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Coenzyme Models. XIII. Micellar Catalysis of 1,4-Dihydronicotinamide Reduction of Isoalloxazines and Acridinium Ion

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Synopsis. The 1,4-dihydronicotinamide reduction of isoalloxazines and acridinium ion was examined in cationic (CTAB, CPyB), anionic (SDS), and nonionic (POOA, Brij-35) micelles. The micellar effect is accounted for by micellar stabilization (or destabilization) of the micelle-bound species.

Micellar catalysis provides suitable model systems of enzymatic catalysis, since micelles can influence rates and equilibria of biologically important reactions.[1-4] Very recently, Hadju and Sigman[5,6] reported that *N*-(2-carboxybenzyl)-1,4-dihydronicotinamide and *N*-(*cis*-2-carboxycyclopentyl)-1,4-dihydronicotinamide where the carboxylate moiety is closely fixed near the nicotinamide moiety nonenzymatically reduce *N*-methylacridinium ion in acetonitrile 150—300 times faster than corresponding dihydronicotinamides, but the significant difference cannot be found in an aqueous solution. The result prompted us to investigate the NADH (and model) dependent reactions in micellar systems, since micelles can provide charged environment on the surface and apolar environment in the core. This paper deals with micellar catalysis of 1,4-dihydronicotinamide reduction of isoalloxazines and acridinium ion (Eqs. 1 and 2). We found that the reactions are subject to micellar catalysis, especially to micellar rate inhibition.

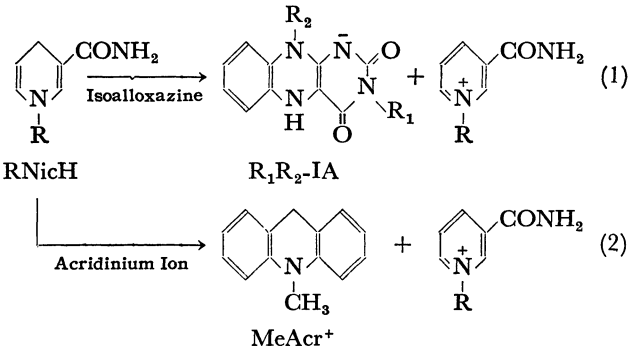

Figure 1 shows the second-order rate constants (k_2) for the reaction of BzlNicH ($R=CH_2C_6H_5$) and MeEt-IA ($R_1=CH_3$, $R_2=C_2H_5$) plotted against surfactant concentrations. The plots for three ionic surfactants (CATB, CPyB, and SDS)** gave sigmoidal curves followed by gradual rate suppression. Since the surfactant concentration where a considerable rate enhancement occurs is almost equal to the CMC for each surfactant (4×10^{-4} M for CTAB, 3×10^{-4} M for CPyB, and 3×10^{-3} M for SDS), the rate enhancement observed is attributable to the micelle formation. It is seen from Fig. 1 that the rate enhancement is transient and the magnitude is of 1.4—2.3 fold at optimal surfactant concentrations. On the other hand, the reaction rate

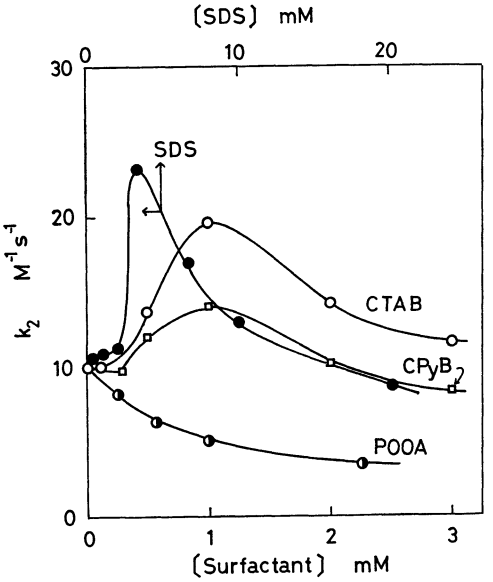

Fig. 1. Second-order rate constants for the reaction of BzlNicH and MeEt-IA plotted as a function of surfactant concentrations. pH 8.5 with 0.02 M phosphate, $\mu=0.02$ with KCl.

was simply retarded with increasing POOA** concentration. The contrasting influence observed between the ionic micelles and the nonionic micelle implies that the ionic environments (both cationic and anionic) are favorable to the reaction.

