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NAD(P)$^+$-NAD(P)H Model. 47. Mechanism of the Formation of 1,4-Dihydronicotinamide in the Reaction of Pyridinium Salt and Amine

Atsuyoshi OHNO,* Satoshi USHIDA, and Shinzaburo OKA
Institute for Chemical Research, Kyoto University, Uji, Kyoto 611

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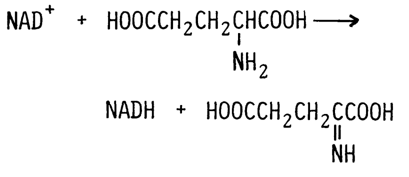
An amine reduces 1-benzyl-3-carbamoylpyridinium ion (BNA$^+$) into 1-benzyl-1,4-dihydronicotinamide (BNAH) in aqueous solution. The mechanism of reduction has been elucidated. The covalently bound adduct of the amine and BNA$^+$ at the 6-position is found to be the true reductant. The reduction with formate proceeds through the direct "hydride-transfer" mechanism. Selectivity of nucleophilic attack on the 4- and 6-positions of BNA$^+$ is discussed in terms of hard-soft-acid-base (HSAB) principle.

The biological reduction of NAD$^+$ coupled with oxidation of substrates such as alcohols and amines takes place smoothly. On the other hand, only a few results on the non-enzymic reduction of pyridinium salts, model compounds for NAD$^+$, by organic substrates have been accumulated. As a model for an alcohol dehydrogenase, Ohnishi and Kitami reported the reduction of pyridinium salts by alkoxides.[1] However, the mechanism of the reaction seems to be different from that of the dehydrogenase, because, in their investigation, a direct hydrogen-transfer from an alkoxide to pyridinium salt was not observed. Recently we reported that 1-benzyl-1,4-dihydronicotinamide (BNAH) is produced[2] by the reaction of 1-benzyl-3-carbamoylpyridinium ion (BNA$^+$) with α-ketols such as glyceraldehyde and glycolaldehyde and that the mechanism is different from that suggested for the reaction catalyzed by glyceraldehyde 3-phosphate dehydrogenase.[3] In the reduction of BNA$^+$ with glycolaldehyde, addition of glycolaldehyde to the 4-positon of BNA$^+$ takes place initially, and the adduct acts as the real reductant for another molecule of BNA$^+$.[3]

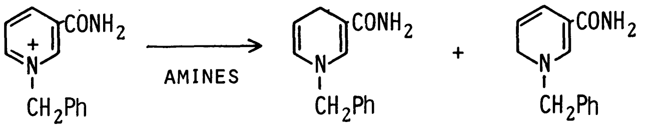
By using a variety of amines and ammonia, Ohnishi reported the formation of BNAH from BNA$^+$.[4] The mechanism of the reduction, however, has remained unclear. In enzymic systems, the reduction of NAD$^+$ by amine is found in the reaction catalyzed by glutamate dehydrogenase,[5] where, according to the suggested mechanism, glutamate is oxidized to 2-iminoglutarate with simultaneous formation of NADH.[6]



$$NAD^+ + HOOCCH_2CH_2\underset{NH_2}{CH}COOH \longrightarrow NADH + HOOCCH_2CH_2\underset{\underset{NH}{\|}}{C}COOH \qquad (1)$$

It is interesting to compare the mechanism of non-enzymic formation of 1,4-dihydropyridine by amines with that of enzymic process, not only from the view point of a simple comparison of the two systems but also from that for understanding biochemical reactions as well as to extend biomimetic reactions. We now report a mechanism of the formation of BNAH in the reaction of BNA$^+$ with amines.

