Coenzyme Models. II. Effect of Micellar Environments on the Acid-Catalyzed Hydration of 1,4-Dihydropyridine Derivatives[1)]

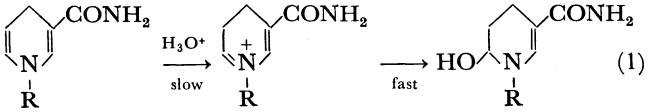
Seiji SHINKAI, Reiko ANDO, and Toyoki KUNITAKE

Department of Organic Synthesis, Faculty of Engineering, Kyushu University, Fukuoka 812

(Received February 8, 1975)

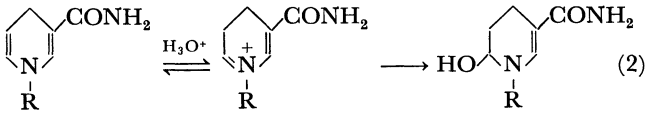
An effect of micelles on the acid-catalyzed modification reaction of two dihydropyridines is reported in connection with the NADH function bound to alcohol dehydrogenase, and the hydration equilibrium of dihydronicotinamides was found to be quite susceptible to the ionic environment. The absorption maximum at 357 nm of 1-benzyl-1,4-dihydronicotinamide(NBzNH) was not affected by the presence of cationic micelles, while that of 1-lauryl-1,4-dihydronicotinamide(NLaNH) shifted by 5—6 nm to shorter wavelengths, probably due to the neighboring cationic charge. The hydration rates for dihydronicotinamides were prompted by NaLS micelle and retarded by CTAB micelle. The rate augmentation in the NaLS micelle, compared with the non-micellar system, was 1.7-fold for NBzNH and 33-fold for NLaNH. On the other hand, CTAB micelle pronouncedly depressed the hydration rate of NLaNH, which is 414-fold slower than in NaLS. The micelle of 1-lauryl-3-carbamoylpyridinium(NLaN), which forms the charge transfer complex with NLaNH, was found to inhibit the acid-catalyzed hydration more efficiently(1400-fold slower). Thus, it is suggested that the ammonium ion adjacent to the dihydronicotinamide group in alcohol dehydrogenase-NADH complex performs some biological functions.

NADH and its model compounds, 1,4-dihydronicotinamide derivatives, undergo rapid structural alternations in acidic solutions, and their UV spectra result in a shift of the characteristic absorption band in the 340—360 nm region downward to around 290 nm with tight isosbestic points.[2)] Metzler and co-workers have studied the acid transformation of NADH and NPrNH[3)] in the presence of a series of acids, and established that the observed spectral shift of NPrNH is completely reversible until the much slower secondary decay takes place.[4)] They claimed acid-catalyzed hydration for the initial acid modification reaction (Eq. 1 or 2).



(1)

or



(2)

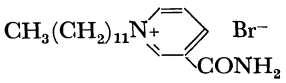
The 290 nm-absorbing compound was referred to as the "primary acid modification compound," and was later identified as 6-hydroxy-1,4,5,6-tetrahydronicotinamide by X-ray crystallography.[5)] This compound is also unstable in acid, and the absorption at 290 nm disappears through the much slower, so-called "secondary acid reaction."

This reaction may be biologically important in connection with the fact that NADH bound to glyceraldehyde-3-phosphate dehydrogenase is characterized by a change in the UV spectrum similar to that which accompanies the primary acid reaction.[6,7)] Reaction schemes (1) and (2) involve the formation of a cationic intermediate that is established to be a rate-determining step in aqueous media.[5)] One would anticipate that the anionic environment would result in the acceleration of the apparent hydration rate as seen in acetal hydrolyses.[8,9)] On the other hand, the cationic environment would retard the hydration rate. If this expectation is correct, it is conceivable that the lysine residue of the alcohol dehydrogenase which is postulated to exist very close to the bound NADH[10)] performs some biological function by affecting the NADH reactivity.

In this paper we describe the effect of ionic micelles on the apparent hydration rate of acid-modifications of a few 1,4-dihydropyridines. Sodium laurylsulfate (NaLS), cetyltrimethylammonium bromide (CTAB) and 1-lauryl-3-carbamoylpyridinium bromide (NLaN) were used as surfactants. In order to study the micellar effect we chose 1-lauryl-1,4-dihydronicotinamide (NLaNH) in addition to 1-benzyl-1,4-dihydronicotinamide (NBzNH).