The micellar catalysis is substantiated more clearly by the reactions involving HxdecBu-IA ($R_1=C_{16}H_{33}$, $R_2=C_4H_9$) and DodNicH ($R=C_{12}H_{25}$), for these

Table 1. Second-order rate constants ($M^{-1}\,s^{-1}$) for the reaction of 1,4-dihydronicotinamides with isoalloxazines[a]

Surfactant	BzlNicH+ MeEt-IA	BzlNicH+ HxdecBu-IA	DodNicH+ MeEt-IA
None	10.0	10.0[b]	48.2[c]
CTAB	20—11	9.92 (3 mM)	4.00 (3 mM)
CPyB	14—8	14.1 (3 mM)	2.86 (3 mM)
SDS	23—9	1.16 (10 mM)	99.5 (10 mM)
POOA	10—3	7.36 (3.3 mM)	18.6 (3.3 mM)

a) 30 °C, $\mu=0.02$, pH 8.5 with 0.02 M phosphate, 3 vol% ethanol, [dihydronicotinamide] = $(2.2-5.0)\times10^{-4}$ M, [isoalloxazine] = $(2.1-4.0)\times10^{-5}$ M. The numbers in parentheses indicate surfactant concentrations. b) MeEt-IA was used instead of HxdecBu-IA. c) PrNicH was used instead of DodNicH.

** CATB: cetyltrimethylammonium bromide, CPyB: cetylpyridinium bromide, SDS: sodium dodecylsulfate, POOA: polyoxyethylene ($n=10$) oleyl alcohol.

compounds are bound to the micellar phase as shown by their spectral changes.[7,8] The second-order rate constants determined at a given surfactant concentration above the CMC are listed in Table 1. The reaction of BzlNicH with HxdecBu-IA (hydrophobic isoalloxazine) was markedly retarded on the addition of 10 mM SDS, while the addition of other surfactants was less significant. On the contrary, the reaction of DodNicH (hydrophobic dihydronicotinamide) with MeEt-IA was suppressed by the cationic micelles, and the SDS micelle accelerated the reaction by a factor of *ca.* 2. Evidently, the micellar effect in these systems reflects the electrostatic influence on the micelle-bound reactant. The examination of Table 1 also reveals that micelles result in the profound inhibitory effect on the dihydronicotinamide reduction, while the accelerative effect is rather small.

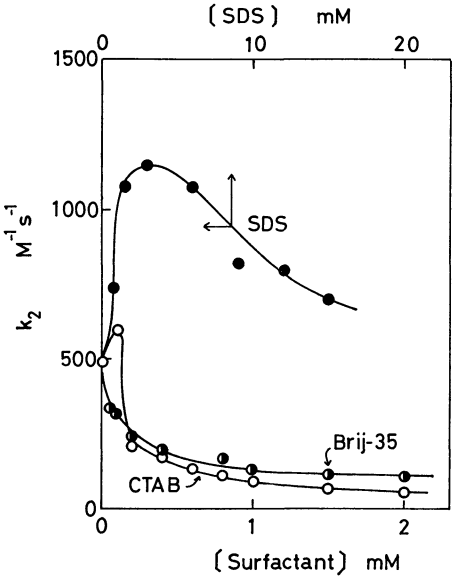

Fig. 2. Second-order rate constants for the reaction of Cl_2BzlNicH and MeAcr$^+$ plotted as a function of surfactant concentrations. pH 7.1 with 0.02 M phosphate, μ=0.02 with KCl.

Figure 2 shows the second-order rate constants for the reaction of Cl_2BzlNicH (R=2,4-dichlorobenzyl) and *N*-methylacridinium ion plotted against surfactant concentration. Plots of k_2 against the concentration of SDS gave the accelerative dependence upon the surfactant concentration, giving rise to the largest rate augmentation of 2.7 fold at the optimal SDS concentration (3 mM). In contrast, k_2 in CTAB and Brij-35 simply descended with increasing surfactant concentrations. The greatest rate suppression caused by the CTAB micelle was 8.7 fold.

The foregoing results establish that the micellar effect in the present system is attributable to the stabilization (or destabilization) of developed charge in the micellar phase. This suggests that NADH dependent reactions which involve the change in the transition state can be controlled by the ionic microenvironments.

Experimental

Materials. *N*-(2,4-Dichlorobenzyl)-1,4-dihydronicotinamide was prepared from *N*-(2,4-dichlorobenzyl)-nicotinamide according to the method of Kim and Chaykin;[9] mp 125—128 °C. Found: C, 54.87; H, 4.24; N, 9.91%. Calcd for $C_{13}H_{12}N_2OCl_2$: C, 55.12; H, 4.24 N, 9.89%. Preparations of other compounds were previously described.[7,8]

Kinetics. All the kinetic measurements were carried out at 30±0.1 °C at a calculated ionic strength (μ=0.02 with KCl) unless otherwise stated. The kinetic runs involving isoalloxazines were performed under strictly anaerobic(N_2) conditions in order to avoid air reoxidation of the reduced products. The progress of the reaction was followed spectrophotometrically by monitoring the disappearance of isoalloxazines (433 nm for MeEt-IA and 440 nm for HxdecBu-IA) and MeAcr$^+$ (420 nm). The reactions were first-order both in dihydronicotinamides and in oxidizing agents.

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