Results and Discussion



BNA$^+$ BNAH (2)

Table 1. Reaction of BNA$^+$ and amines[a]

BNA$^+$/M	Amine/M	Solvent	Reaction time/h	Yield of BNAH/%[g]
0.50[b]	1.60 (AE[d])	H_2O[f]	12	10
0.22[b]	1.88 (DA[e])	H_2O-MeOH	22	8
0.22[b]	1.88 (AE)	H_2O-MeOH	22	9
0.22[c]	1.88 (DA)	CH_3CN	50	18

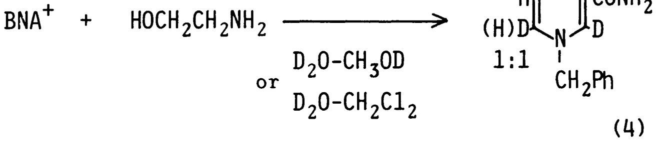
a) At room temperature in the dark. b) Counter anion is Cl^-. c) Counter anion is ClO_4^-. d) 2-Aminoethanol. e) Diethylamine. f) In the presence of CH_2Cl_2 (bi-phase system). g) Yields based on BNA$^+$ were determined on NMR using p-nitrotoluene as an internal standard.

According to a literature reported by Ohnishi,[4] BNAH is formed in the reaction of BNA$^+$ and excess of amine in a yield of 18% in acetonitrile and 5% in water, respectively. We re-examined the reaction under similar conditions and obtained the results listed in Table 1. The reduction products were confirmed with an NMR spectrum to be almost consisted of the 1,4-dihydro isomer (more than 90%) with contamination of small amount of the 1,6-dihydro derivative.

Among informations to elucidate the mechanism, the most important one is that which clarifies the

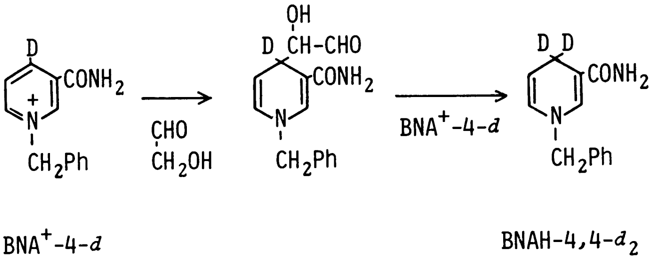
(3)

BNA$^+$



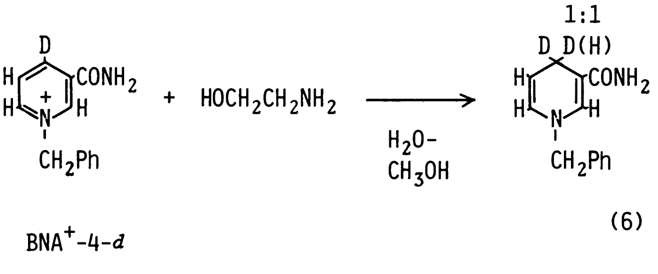
(4)

origin of hydrogen at the 4-position of BNAH produced. First of all, the reactions with 3-amino-1-propanol-3,3-d_2 and 2-aminoethanol in D_2O–CH_3OD (50/50 v/v) were carried out.[7] As shown in Eqs. 3 and 4, both hydrogens at the 4-position of the BNAH were "H", which indicates that the hydrogen comes neither from the substrate nor from the solvent. The latter reaction yields BNAH-2,6-d_2. The contamination of deuteriums in BNAH is due to H–D exchange in BNA$^+$.[8] The H–D exchanges at the 2- and 6-positions of BNA$^+$ was confirmed by the observation of disappearance of NMR signals from the protons at 2- and 6-positions of BNA$^+$ in an alkaline buffer solution. Roberts and his co-workers have claimed, in a recent papaer,[9] partial incorporation of deuterium at the 2-position of BNAH in the reduction by sodium dithionite in D_2O suggesting bimolecular isomerization and reisomerization between BNA$^+$ and BNAH. However, it should be noted that BNAH-2-*d* is formed without isomerization.



