Coenzyme Models. VII. Micellar Catalysis of Acid-Catalyzed Hydration of 1-Dodecyl-1,4-Dihydronicotinamide*

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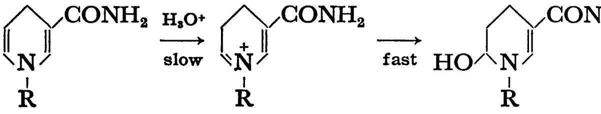
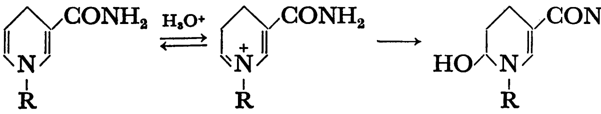
The acid-catalyzed hydration reaction of 1-dodecyl-1,4-dihydronicotinamide was examined in cationic(CTAB) and anionic (SDS) micelles. The H_3O^+-catalysis(k_H) was negligibly small in the presence of the CTAB micelle and 225—521 M^{-1} s^{-1} in the presence of the SDS micelle, the difference being about 10^7-fold. The catalysis by carboxylic acids (k_{HA}) was considerably suppressed in the CTAB micelle, but was promoted in the SDS micelle, suggesting the development of a cationic charge on a nitrogen atom of dihydropyridine at the rate-determining step. Brönsted plots for log k_{HA} in the CTAB and SDS micelles *vs.* pK_a of carboxylic acids gave straight lines with the slope of −0.8, the line for SDS being higher by two orders of magnitude than that for CTAB. These results indicate that a cationic charge (*e.g.*, the lysine residue of alcohol dehydrogenase located very close to the bound NADH) may protect the NADH from hydration decay.

It has been established that NADH (reduced form of nicotinamide adenine dinucleotide) and its model compounds, 1,4-dihydronicotinamide derivatives rapidly decompose in aqueous acidic media.[1] The decomposition causes shifts of the characteristic UV absorption band of dihydronicotinamides in the 340—360 nm region downward to around 290 nm with isosbestic points.[1] The 290 nm-absorbing compound was later confirmed to be a 6-hydroxy-1,4,5,6-tetrahydronicotinamide by X-ray crystallography.[2] The following reaction scheme (Eq. 1 or 2) was thus suggested by Johnston and others,[1] in which the protonation of dihydronicotinamide is involved in the rate-determining step.



(1)

or



(2)

The biological importance of this reaction was pointed out by Meinhart and co-workers,[3] since NADH bound to glyceraldehyde-3-phosphate dehydrogenase is characterized by a change in the UV spectrum similar to that of the acid-catalyzed hydration of dihydronicotinamides.

In the previous paper,[4] we demonstrated a striking effect of micelles on the acid-catalyzed hydration of 1,4-dihydronicotinamide derivatives. The hydration rates were accelerated by an anionic micelle of sodium dodecylsulfate (SDS), in accord with the fact that the anionic environment would result in the acceleration of the protonation step in the hydration reaction as seen in acetal hydrolyses.[5,6] On the contrary, a cationic micelle of cetyltrimethylammonium bromide (CTAB) pronouncedly depressed the hydration rate of 1-dodecyl-1,4-dihydronicotinamide (NDNH; R=$CH_3(CH_2)_{11}$–); the rate being 414-fold slower than that in SDS. To our knowledge this was the first example that the reaction involving NADH analogs is subject to the microenvironmental effects.

In this paper, we wish to report further investigations on the micellar catalysis of the hydration reaction, which was conducted in order to evaluate the respective rate constants for three catalytic species, water, oxonium ion, and buffer acid in the presence of micelles. NDNH was employed as substrate, since it undergoes the largest micellar effect in the hydration reaction.[4] Surfactants chosen were CTAB and SDS.

Surfactants: $CH_3(CH_2)_{11}SO_4Na$ (SDS)
$CH_3(CH_2)_{15}N^+(CH_3)_3Br^-$ (CTAB)

Experimental

Materials. Surfactants were products of Wako Pure Chemical Ind. SDS (biochemical use) was used without further purification. CTAB was recrystallized from ethanol before use. Preparation of NDNH was reported previously.[4]

