BULLETIN OF THE CHEMICAL SOCIETY OF JAPAN, VOL. 48(6), 1918—1921 (1975)

Coenzyme Models. III. Enhanced Charge Transfer Interaction between the Oxidized and Reduced Forms of Nicotinamide in Micellar and Polymeric Systems. NAD Coenzyme Model[1]

Seiji Shinkai, Kiyoshi Tamaki, and Toyoki Kunitake

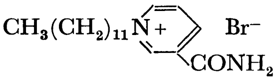
Department of Organic Synthesis, Faculty of Engineering, Kyushu University, Fukuoka 812

(Received February 8, 1975)

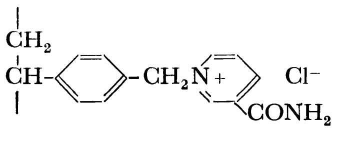
The micelle of 1-lauryl-3-carbamoylpyridinium bromide(NLaN) and poly-1-(*p*-vinylbenzyl)-3-carbamoylpyridinium chloride (PVCC) were shown to form charge transfer complexes with dihydronicotinamides and several aromatic compounds in aqueous media. A broad charge transfer absorption at longer wavelengths was easily detected, and the association constant of 1 : 1 complexes which was estimated from the CT band was enhanced by 6.2—220 times in the NLaN micelle and by 14—66 times in the polymeric system, compared with non-micellar, monomeric system. The enhancement of the interaction between NLaN and its reduced from (NLaNH) was similar in the CTAB micelle and in a nonionic micelle. The increased electron-acceptabilities of the pyridinium group in micellar and polymeric systems were discussed in reference to NAD coenzyme.

The oxidized and reduced forms of flavin mononucleotide produce a charge transfer complex which is a precursor of the semiquinone-type free radical.[2] The charge transfer interaction is similarly expected to exist in NAD coenzyme, and, in fact, a new broad absorption which appears to be due to a 1 : 1 charge transfer complex between NAD and NADH was observed at the longer wavelength.[3] In the nonenzymatic system the charge transfer interaction could be recognized only at abnormally high concentrations (0.14—1.5 M),[3] in the intramolecular charge transfer systems,[4,5] and in the frozen system at −78 °C.[6] Though it is quite important to prove the existence of the charge transfer interaction in connection with the hydrogen transfer reaction involving NAD coenzyme, the conditions employed in the previous model systems seem to be far from the original milieu where the enzymic reaction proceeds. Thus, it occurs to us that the charge transfer interaction may be more easily detected in microheterogeneous systems such as micelles and water-soluble polyelectrolytes. This paper describes charge transfer interactions of poly-1-(*p*-vinylbenzyl)-3-carbamoylpyridinium chloride(PVCC) and 1-lauryl-3-carbamoylpyridinium bromide (NLaN) with dihydronicotinamides (NBzNH, NLaNH) and several aromatic compounds, and the results obtained were compared with the corresponding monomeric compound.

Acceptor:

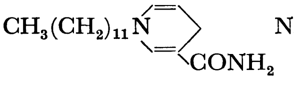


NLaN

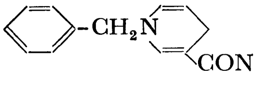


PVCC

Donor:



NLaNH



NBzNH

Experimental

Materials. *p*-Chloromethylstyrene was prepared according to the method of Miyake and Tanimoto,[7] bp 58—61°C/0.13 mmHg (lit,[7] 58—62 °C/0.4 mmHg).

1-(*p*-Vinylbenzyl)-3-carbamoylpyridinium chloride (VCC) was prepared by quaternization of nicotinamide by *p*-chloromethylstyrene in methanol. Recrystallization of the resulting precipitates from methanol gave colorless needles; yield 86—92%, mp 235—238 °C. Found: C, 64.16; H, 6.21; N, 10.16%. Calcd for $C_{15}H_{15}N_2OCl \cdot 0.33\ H_2O$: C, 64.16; H, 6.35; N, 9.88%.

Polymerizations of VCC were conducted using potassium persulfate as initiator at 65 °C in water solvent. After 24 hr viscous polymer solutions were poured into acetone, and the recovered polymers were purified by dialysis to give white powders, yield 90%.

The syntheses of NLaN and NLaNH were described in the accompanying paper.[8] Other materials used in this paper were products of Wako Pure Chemical Ind.

Electronic Spectra. Absorption spectra were taken on a Hitachi 124 spectrophotometer at a slow scan speed to permit accurate determination of extinction coefficients. Spectra were recorded at 30 °C in aqueous solutions adjusted to pH 9.30 with 0.02 M borate buffer.