(5)

It is well known that nucleophiles attack easily the pyridinium salt to afford adducts that have dihydropyridine structure.[10] Such adducts sometimes have abilities to reduce pyridinium salt. For example, the adduct of BNA$^+$ and glycolaldehyde reduces BNA$^+$ to form BNAH.[3] The mechanism of this reaction was confirmed by the finding that BNAH-4,4-d_2 was formed from BNA$^+$-4-*d* with glycolaldehyde in protic solvents. The reaction with amine may undergo with the same mechanism and we examined the possibility. When BNA$^+$-4-*d* was reacted with 2-aminoethanol in H_2O–CH_3OH the obtained dihydropyridine was an about 1:1 mixture of BNAH-4-*d* and BNAH-4,4-d_2 (Eq. 6). On the other hand, when the reaction was carried out in H_2O in the presence of CH_2Cl_2 (bi-phase reaction), the product obtained from the organic layer was BNAH-4-*d* (Eq. 7). If the reductant were the adduct at the 4-position of BNA$^+$, the product should be BNAH-4,4-d_2. Therefore, the above result is inconsistent with the mechanism for the reduction with glycolaldehyde. Because a large amount of absorption at around 350 nm due to the dihydropyridine structure was observed during the reaction, we assumed that the hydrogen transfered from the 6-position of BNA$^+$ through an adduct (**1**) (Eq. 8). Similar idea has appeared in a literature.[11] In order to ascertain the hypothesis, BNA$^+$-2,6-d_2 and BNA$^+$-2,4,6-d_3 were reacted with $DOCH_2CH_2ND_2$ in D_2O in the presence of CH_2Cl_2 (Eqs. 9 and 10). In both cases, incorporated hydrogens at the 4-position were deuterium. Since it has been confirmed that these hydrogens do not come from the solvent, the result clearly indicates that the origin of the hydrogen at the 4-position of BNAH is the 2- or 6-position of BNA$^+$. Whereas the hydrogen at the 2-position of BNA$^+$ can easily be exchanged by deuterium in D_2O under the basic conditions, the exchange at the 6-position is rather slow.[8] On the basis of the result from the reaction in D_2O (Eq. 4) that no deuterium incorporation took place within the limitation of NMR spectroscopy, the possibility of the transfer from the 2-position is excluded or is estimated to be a very minor path, if any. That is, only from the 6-position, the hydrogen-transfer is expected.



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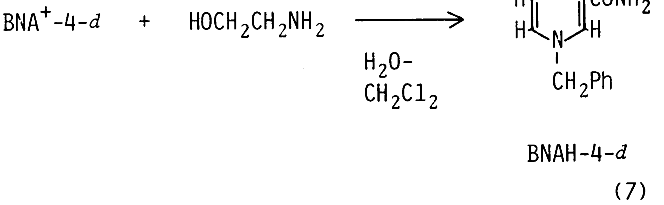

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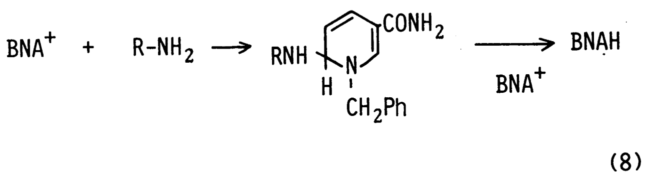

(8)

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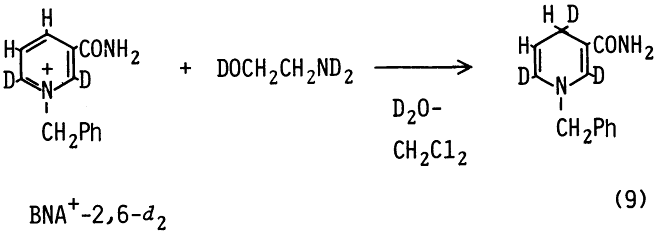

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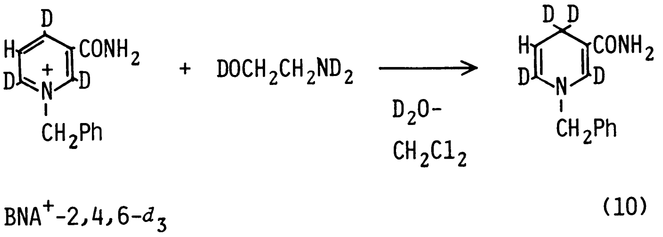

(10)

In spite that the hydrogen at the 6-position of BNA$^+$ exchanges with a proton from solvent, no deuterium incorporation onto the 4-position was observed in the reaction shown in Eq. 4. This result stems from the fact that the proton exchange and the reaction of **1** with BNA$^+$ proceeds comparably in rate so that the amount of **1**-6-*d* is not so large. Even though the **1**-6-*d* is produced in certain amount, kinetic isotope effect[11] prefers the "H"-transfer from **1** than "D"-transfer from **1**-6-*d*.