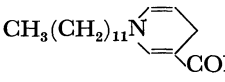
Surfactants: $CH_3(CH_2)_{11}SO_4Na$ (NaLS)

$CH_3(CH_2)_{15}N(CH_3)_3Br$ (CTAB)

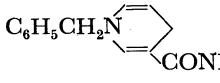


(NLaN)

Dihydropyridines:



(NLaNH)



(NBzNH)

Experimental

Materials. Surfactants were products of Wako Pure Chemical Ind., NaLS(biochemical use) was used without further purification, and CTAB was recrystallized from ethanol before use.

NBzNH was prepared according to the method of Kim and Chaykin,[5)] and recrystallized from ethanol-water, yellow needles, mp 110—112 °C (lit,[5)] mp 110—112 °C). NLaN was obtained from lauryl bromide and nicotinamide[11)], and reduced to NLaNH with sodium dithionite by the procedure used to prepare NPrNH.[12)] The yellow precipitates were collected and recrystallized twice from petroleum ether-ether, mp 45—48 °C. An NMR spectrum taken in $CDCl_3$ was identical to that of NPrNH except for the resonance of the 1-alkyl group: $CH_3(CH_2)_{10}$–, 0.8—1.6 ppm, 23.1H; N–CH_2- and C_4–H_2, 3.0—3.2 ppm, 4.2H; C_5–H, 4.5—4.8 ppm, 1.0H; C_6–H and NH_2, 5.4—5.8 ppm, 2.8H; C_2–H, 7.0 ppm, 1.0H. This dihydropyridine was unstable to heat and slowly decomposed to the orange-colored oil even at room tempera-

ture. The crystals were preserved in a refrigerator, and stock solutions for kinetic measurements were prepared on the day of use.

Kinetics. The kinetic and spectroscopic measurements were carried out at 30±0.1 °C at a calculated ionic strength (with KCl) using a Hitachi 124 spectrophotometer. Reaction rates of hydration (NBzNH and NLaNH) were estimated by following the increase of absorbance at 292 nm or the decrease of absorbance at 357 nm, and both methods provided the identical result. The pseudo first-order rate constants were determined from the time-course of reaction thus obtained.

Results

Electronic Spectra. In order to assess the environmental effect of micelles on the absorption maxima of dihydronicotinamides, spectra of NLaNH and NBzNH were taken in various solvents (Table 1). The absorption maximum of NLaNH could not be determined in water because of the sparing solubility. If the hypothetical λ_{max} value of NLaNH in water is assumed to be the same as that of NPrNH in water, λ_{max} of NLaNH in cationic micelles is estimated to shift to the shorter wavelength by 5—6 nm by transferring from the aqueous to micellar phase. A similar hypsochromic shift was observed in ethanol.

Table 1. Electronic spectra of dihydropyridines in various media.[a]

Medium	λ_{max} (nm)		
	NLaNH	NPrNH	NBzNH
Diethyl ether			340[c]
Methanol		354[b]	
Ethanol	355		354[c]
52wt% Methanol-water		362[b]	
Water		362[b]	357[c]
CTAB micelle	356		357
NLaN micelle	357		357

a) 30 °C. b) S. Shinkai and T. C. Bruice, *Biochemistry*, **12**, 1750 (1973). c) From Ref. 10.

The λ_{max} value of NBzNH in water was not affected by the addition of cationic surfactants. Presumably, the equilibrium constant for the association of NBzNH with surfactants is rather small, resulting in the smaller micellar effect.

Hydration of 1-Benzyl-1,4-dihydronicotinamide (NBzNH). The absorption spectrum of the acid modification product of NBzNH are essentially the same as that of reference 4 with tight isosbestic points. In Fig. 1, pseudo first-order rate constants (k_{obsd}) for the hydration of NBzNH in the phosphate buffer were plotted against the concentration of surfactants, CTAB and NaLS. The concentration-rate profile is multiphasic. The rate constants are independent of the surfactant concentration below cmc, while they rapidly rise or descent with increasing concentration of surfactants above cmc. From Fig. 1 the cmc is estimated to be 4×10^{-4} M for CTAB and 3×10^{-3} M for NaLS under the reaction condition employed (lit,[13] cmc at 25 °C for CTAB, 9.2×10^{-4} M; for NaLS, 8.1×10^{-3} M).