Association Constants. Association constants (K) in polymeric systems were determined by varying the concentration of PVCC while maintaining the concentration of donor compounds(D) constant. Data thus obtained were plotted according to Eq. 1 which holds for formation of a 1 : 1 complex,[9]

$$\frac{\Delta \mathrm{OD}}{[\mathrm{PVCC}]} = K \cdot \varepsilon[\mathrm{D}] - K\Delta \mathrm{OD} \qquad (1)$$

where [PVCC] represents the concentration of the VCC unit in the polymer, ε is the apparent molar absorption coefficient of the complex, and ΔOD represents the difference of optical density between the observed value and the sum of the individual absorptions. From the least-squares procedure of ΔOD *vs.* ΔOD/[PVCC] plots, slopes and intercepts were evaluated, and K and ε values are determined according to Eq. 1.

In micellar systems it was experimentally difficult to attain the conditions mentioned above, so that Eq. 1 was modified to Eq. 2 and ΔOD values were measured at two different NLaN concentrations.

$$\Delta \mathrm{OD} = \frac{\varepsilon K}{\left(K + \frac{1}{[\mathrm{NLaN}]}\right)} \cdot [\mathrm{D}] = A \cdot [\mathrm{D}] \qquad (2)$$

From the slope of [D] *vs.* ΔOD plots at different NLaN concentrations were determined two A values, the simultaneous equation of which gave K and ε values.

Results and Discussion

Charge Transfer Absorption Spectra. The absorption spectrum of NLaNH obtained in a micellar solution of cetyltrimethylammonium bromide (CTAB: 2.50×10^{-2} M) was essentially the same as that in absolute ethanol. On the other hand, the addition of NLaNH to a NLaN-micellar solution provided a colored solution (yellow-light orange). A new, diffused absorption appeared at 430—550 nm (Fig. 1) as previously reported for NAD[3)] and for 1-benzyl-3-carbamoylpyridinium[9)] in non-micellar systems at higher concentrations. In the NLaN-micelle, addition of 10^{-3}—10^{-4} M of NLaNH was found to be sufficient to detect the charge transfer absorption. According to Ludowieg and Levy,[3)] the charge transfer absorption between 1-propyl-3-carbamoylpyridinium chloride (NPrN) and its reduced form (NPrNH) is very susceptible to the dilution effect. Although mixing of a 10^{-2} M colorless solution of NPrN with a 10^{-2} M light-yellow solution of NPrNH gave a solution with little increase in color intensity, an instantaneous orange color was observed at higher concentrations (0.7—1.5 M).[3)] Therefore, the enhanced charge transfer interaction observed in the present micellar system may be associated with increased concentrations of the nicotinamide groups of NLaN and NLaNH in the thin Stern layer. The intensity of the charge transfer absorption was proportional to the concentration of NLaNH, when the concentration of NLaN was maintained constant under the condition of $[\text{NLaN}] \gg [\text{NLaNH}]$. Thus, Eq. 2 is applicable to the present system, *i.e.*, the 1 : 1 complex is formed between NLaN and NLaNH even in a micellar system.

The interaction between NLaN and NLaNH was enhanced to a similar extent in a nonionic micelle of polyoxyethylene (10) oleyl alcohol as well as in CTAB micelle, and the spectra observed were almost the same as that observed in NLaN micelle (Fig. 1). Evidently, the ionic environment, characteristic of ionic micelles, is not obligatory to enhanced interaction. The concentration effect must play a decisive role for the enhancement of the charge transfer interaction in micellar systems.

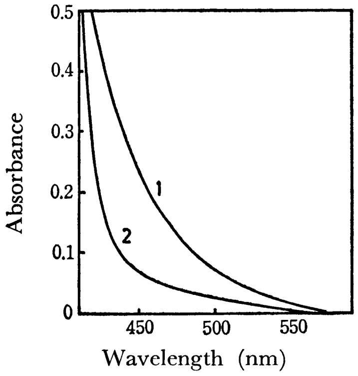


Fig. 1. Absorption spectra of NLaNH in micellar systems.
$[\text{NLaNH}] = 4.0 \times 10^{-3}$ M, 30 °C, pH 9.30 with 0.02 M borate, 8 vol % ethanol.
1: $[\text{NLaN}] = 1.56 \times 10^{-2}$ M, 2: $[\text{CTAB}] = 2.50 \times 10^{-2}$ M.