Kinetics. The kinetic measurements were carried out at 30±0.1 °C at a calculated ionic strength (with KCl) using a Hitachi 124 spectrophotometer. The reaction was initiated by mixing NDNH (alcohol solution) with a buffered, aqueous solution of surfactant, and the rate of hydration was estimated by monitoring the disappearance of absorbance at 357 nm, which is characteristic of 1,4-dihydronicotinamides. The pseudo-first-order rate constants (k_{obsd}) thus obtained can be satisfactorily described by Eq. 3, in which k_0 is the reaction rate with water, and k_H and k_{HA} are the catalytic constants for oxonium ion and buffer acid, respectively.

$$k_{obsd} = k_0 + k_H[H_3O^+] + k_{HA}[HA] \tag{3}$$

One can separately determine $k_0+k_H[H_3O^+]$ and k_{HA} from the slope and the intercept of k_{obsd} *vs.* [HA] plots.[1] Although it was desirable to lower the ionic strength as much as possible in order to obtain large micellar effects, the data measured in the absence of added salts did not give linear plots: the lower the buffer concentration, the faster is the rate of hydration. When ionic strength (defined here as $[A^-]+[KCl\ added]$, the concentration of surfactants was always held constant above their CMC) was maintained constant, the plots showed excellent linear relationships ($r>0.99$). Thus, the two kinetic term could be determined by the least-squares method.

Results

Hydration Reaction in the Presence of the CTAB Micelle. In Fig. 1, pseudo-first-order rate constants (k_{obsd}) for the

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* Contribution No. 394 from this Department.

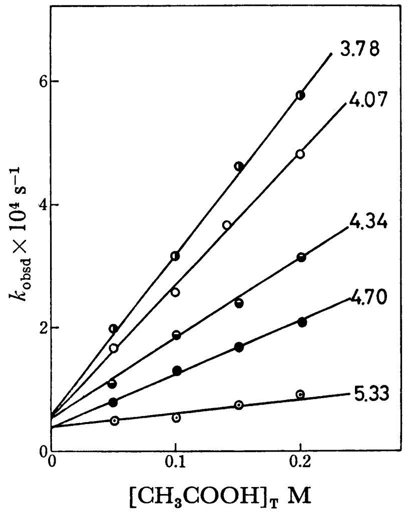


Fig. 1. Pseudo-first-order rate constants for hydration of NDNH in the presence of 1.0×10^{-3} M CTAB plotted as a function of the concentration of acetic acid. 30 °C, $\mu=0.2$, 8.7 vol% ethanol. Numbers in Figure denote pH values.

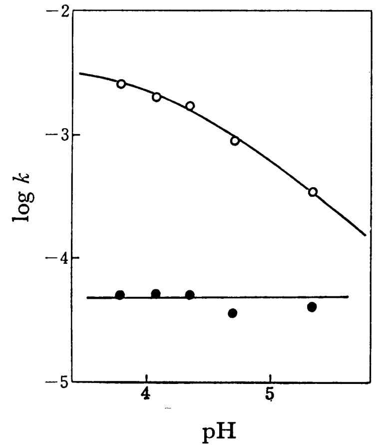


Fig. 2. pH *vs.* log $k_{HA} \cdot [CH_3COOH]_T$ (○) and log $(k_0+k_H[H_3O^+])$ (●) for the CTAB catalysis.

hydration of NDNH in the presence of the CTAB micelle were plotted against the total concentration of acetic acid, $[CH_3COOH]_T=[CH_3COOH]+[CH_3COO^-]$. The concentration of surfactant ($[CTAB]=1.0 \times 10^{-3}$ M) and ionic strength ($\mu=0.2$) were kept constant. The slope, which corresponds to the buffer-catalyzed term (Eq. 3), increased with lowering pH. In contrast, the intercepts which are equal to the sum of k_0 and $k_H[H_3O^+]$ were found to be essentially independent on pH. Figure 2 gives the plots of logarithmic rate constants against pH. The apparent, buffer-catalyzed term (k_{HA}') showed a kinetic pK_a at around 4.60, which is in accord with the potentiometric pK_a value (4.62) of acetic acid. Thus, the true second-order rate constant (k_{HA}) for the undissociated species of acetic acid can be related to k_{HA}' by Eq. 4,

$$k'_{HA} = \frac{a_H}{K_a+a_H} k_{HA} \tag{4}$$

where K_a denotes the acid dissociation constant of acetic acid. The solid curve in Fig. 2 was depicted from Eq. 4 by using pK_a=4.62 and $k_{HA}=3.81 \times 10^{-3}$ M^{-1} s^{-1}.

Since the $k_0+k_H[H_3O^+]$ term is not dependent on pH, the k_H term must be negligibly small in the presence of the CTAB micelle, and the k_0 term is equal to 5.0×10^{-5} s^{-1} under the given condition.