The existence of the NaLS micelle raises the reaction

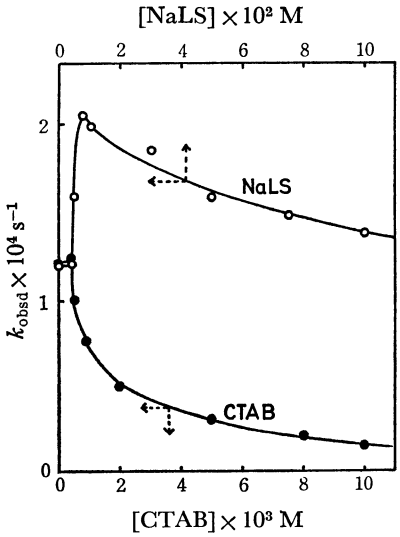


Fig. 1. Pseudo first-order rate constants for the acid-catalyzed hydration of NBzNH in aqueous solution. 30 °C, pH 6.30 with 0.01 M phosphate.

rate, and a rate augmentation of 1.7-fold is observed at the optimal surfactant concentration. Since the rate enhancement is slight, it is presumed that most of NBzNH is dissolved in the bulk phase and that the small amount of NBzNH bound to the micelle contributes to the rise of the total reaction rate.

On the contrary, CTAB depressed the hydration rate above the cmc, and the rate constant is 8.4-fold smaller at [CTAB]$=10^{-2}$ M, compared with that obtained without surfactant.

Hydration of 1-Lauryl-1,4-dihydronicotinamide (NLaNH). NBzNH is moderately soluble in aqueous solution (solubility: *ca.* 10^{-2} M), while NLaNH is sparingly soluble in water. In fact, precipitation of NLaNH (1.67×10^{-4} M) was recognized with less than 3.0×10^{-3} M of NaLS. Therefore, it would be reasonable to infer that the acid-catalyzed hydration reaction of this dihydronicotinamide entirely proceeds in the micelle. The surfactant concentration-rate profile for NLaNH is shown in Fig. 2. In the CTAB micelle the decay of NLaNH was extremely inhibited, so that

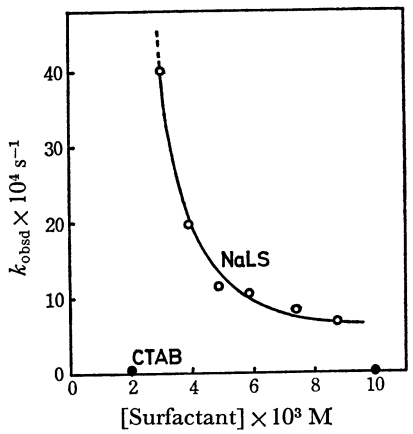


Fig. 2. Pseudo first-order rate constants for the acid-catalyzed hydration of NLaNH in aqueous solution. 30 °C, pH 6.30 with 0.01 M phosphate, [NLaNH]$=1.67\times10^{-4}$ M.

the first-order rate constant was calculated by means of the Guggenheim's equation: $k_{obsd}=9.7\times10^{-6}$ s^{-1} ($t_{1/2}$= *ca.* 20 hr) at [CTAB]$=1.0\times10^{-2}$ M. This reaction rate is about 12-fold slower than that of NBzNH in the non-micellar system. Since the NADH- or 1,4-dihydronicotinamide-mediated reduction reaction is generally much faster,[2] it may be said that dihydronicotinamides in the CTAB cationic micelle are almost completely stabilized in weakly acidic solutions.

In the accompanying paper we showed that NLaNH forms the charge transfer complex with NLaN in micellar systems (association constant, $K=22$—45 M^{-1}).[14] The hydration reaction in NLaN micelle is of much interest from the biological standpoint because the glyceraldehyde-3-phosphate dehydrogenase-NAD complex involves the charge transfer interaction between the 3-carbamoylpyridinium group of NAD and the enzyme residue, probably an indole nucleus.[15] The pseudo first-order rate constant for the hydration reaction of NLaNH was estimated to be 2.8×10^{-6} s^{-1} at [NLaN]$=1.56\times10^{-2}$ M, which is 3.5 times slower than in CTAB micelle. It seems, therefore, that the charge transfer interaction inhibits the acid-catalyzed hydration reaction more efficiently.

In NaLS micelle the hydration rates were conspicuously accelerated (Fig. 2). Shiny crystals of NLaNH were recognized in the presence of NaLS below 3.0×10^{-3} M. The rate rapidly increases as the surfactant concentration approaches the cmc, and, at [NaLS]$=3.0\times10^{-3}$ M, a remarkable rate augmentation of 414-fold (relative to that in the CTAB micelle) was observed. This enhanced rate is 33-fold greater than the hydration rate of NBzNH in non-micellar solution. The protonated intermediate (or the transition state) of the hydration reaction should be significantly stabilized by the anionic environment consisting of the NaLS micelle.