Enhanced charge transfer interactions with NLaN and with PVCC were also observed for naphthalene, acenaphthene, adenosine and indole derivatives. Complexes with indole derivatives showed strong charge transfer absorptions at 320—450 nm and a difference spectrum indicated absorption maxima at 303—324 nm ($\varepsilon = 2600$—900, Fig. 2).[10)] These spectra are of particular interest because of their similarity to the spectrum of the NAD-glyceraldehyde-3-phosphate dehydrogenase complex (λ_{max} 360 nm).[11)] This enzyme is the only one which is known to form a charge transfer complex between NAD and the enzyme residue, probably an indole nucleus. Velick[12)] suggested that the NAD coenzyme is bound at the top or bottom of a tryptophan stack in the enzyme. A similar spectrum is observed in a model compound, 1-(β-indolylethyl)-3-carbamoylpyridinium chloride, which possesses the intra-

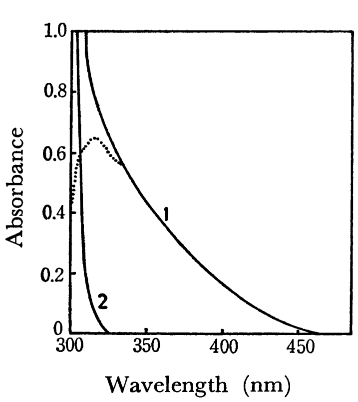


Fig. 2. Absorption spectra of (3-indolyl)acetic acid in micellar systems. The dotted curve indicates a difference spectrum (λ_{max} 317 nm).
$[\text{(3-Indolyl)acetic acid}] = 1.0 \times 10^{-3}$ M. Other conditions are the same as a note of Fig. 1.

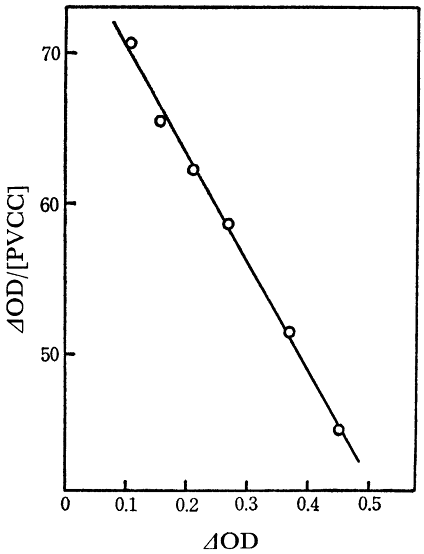


Fig. 3. Spectrophotometric determination of the association constant for the complex of indole with PVCC in water.
30 °C, pH 9.30 with 0.02 M borate, $[\text{indole}] = 3.0 \times 10^{-3}$ M. See text (Experimental, Eq. 1) for details of the method.

molecular interaction between 3-carbamoylpyridinium and indole moieties (λ_{max} 325 nm, $\varepsilon = 1000$).[4,5]

Association Constants. Association constants were determined according to the method of Cilento *et al.*[9,13,14] as described in the experimental section. The wavelengths employed are the same as in their system. The plots of ΔOD *vs.* ΔOD/[PVCC] in the polymeric systems gave good linear relationships and the correlation coefficients were better than 99% (Fig. 3). Association constants (K) and molar absorption coefficient (ε) determined using the least squares method are recorded in Table 1. It is seen that ε values are one half to two thirds of those of monomeric 3-carbamoylpyridinium.[9,13,14] On the other hand, the association constant was enhanced by 14—66 times. These results indicate that PVCC provides a better environment in forming the charge transfer complex compared to the monomeric compound and that a 1 : 1 complex is formed between a donor molecule and a VCC unit in the polymer. In the polymeric system two VCC units could interact with a donor molecule from both sides. However, this is not the case. And the better acceptor property observed may suggest that adjacent VCC units function to increase the electron-acceptability of the complex-forming VCC unit. As expected, the association constant of (3-indolyl)acetic acid was the greatest.

The plots of [D] *vs.* ΔOD for micellar systems gave linear relationships (Fig. 4), and association constants and molar absorption coefficients were computed from Eq. 2. The result is summarized in Table 2. Association constants for indole derivatives were enhanced by only 6—10 times in NLaN micelle, compared with non-micellar system. The enhancement was greater in the polymeric system than in the micellar system. On the other hand, association constants for naphthalene and acenaphthene were enhanced by 129—220 times in NLaN micelle. This large augmentation would be associated with solubilization by micellar systems, that is, water-insoluble aromatics like naphthalene and acenaphthene should be fixed in and/or near the Stern layer of NLaN micelle, resulting in strong charge transfer interactions. These aromatic compounds are insoluble in the aqueous solution of PVCC.