Hydration Reaction in the Presence of the SDS Micelle. It has been noted that the hydration rate of 1,4-dihydropyridine derivatives is conspicuously accelerated in the presence of the SDS micelle at extremely low ionic strength.[4] However, this rate acceleration by the SDS micelle is suppressed at higher ionic strengths (Fig. 3), and the hydration rate of NDNH in the SDS micelle at $([CH_3COOK]+[KCl])>0.01$ is not much different from that of 1-propyl-1,4-dihydronicotinamide in a non-micellar system (*vide post*).

Buffer dilution and pH change were carried out at $([CH_3COOK]+[KCl])=0.03$. The pH of the reaction mixture was confirmed not to vary from pH measurements before and after the reaction. At lower ionic strengths, it was extremely difficult to maintain the pH

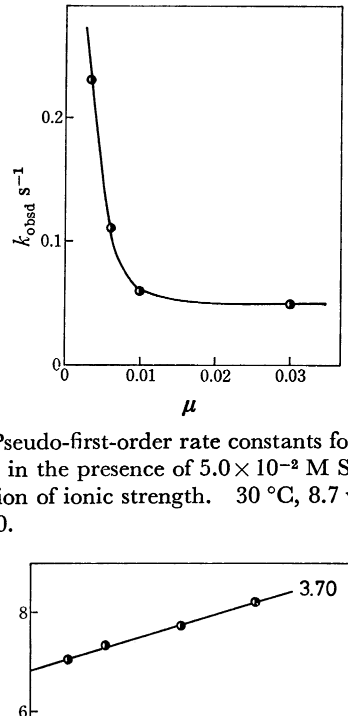


Fig. 3. Pseudo-first-order rate constants for hydration of NDNH in the presence of 5.0×10^{-2} M SDS plotted as a function of ionic strength. 30 °C, 8.7 vol% ethanol, pH 3.80.

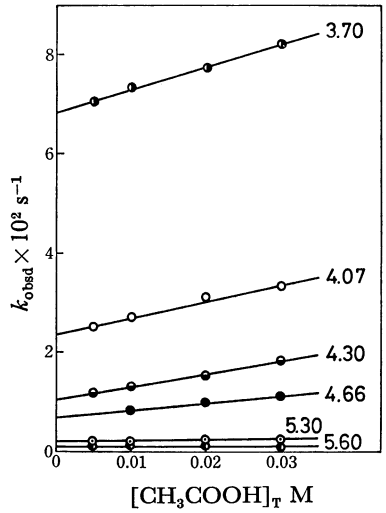


Fig. 4. Pseudo-first-order rate constants for hydration of NDNH in the presence of 5.0×10^{-2} M SDS plotted as a function of the concentration of acetic acid. 30 °C, $\mu=0.03$, 8.7 vol% ethanol. Numbers in figure denote pH values.

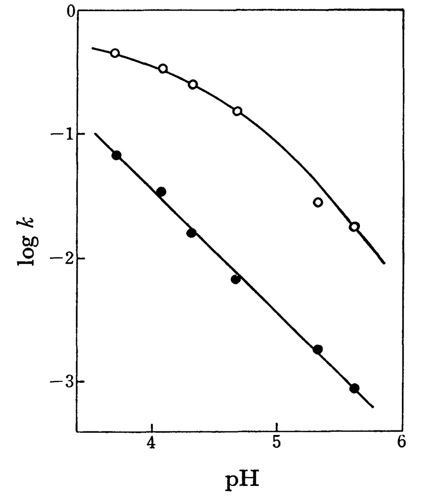


Fig. 5. pH *vs.* log $k_{HA}\cdot[CH_3COOH]_T$ (○) and log $(k_0+k_H[H_3O^+])$ (●) for the SDS catalysis.

of the reaction system constant. The results are illustrated in Fig. 4. Excellent linear correlations are demonstrated between k_{obsd} and $[HA]_T$ (correlation coefficient better than 0.99 in all cases). The hydration reaction in the presence of the SDS micelle is catalyzed by both H_3O^+ and HA, since both of the slope and the intercept increase by lowering pH of the reaction system in Fig. 4. The k_{HA}'-pH plots of Fig. 5 showed a kinetic pK_a at 4.68, which again coincides with the potentiometric pK_a of acetic acid (4.62). The theoretical curve in Fig. 5 was obtained from Eq. 4 for $k_{HA}=0.581$ M^{-1} s^{-1} and $pK_a=4.68$, which produced the best fit to the experimental plots.