Discussion

Absorption Maxima of Dihydronicotinamides. The absorption maximum of dihydropyridines is known to shift depending on the environment. This phenomenon is of particular interest in connection with the observation that the complex of alcohol dehydrogenase and NADH has an absorption maximum at 325 nm in contrast to that of NADH at 340 nm.[16] This observation has been repeated and extended to NADH analogs and NAD-addition products.[17,18] Although the shift to shorter wavelengths is also brought forth by non-polar environments, Kosower rather claimed that a positively-charged nitrogen(probably ammonium ion of a lysine residue) which is located 3 Å apart from the nitrogen of the dihydropyridine ring produces the shift by destabilization of the polar excited state of the dihydropyridine.[10]

In the present study λ_{max} of NLaNH shifted by 6 nm to shorter wavelengths in the CTAB micelle compared with NPrNH in water (Table 1). NLaN efficiently forms the charge transfer complex with NLaNH in micellar systems,[14] and the complex probably possesses the face-to-face orientation. Despite the expectation that the remarkable hypsochromic shift may occur, the observed shift was only 5 nm in the micelle of NLaN. Therefore, the ring nitrogen of NLaNH may not be located very close to the cationic charge of surfactant molecules enough to perturb the spectrum.

Hydration Rates of Dihydronicotinamides. Although the hypsochromic shift of dihydronicotinamides caused by the adjacent cationic charge of the surfactant is only 5—6 nm, it is interesting to assess the acid-catalyzed hydration rates in reference to the shifts.

The experimentally observed first-order rate constants for the hydration of a dihydronicotinamide can be satisfactorily described by a general acid catalysis equation (3), in which k_0 is the reaction rate with water and k_H and k_{HA} are the catalytic constants of hydronium ion and buffer acid, respectively.[4]

$$k_{obsd} = k_0 + k_H[H_3O^+] + k_{HA}[HA]$$

Since the k_0 term is negligible in comparison with other terms,[4] one can consider that hydronium ion and buffer acid catalyze the hydration reaction. Under the condition employed, the hydration reaction is mainly catalyzed by $H_2PO_4^-$ ion,[4] that is, k_{HA} [HA]$>$ k_H [H_3O^+]. The CTAB micelle would localize $H_2PO_4^-$ ion around the Stern layer, while H_3O^+ ion would be expelled out of the micellar surface. Thus, one may expect that the hydration rates would be accelerated by concentrated $H_2PO_4^-$ ion. On the contrary, the presence of CTAB micelle depressed the reaction rates, so that the retardation should be caused by the cationic micelle itself. It is concluded, therefore, that a cationic micelle destabilizes a positively-charged intermediate (or the transition state to form the intermediate; Eqs. 1 and 2). A similar discussion would be possible to account for the acceleration effect by an anionic micelle. Thus, the effect of the cationic and anionic micelles on the hydration reaction is consistent with the previous proposition by Kim and Chaykin[5] that the protonation of dihydronicotinamides is involved in the rate-determining step.

The micellar effect on the hydration rate was most pronounced in the case of NLaNH. Though the decay of NLaNH is extremely depressed in CTAB micelle, it would not mean the dihydronicotinamide portion of NLaNH to be in the apolar core of the micelle. The hydration reaction is accelerated by the presence of NaLS micelle, and the acceleration phenomenon could not be expected for the functional group sinking in the micelle core. The dihydronicotinamide group probably exists in the Stern layer or at the vicinity. Our recent observation that NLaNH efficiently forms the charge transfer complex with the 3-carbamoylpyridinium group of NLaN in CTAB-micelle[14] would support this inference.

The relative rate augmentation and depression, compared with NBzNH in non-micellar system, are listed in Table 2. The surfactant concentration, where the rate difference is the greatest, was employed to demonstrate the susceptibility of the hydration reaction to the ionic environment. The conspicuous micellar effect is seen in the case of NLaNH. The NaLS micelle accelerates the hydration rate of NLaNH by 33-fold. This augementation corresponds to 414-fold of that in CTAB micelle, and, in fact, 1400-fold of that in NLaN micelle.