In the reaction described in Eq. 6, the product was a mixture of BNAH-4-*d* and BNAH-4,4-d_2. The result is accounted for by transhydrogenation between initially produced BNAH-4-*d* and BNA$^+$-4-*d*. The isotope effect of 6[12] suggests that hydrogen dissociates six times as selectively as deuterium does from the 4-positions of BNAH-4-*d* and BNAH-4,4-d_2 accumulating BNAH-4,4-d_2 after repetition of bimolecular isomerization. The easiness of transhydrogenation was ascertained by an experiment; when BNAH-4-*d* was reacted with about tenfold excess of BNA$^+$-4-*d* for 16 h, the recovered dihydropyridine was found to be solely composed of BNAH-4,4-d_2.

Therefore, it is concluded that the adduct of amine at the 6-position of BNA$^+$ (**1**) reduces another molecule of BNA$^+$ to afford BNAH as shown in Eq. 8. The conclusion shows an interesting contrast between the reactions of α-ketols and those of amines. The result also discards the possibility of direct hydrogen-transfer from amines.

Adduct formation from pyridinium salts with many kinds of nucleophiles such as cyanide,[13] thiols,[14] and enolates[15] have been reported.[10] The attack by these nucleophiles is generally thought to occur at the 4-position.[10,16] In contrast, the additions by amine and hydroxide ion[17] take place at the 6-position. Similar reaction centers[18] are, thus, selectively discriminated by nucleophiles depending on their own property. The selectivity can be interpreted in terms of the hard-soft-acid-base (HSAB) principle.[19] Because of adjcent cationic center, the 6-position is harder center than the 4-position for the nucleophilic attack.

In the present system the product had mainly (more than 90%) 1,4-dihydro structure. It is reported[20] that a small amount of pyridinium salt catalyzes the equilibrium between 1,4- and 1,6-isomers with large predominance of the 1,4-isomer. Therefore, it is reasonable that the reaction in an aqueous medium produces an equilibrium mixture containing a large excess of the 1,4-isomer. However, even when the reaction proceeded in a bi-phase system, where the dihydropyridine produced was extracted immediately into organic phase, the predominant product was the 1,4-isomer (more than 97%). Moreover, in the reaction of BNA$^+$-4-*d* in the bi-phase system, in which there takes place no equilibration between BNA$^+$ and BNAH, the extracted dihydropyridine was BNAH-4-*d* with 1,4-selectivity. These results indicate that the selectivity for the 1,4-isomer is not due to thermodynamic control but to the kinetic preference of transhydrogenation from **1** to the 4-position of BNA$^+$. Thus, based on the HSAB principle discussed above, the transferring "hydride" might be a soft species.

Most substrates that reduce NAD$^+$ in enzymic systems are nucleophiles. As mentioned above, such nucleophiles attack the pyridinium ion to form covalently bound adducts in organic reaction systems. The formation of these adducts seems to be one of the major reasons for the difficulty to achieve the biomimetic reduction of pyridinium ion by a direct hydrogen-transfer from organic substrates.