Table 1. Association constants of PVCC[a]

Donor	λ[b] (nm)	NBzN[c] ε	NBzN[c] K (M^{-1})	PVCC ε	PVCC K (M^{-1})
NBzNH	440	307	3.76	180	51
Adenosine	325	145	2.29	100	140
Indole	380	890	2.21	360	72
(3-Indolyl)acetic acid	380	1060	4.09	440	271

a) 30 °C, pH 9.30 with 0.02 M borate buffer. b) Wavelength employed to evaluate ΔOD values of Eq. 1. c) Determined by Cilento *et al.*[9,13,14]

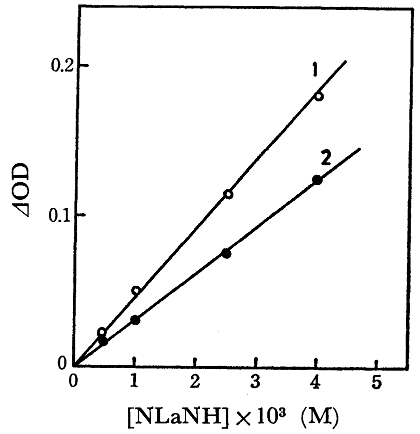


Fig. 4. Spectophotometric determination of the association constant for the complex of NLaNH with NLaN.
30 °C, pH 9.30 with 0.02 M borate, 8 vol % ethanol. 1: [NLaN] = 1.56×10^{-2} M, 2: [NLaN] = 1.00×10^{-2} M. See test (Experimental, Eq. 2) for details of the method.

Association constants of aromatic compounds with NLaN decreased in CTAB and nonionic micelles. On the other hand, those of NLaNH with NLaN increased, or were unaffected in these micelles (Table 2). Presumably, the formation of a charge transfer complex between NLaNH and NLaN is favored because both of them possess the surfactant structure.

NAD Coenzyme Model. The foregoing observations establish that micellar and polymeric systems provide favorable environmentals in forming the 1 : 1 charge transfer complex between NLaN and donors including NLaNH, indole derivatives, and other aromatic compounds. These findings imply that these systems would act as a proper NAD coenzyme model, since it has been known that the interaction between pyridine

Table 2. Association constants of NLaN in micellar systems[a]

Donor	λ (nm)	NBzN[c] ε	NBzN[c] K (M^{-1})	Micellar system ε	Micellar system K (M^{-1}) NLaN	Micellar system K (M^{-1}) CTAB	Micellar system K (M^{-1}) Non-ion
NLaNH	440	307	3.76[b]	180	22	46	23
Naphthalene	326	850	0.17	490	38	9.2	4.6
Acenaphthene	330	1550	0.32	1040	41	19	1.9
Indole	380	890	2.21	600	14	5.6	4.9
(3-Indolyl)acetic acid	380	1060	4.09	690	41	14	11
DL-Tryptophan	380	650	2.52	420	22	13	—

a) 30 °C, pH 9.30 with 0.02 M borate, 8 vol% ethanol. b) Association constant between 1-benzyl-3-carbamoylpyridinium and 1-benzyl-1,4-dihydronicotinamide.[14] c) Determined by Cilento *et al.*[9,13,14]

and adenine rings produced by the folded conformation plays an important role to adjust the oxidation-reduction reactivity in the enzymic system.[15,16] It would be interesting to assess the effect of such an interaction on the oxidation-reduction reaction in a model system.

References

1) Contribution No. 345 from this department.

2) Q. H. Gibson, V. Massey, and N. M. Atherton, *Biochem. J.*, **85**, 369 (1962).

3) J. Ludowieg and A. Levy, *Biochemistry*, **3**, 373 (1964).

4) S. Shifrin, *Biochim. Biophys. Acta*, **81**, 205 (1964).

5) Idem, *Biochemistry*, **3**, 829 (1964).

6) M. J. Spiegel and G. R. Drysdale, *J. Biol. Chem.*, **235**, 2498 (1960).

7) T. Miyake and S. Tanimoto, *Yuki Gosei Kagaku Kyokai Shi*, **31**, 1050 (1973).

8) S. Shinkai, R. Ando, and T. Kunitake, This Bulletin, **48**, 1914 (1975).

9) G. Cilento and S. Schreier, *Arch. Biochem. Biophys.*, 107, 102 (1964).

10) A. Difference spectrum was obtained by subtracting the absorption of indole in CTAB micelle from that in NLaN micelle.

11) E. M. Kosower, *J. Amer. Chem. Soc.*, **78**, 3497 (1956).

12) S. F. Velick, *J. Biol. Chem.*, **233**, 1455 (1958).

13) G. Cilento and D. L. Sanioto, *Arch. Biochem. Biophys.*, **110**, 133 (1965).

14) G. Cilento and P. Tedeschi, *J. Biol. Chem.*, **236**, 907 (1961).

15) G. Weber, *Nature*, **180**, 1409 (1957).

16) S. Shifrin and N. O. Kaplan, *ibid.*, **183**, 1529 (1959).