaction was fast enough to occur 10 times before the BNAH is extracted, BNAH-d_2 might be accumulated in 80% of total BNAH. However, the transhydrogenation is not so fast: when the solution of BNAH-*hd* is added to the aqueous solution of BNA+-*h* dropwise at the reflux temperature of chloroform recovered dihydropyridine in chloroform layer was BNAH-*hd*. If the transhydrogenation occured, BNAH-h_2 should be extracted from the reaction mixture.

This result leads us to the conclusion that the transhydrogenation shown by Mechanism A does not occur before the extraction of BNAH. Moreover, it is also to be referred that the extracted BNAH had no ability for the transhydrogenation: the fact was confirmed by

recovering 90% of BNAH-*hd* after the reflux of aqueous solution of BNA^+-*h* in the presence of a chloroform solution of BNAH-*hd* for 18 h under vigorous stirring.

Alternative mechanism that can explain the result is that the true reductant is an adduct of BNA^+ with **3** (Mechanism B). Burton and Kaplan already reported that, with **2**, **3**, **4**, and so on, NAD^+ forms adducts containing 1,4-dihydropyridine structure[11] and that the adduct has an ability to reduce potassium hexacyanoferrate(III),[18] although they did not isolate NADH from this reaction system.

The same type of adducts were afforded by the reaction of BNA^+ and **2**—**6**. During the reaction colored product with an absorption maximum at around 365 nm was formed in aqueous layer. This product which is not extracted to chloroform is expected to be 1,4-dihydropyridine derivative **11** or **12** as proposed by Burton and Kaplan. It was confirmed that this adduct reduced 1-methylacridinium iodide and BNA^+ in aqueous solution. From the reaction mixture BNAH was isolated by extraction with chloroform and remaining BNA^+ was precipitated as the salt of tetraphenylborate. A number of examples concerning such adducts of pyridinium salts with nucleophiles, especially with carbonyl compounds that have an α-hydrogen, are known.[2,19] It is reported that among them the adduct with pyruvate has an ability to reduce pyridinium salt.[20]

Table 2. Dependency of yields of BNAH on the concentrations of reactants[a]

$[BNA^+]$/M	[**2** or **3**]/M	Yield/%[b]
0.01	0.01(**2**)	21[c]
0.01	0.02(**2**)	20[c]
0.01	0.05(**2**)	17[c]
0.02	0.02(**2**)	24[c]
0.05	0.05(**2**)	26[c]
0.01	0.01(**3**)	22[d]
0.02	0.01(**3**)	29[d]
0.05	0.01(**3**)	37[d]
0.10	0.01(**3**)	45[d]

a) The reaction was carried out in 0.05 M $Na_2B_4O_7$ in the presence of chloroform at its reflux temperature. b) Determined by the intensity of an absorbance at 355 nm. c) Based on BNA^+. d) Based on **3**.

According to the Mechanism B, the yield of the BNAH might increase to 50% when equimolar BNA^+ and **2** are used. However, the yield was not so high as expected as listed in Table 1 because decomposition of the adduct in aqueous media competes with its oxidation. Table 2 shows that the yield of BNAH based on **3** increases as the increase in the concentration of BNA^+. On the other hand, the increase in the concentration of **2** exerts no effect toward the yield. The reaction in relatively concentrated solution affords BNAH in slightly high yield. These results are consistent with the idea that the transhydrogenation from the adduct to BNA^+ is slow enough to compete with its degradation.

In conclusion, by the reaction of BNA^+ with glyceraldehyde or its analogues, BNAH is produced with a high 1,4-selectivity. The mechanism is, however, different from that suggested for the reaction catalyzed by GAPDH.

Experimental

Instruments. UV and NMR spectra were obtained on a Union Giken SM-401 and a JEOL JNMFX-100, respectively.

Materials. BNA^+, PNA^+, and DNA^+ were prepared by quarterization of nicotinamide with benzyl chloride, propyl bromide, and 2,6-dichlorobenzyl bromide, respectively.

BNA^+-*d* was prepared from BNA^+ by twice repetition of reduction with sodium dithionite in D_2O and oxidation by Malachite Green.[21] Deuterium content in BNA^+ was 91± 1%.

2-Hydroxypropanal was synthesized by the condensation of dithiane and acetaldehyde followed by the dethioacetalization with mercury(II) chloride and mercury(II) oxide.[22]

Deuterated glycolaldehydes were prepared by the condensation of 1,3-dithiane and formaldehyde by an appropriate combination of deuterated materials (perdeuterioparaformaldehyde from Merck Inc. and 1,3-dithiane-2,2-d_2 synthesized by three times proton exchange using butyl lithium and D_2O), followed by dethioacetalization with mecury(II) chloride and mercury(II) oxide.

Reaction of BNA^+ and **2**. *A Typical Procedure*: BNA^+ (50 mg) and **2** (16 mg) were dissolved in 20 cm^3 of 0.05 M aqueous solution (1 M = 1 mol dm^{-3}) of sodium tetraborate. The solution was heated in the presence of 20 cm^3 of chloroform at its reflux temperature. After confirming that the intensity of the absorption of extracted BNAH in the organic layer did not increase any more, the chloroform layer was separated, dried over anhydrous sodium sulfate and evaporated to afford the reduced product. The product was identified as BNAH on an NMR spectrometer and the yield of BNAH was measured on a UV-spectrometer by means of absorbance of the chloroform solution at 355 nm(ε=6100)and/or on an NMR spectrometer using *p*-nitrotoluene as an internal standard.

^1H NMR ($CDCl_3$, δ from TMS): 3.16 (m, 2H), 4.25 (s, 2H), 4.75 (dt, 1H), 5.75 (m, 1H), 5.59 (bs, 2H), 7.16 (s, 1H), and 7.28 (s, 5H). The amount of 1,6-isomer measured on the NMR signal from benzyl proton at δ=4.19 was less than 3%.

Reaction of BNA^+-d and **3**. The reaction was carried out in a similar manner as described above. The NMR spectrum of the reduced product was identical with that of BNAH-d_2. No signal for the hydrogen on the 4-position (δ=3.16) was observed and the splitting of the signal for the hydrogen on the 5-position due to the hydrogens on the 4-position disappeared.

Reactivity of the Adduct. The reaction of BNA^+ (50 mg) and **3** (12 mg) was carried out and stopped after an appropriate reaction time. From the aqueous layer BNAH was removed by extraction and remaining BNA^+ was removed as a precipitate of tetraphenylborate. The solution exerted a UV-absorption at around 365 nm which disappeared quantitatively by the addition of 1-methylacridinium iodide. To the aqueous layer, BNA^+ (50 mg) was added again and further reduction was undergone as described above in the typical procedure. BNAH was extracted to the chloroform layer about half amount as compared with the case without interruption.

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