Although the formation of 1,4-dihydropyridine derivative was observed in the reactions of α-ketols and amines, it has been elucidated that the mechanism is different from that proposed for enzymic reactions. Only one example for direct hydrogen-transfer from a substrate to NAD$^+$-models was observed in the reduction with formate. Gizzi and Joullie reported a direct deuterium-transfer to 1-(*p*-fluorobenzyl)-3-carbamoylquinolinium chloride in the reduction with DCOOH.[21] We also confirmed the deuterium-transfer in the reduction of BNA$^+$ with DCOOK[22] under the conditions reported by Ohnishi and Tanimoto.[23]

In the active site of enzymes, the formation of covalently bound adduct between NAD$^+$ and a substrate is prohibited because of proper orientation of two reactants. Even though the adduct is formed, it cannot be a reductant for another molecule of NAD$^+$ because of the interference of the enzyme wall. Therefore, in the model system for the reduction of pyridinium salt, the reactants should be designed to suppress the adduct formation as well as the transhydrogenation from the adduct. Facile reductions coupled with oxidation of alcohols or amines have been performed in the case of pyrido[2,3-*d*:6,5-*d'*]dipyrimidine,[24] hydroxyacridinium salt,[25] and deazaflavines.[26] The reason for the facility is thought to be based on the ring structure that have an ability to accept a "hydride" compared to the attack by a nucleophile. In fact, in a certain case,[27] the desired reaction is suppressed by adduct formation, which shows the importance of proper design for model molecules. Unfortunately, these compounds are not direct models for NAD$^+$ where pyridinium ion participates. Further investigations for the simulation of reduction of NAD$^+$ by the use of pyridinium salts are desired.

Experimental

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

Materials. 3-Amino-1-propanol-3,3-d_2 was prepared from 3-hydroxypropionitrile by the reduction with lithium aluminum deuteride in tetrahydrofuran. ^{1}H NMR ($CDCl_3$; δ from TMS): 1.70 (t, 2H), 2.70 (bs, 3H), and 3.77 (t, 2H).

BNA$^+$-4-*d* was prepared from BNA$^+$ by repetition of reduction by sodium dithionite in D_2O and oxidation by Malachite Green[28] for three times. Deuterium content at the 4-position was more than 97%. BNA$^+$-2,6-d_2 and BNA$^+$-2,4,6-d_3 were prepared as follows: The solution of 100 mg of BNA$^+$ (or BNA$^+$-4-*d*) and 100 mg of anhydrous sodium tetraborate in 1 cm^3 of D_2O was placed at 60 °C until the NMR signals from the protons at the 2- and 6-positions disappeared. It took about an hour for the disappearance of the proton at the 2-position and about several hours for the one at the 6-position. Water was removed by evaporation and obtained solid was washed with dichloromethane for three times. BNA$^+$-2,6-d_2 (or BNA$^+$-2,4,6-d_2) was extracted with methanol from the solid and used without further purification.

Reaction of BNA$^+$ and Amine. A Typical Procedure: To the solution of BNA$^+$ (41.5 mg) in a 1:1 (v/v) mixture of H_2O–CH_3OH (0.75 cm^3) 87×10^{-6} cm^3 of 2-

aminoethanol was added. Methanol was added to keep the solution homogeneous. The solution was stirred for 20 h at room temperature in the dark. Reduced products were extracted by 10 cm^3 of ether for three times and collected by removing the solvent. The NMR signals from the product were identical with those from the authentic sample of BNAH. The yield of BNAH was measured on an NMR spectrometer using *p*-nitrotoluene as an internal standard. NMR signals from the products obtained under various conditions are listed in Table 2. Oxidized **1** or decomposed products from BNA$^+$ could not be isolated because of complication.

Table 2. Type of Coupling and Amount of Protons Attached to the Dihydropyridine Ring in ^{1}H NMR Spectra of the Products

No. of Eq.	Position of the proton[a]			
	4- (3.16)	5- (4.75)	6- (5.75)	2- (7.16)
3	m (2H)	dt (1H)	d (1H)	s (1H)
4	dd (2H)	m (1H)	m (0.5H)	—
7	d (1H)	dd (1H)	d (1H)	s (1H)
9	d (1H)	d (1H)	—	—
10	—	s (1H)	—	—

a) Chemical shifts (δ from TMS in $CDCl_3$) of each position are represented in parentheses. Each product shows other signals at δ=4.25 (s, 2H), 5.3—5.6 (bs, 2H), and 7.28 (s, 5H). dd: double doublet, dt: double triplet.

For the reaction in bi-phase system, dichloromethane as a solvent for extraction was added to an aqueous solution of BNA$^+$ and the amine.

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