Figure 5 also gives log $(k_0+k_H[H_3O^+])$ plotted against pH. The linear relation of slope of −1.0 indicates that the H_3O^+–catalysis is predominant, and the following rate constants are obtained: $k_0\approx 0$ s^{-1} (negligibly small compared with the $k_H[H_3O^+]$ term) and $k_H=341$ M^{-1} s^{-1}.

The rate constants determined by the acetate-buffer dilution and by the pH change are summarized in Table 1.

Hydration Catalyzed by Miscellaneous Carboxylic Acids. Five different carboxylic acids were examined as buffer in addition to acetic acid, *i.e.* α-chloroacetic acid, malonic acid, decanoic acid, hippuric acid, and isobutyric acid. Buffer dilutions were carried out at one pH at the constant surfactant concentration. From the slope and the intercept were determined the rate constants for each carboxylic acid, and the k_{HA} values were evaluated by using the potentiometric pK_a values. The results are recorded in Table 2. It is seen from Table 2 that the k_H terms are again suppressed by the CTAB micelle. It is also worth mentioning that the k_{HA} values in the presence of the CTAB micelle are decreased by more than 100 times as compared with those in the presence of the SDS micelle.

TABLE 1. RATE CONSTANTS FOR THE HYDRATION REACTION OF NDNH CATALYZED BY ACETIC ACID AT 30 °C

Micelle	k_0 s^{-1}	k_H M^{-1}s^{-1}	k_{HA} M^{-1}s^{-1}
None[a)]	d)	260	0.15
CTAB[b)]	5×10^{-5}	$\sim0(<10^{-4})$	0.00381
SDS[c)]	d)	341	0.581

a) Determined for 1-propyl-1,4-dihydronicotinamide; 25 °C, $\mu=0.48$.[1)] b) $[CTAB]=1.0\times10^{-3}$ M, $\mu=0.2$ (KCl), 8.7 vol% ethanol. c) $[SDS]=5.0\times10^{-3}$ M, $\mu=0.03$ (KCl), 8.7 vol% ethanol. d) Too small to determine as compared with other terms.

Discussion

Since NDNH is not soluble by itself in aqueous solutions, one may assume that it is solubilized in the micellar phase. This assumption is very important in discussing the micellar catalysis, for partitioning of the substrate between the micellar phase and the bulk phase frequently gives rise to complications of the micellar systems.

Although our previous study showed that an anionic micelle accelerated the hydration reaction and that an cationic micelle decelerated,[4)] the contribution of each catalytic species, water, oxonium ion, and buffer acid, remained ambiguous. The k_0 value of 1-propyl-1,4-dihydronicotinamide in a non-micellar system was previously evaluated to be 2.6×10^{-5} s^{-1} (25 °C)** by Johnston and co-workers.[1)] This value is very close to

TABLE 2. RATE CONSTANTS FOR THE HYDRATION REACTION OF NDNH CATALYZED BY VARIOUS CARBOXYLIC ACIDS AT 30 °C

Carboxylic acid	pK_a[a)]	Rate Constant in SDS		CTAB		
		k_H M^{-1}s^{-1}	k_{HA} M^{-1}s^{-1}	$k_0\times10^5$ s^{-1}	k_H M^{-1}s^{-1}	k_{HA} M^{-1}s^{-1}
1. $ClCH_2COOH$	2.84	521	12.3	5	~0	0.0810
2. $C_6H_5CONHCH_2COOH$	3.10	494	7.67			
3. CH_3COOH	4.62	341	0.581	5	~0	0.00381
4. $CH_3(CH_2)_8COOH$	4.82	304	0.905			
5. *iso*-C_3H_7COOH	4.86	311	0.641	5	~0	0.00203
6. $^-OOCCH_2COOH$	5.65	225	0.0368	3	~0	0.00085

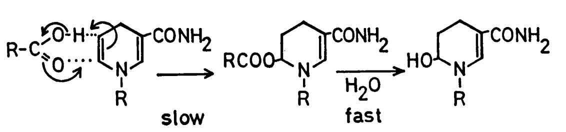
a) Determined potentiometrically in the absence of micelles at 30 °C.

** Johnston *et al.*[1)] reported $k_{H_2O}=4.7\times10^{-7}$ M^{-1} s^{-1}. This value was multiplied by 55 to give k_0(s^{-1}).