The cationic charge of CTAB and NLaN micelles is not located close to the nitrogen of the nicotinadmie

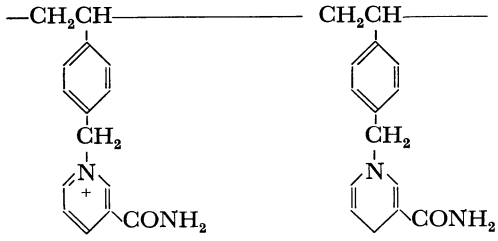
Table 2. Relative hydration rate[a)]

Dihydronicotinamide	Non-micelle	Micelle		
		LaLS	CTAB	NLaN
NBzNH	1.0	1.7	1/ 8.4	
NLaNH		33	1/12.6	1/44

a) 30 °C, pH 6.30 with 0.01 M phosphate. Surfactant concentrations which provide the greatest difference are as follows: for NBzNH, $[NaLS]=8.00\times10^{-3}$ M, $[CTAB]=1.00\times10^{-2}$ M; for NLaNH, $[NaLS]=3.00\times10^{-3}$ M, $[CTAB]=1.00\times10^{-2}$ M, $[NLaN]=1.56\times10^{-2}$ M.

group enough to cause the significant spectral shift. However, it exerts the remarkable inhibitory effect on the hydration. In the complex between NADH and alcohol dehydrogenase, a positively-charged nitrogen in the form of an ammonium ion is located at the distance of 3 Å from the nitrogen of the dihydropyridine ring, which does generate the spectral shift.[10] It is conceivable from the present results that the hydration reaction of the bound NADH to alcohol dehydrogenase would be greatly affected by the presence of such a closely neighboring ammonium ion. At present, however, it is uncertain if any biological function is associated with the presence of such a positive charge. The present work suggests that the positive charge protects the bound NADH from the hydration decay, and that it would enable the dihydronicotinamide group to act as a reducing function over a wide pH range.

Concluding Remark. As described above, the acid-catalyzed hydration of 1,4-dihydronicotinamide is quite susceptible to the influence of the ionic environment. This finding in a model system may be of importance bearing to an attempt to understand the function of the bound NADH to alcohol dehydrogenase. Micelles would be a typical model system such that the dihydronicotinamide group is located close to the ionic group. A similar system is possible in polyelectrolytes containing the dihydronicotinamide function (Eq. 4).[19]



(4)

References

1) Contribution No. 343 from this department.
2) T. C. Bruice and S. J. Benkovic, "Bioorganic Mechanisms," Vol II, New York, N.Y., W. A. Benjamin, (1966) Chapter 9.
3) Abbreviations employed are: nicotinamide adenine dinucleotide and its reduced form, NAD and NADH; 1-R-1,4-dihydronicotinamide, R=Pr(NPrNH), R=benzyl(NBzNH), R=lauryl(NLaNH); 1-lauryl-3-carbamoylpyridinium bromide, NLaN.
4) C. C. Johnston, J. L. Gardner, C. H. Suelter, and D. E. Metzler, *Biochemistry*, **2**, 689 (1963).
5) C. S. Y. Kim and S. Chaykin, *ibid.*, **7**, 2339 (1968).
6) G. M. Rafter, S. Chaykin, and E. G. Krebs, *J. Biol. Chem.*, **208**, 799 (1953).
7) J. O. Meinhart, S. Chaykin, and E. G. Krebs, *ibid.*, **220**, 821 (1956).
8) R. B. Dunlop, G. A. Ghanim, and E. H. Cordes, *J. Phys. Chem.*, **73**, 1898 (1969).
9) B. M. Dunn and T. C. Bruice, *J. Amer. Chem. Soc.*, **92**, 6589 (1970).
10) E. M. Kosower, *Biochim. Biophys. Acta*, **56**, 474 (1962).
11) J. Baumrucker, M. Calzadilla, M. Centeno, G. Lehrmann, M. Uradaneta, P. Lindquist, D. Dunham, M. Price, B. Sears, and E. H. Cordes, *J. Amer. Chem. Soc.*, **94**, 8164 (1972).
12) Y. Paiss and G. Stein, *J. Chem. Soc.*, **1958**, 2905.
13) E. J. Fendler and J. H. Fendler, "Advances in Physical Organic Chemistry," Vol. 8, ed. by V. Gold, Academic Press, New York, N.Y. (1970), p. 271.
14) S. Shinkai, K. Tamaki, and T. Kunitake, This Bulletin, **48**, 1918 (1975).
15) G. Cilento and D. L. Sanito, *Arch. Biochem. Biophys.*, **110**, 133 (1965).
16) H. Theorell and R. K. Bonnicksen, *Acta Chim. Scand.*, **5**, 1105 (1951).
17) J. van Eys, F. E. Stolzenbach, L. Sherwood, and N. O. Kaplan, *Biochim. Biophys. Acta*, **27**, 163 (1958).
18) S. Shifrin, *Biochemistry*, **3**, 829 (1964).
19) T. Kunitake, S. Shinkai, and K. Tamaki, to be submitted.