$k_0=5.0\times10^{-5}$ s^{-1} obtained for NDNH in the presence of the CTAB micelle. Therefore, the k_0 term is little affected by the micelle. On the other hand, the results shown in Table 1 clearly demonstrate that rate suppression observed in the CTAB micelle is largely derived from the lack of the H_3O^+-catalysis. Apparently, oxonium ion cannnot catalyze the hydration reaction of 1,4-dihydronicotinamide if it is trapped in the Stern layer of the CTAB micelle ($k_H\approx0$). It has been noticed that cationic micelles have inhibitory effect upon the acid-catalyzed reactions. Typical examples are seen in the hydrolyses of orthoesters and acetals which involve proton transfer from the acid to the substrate.[7)]

Table 1 shows that the k_{HA} value for acetic acid is *ca.* 40-fold smaller in the CTAB micelle than in the nonmicellar system. This term is associated with the catalysis by neutral species (undissociated acetic acid), so that it is unlikely that this rate suppression is related to the proximity effect of the micelle. Instead, this would reflect the fact that the formation of such a positively charged intermediate be destabilized by the cationic environment of the CTAB micelle.

An alternate reaction mechanism for the k_{HA} term involves a concerted catalysis.[1)] Carboxylic acids can donate both a proton and an anion simultaneously as shown in Eq. 5. This possibility cannot be ruled out kinetically because it is equivalent to Eqs. 1 and 2.



(5)

As can be seen from Eq. 5, the concerted mechanism produces a less charged intermediate. The pronounced lowering of the k_{HA} value observed in the presence of the CTAB micelle is incompatible with this supposition. Therefore, the concerted reaction is unlikely under the present condition. Johnston *et al.*[1)] and Kim and Chaykin[2)] are similarly against the concerted mechanism.

The SDS micelle enhanced both the k_H and k_{HA} values. Apparently, the magnitude of the rate enhancements is not very large because of the use of high ionic strength which suppresses the reaction. The high ionic strength was necessitated due to the high buffer concentration employed. As shown in Fig. 3, the k_{obsd} value rapidly increased with decreasing ionic strength. This rate variation is probably related to the change in the k_H term, for oxonium ion would be adsorbed on the SDS micelle in competition with added salts. Unfortunately, however, the k_H value at the very low ionic strength could not be evaluated (see Experimental section).

Figure 6 gives Brönsted plots for the k_{HA} term in micellar systems. Both plots gave approximately linear relations with the slope of -0.8, and the difference in the intercept between CTAB and SDS was about two orders of magnitude. The SDS micelle provides much more favorable environments for the carboxylic acid-catalyzed hydration reaction than the CTAB micelle. This difference would become more conspicuous by lowering the ionic strength of the reaction system.

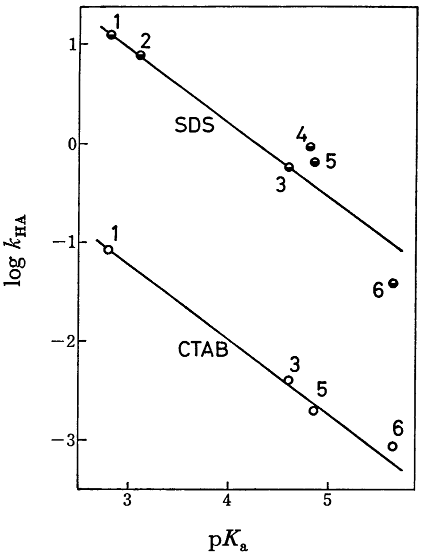


Fig. 6. Brönsted relationship. Numbers in figure correspond to carboxylic acids in Table 2.

The plot for malonic acid (Fig. 6) was lowered by the 0.4 log unit in the relation for the SDS micelle. In contrast, the plot for malonic acid is slightly deviated to the upper part from the CTAB line, the deviation, however, being rather small. These deviations are obviously due to electrostatic repulsion and attraction, respectively.*** and would be more clearly demonstrated at the lower ionic strength.

In conclusion, the acid-catalyzed hydration reaction of 1,4-dihydronicotinamides is very sensitive to the micellar environmental effects. In particular, the k_H value in the presence of the CTAB micelle is suppressed by a factor of 10^7 as compared with the nonmicellar system. Similarly, the reactivity of NADH and its analogs must be greatly affected by the enzymatic microenvironments.

References

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*** pK_{a1}=2.86, pK_{a2}=5.70 at 25 °C[8)]