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Charge-Transfer Complexing Properties of 1-Methyl Nicotinamide and Adenine in Relation to the Intramolecular Interaction in Nicotinamide Adenine Dinucleotide (NAD⁺)

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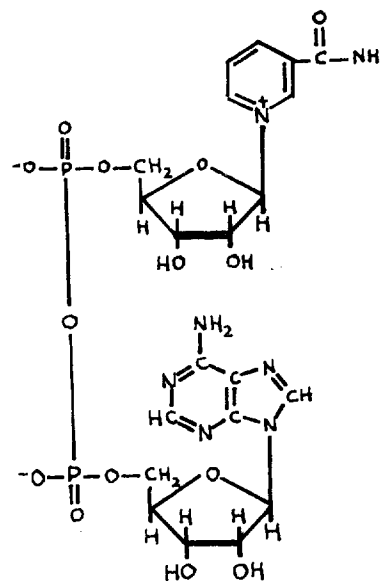
The charge-transfer complexing properties of 1-methyl nicotinamide (MNA), an acceptor, and adenine, a donor, were investigated in water and SDS micellar solutions in relation to the intramolecular interaction in nicotinamide adenine dinucleotide (NAD⁺). The spectral and thermodynamic parameters of MNA-indole and methyl viologen-adenine complex formations were determined, and the data were utilized to evaluate the charge-transfer abilities of MNA and adenine. The electron affinity of nicotinamide was estimated to be 0.28 eV from charge-transfer energy of ~300 nm for MNA-indole. The large enhancement of MNA-indole complexation in SDS solutions by entropy effect was attributed to hydrophobic nature of indole. The complex between adenine and methyl viologen showed an absorption band peaked near 360 nm. The ionization potential of adenine was evaluated to be 8.28 eV from this. The much smaller enhancement of charge-transfer interaction involving adenine than that of indole in SDS solutions was attributed to weaker hydrophobic nature of the donor. The charge-transfer energy of 4.41 eV (280 nm) was estimated for nicotinamide-adenine complex. The spectral behaviors of NAD⁺ were accounted to the presence of intramolecular interaction in NAD⁺, which is only slightly enhanced in SDS solutions. The replacement of nicotinamide-adenine interaction in NAD⁺ by intermolecular nicotinamide-indole interaction in enzyme bound NAD⁺, and guiding role of adenine moiety in NAD⁺ were discussed.

Introduction

The coenzyme nicotinamide adenine dinucleotide (NAD⁺; I) is a major electron acceptor in all known forms of life. Its function as electron acceptor in enzymatic oxidation-reduction reactions necessitates some type of enzyme-coenzyme interactions in which NAD⁺ acts through the reversible reduction of its nicotinamide moiety.

In 1956, Kosower¹ ascribed the moderately intense long wavelength absorption band appearing in the mixtures of NAD⁺ and glyceraldehyde-3-phosphate dehydrogenase (GPD) to a charge-transfer band from a donor to the pyridine cation of NAD⁺. Cilento and Guisti² found that tryptophan gave rise to charge-transfer band with 1-benzyl nicotinamide, which was similar in both contour and intensity to the spectrum of GPD in the presence of NAD⁺. Extensive studies on charge-transfer complex formation between NAD⁺ or its model compounds and indole derivatives in homogeneous solutions¹⁻⁴, and in the presence of micelle⁵, vesicle⁶, and polyelectrolyte⁷ have been followed.

Besides nicotinamide moiety, NAD⁺ bears an adenine moiety, which can act as an electron donor. The intramolecular interaction between nicotinamide and adenine moieties



I. NAD⁺

in NAD⁺ is possible and the interaction, if any, would affect coenzyme activity of NAD⁺. In fact, the charge-transfer interaction between 1-substituted nicotinamide and adenine

derivatives were reported^{8,10b,13}. Thus, the intramolecular charge-transfer interaction in NAD⁺, consequence of this interaction on conformation and activity of NAD⁺, and the role of adenine moiety in NAD⁺ have attracted much attention in last twenty years.

Intensive investigations on conformation of NAD⁺ have been carried out by use of model compounds of NAD⁺. X-ray crystal structure analysis of them suggested that NAD⁺ can adopt a stacking structure (folded conformation), in which the adenine and nicotinamide rings are stacked⁹. This structure was believed to be stabilized by, at least in part, the intramolecular charge-transfer interaction. Spectroscopic¹⁰ and NMR¹¹ studies on NAD⁺ and its model compounds led to conclusion that NAD⁺ adopts the folded conformation in aqueous solution. Recent thermodynamic studies implied that NAD⁺ exists in an equilibrium between an open conformation and the folded one with about 40% for the latter^{12,13}.

On the other hand, the x-ray crystallographic studies on lithium salt of NAD⁺ suggested open conformation for the coenzyme¹⁴. X-ray investigations also demonstrated that NAD⁺ bound to a number of dehydrogenase exist in an open form, with the adenine group is inserted into a hydrophobic pocket of the protein¹⁵.

On these backgrounds, it was of interest to examine, closely, the charge-transfer properties of nicotinamide and adenine moieties of NAD⁺, and to compare the properties with those of methyl viologen, a well-known strong electron acceptor, and indole, respectively. In this paper, we described the results of studies on these aspects in an aqueous and a micellar solutions. The intramolecular charge-transfer interaction in NAD⁺ and, thus, the conformational aspect of the coenzyme have also been studied and was described.

Experimental

1-methyl-3-carboxamide pyridinium (1-methyl nicotinamide:MNA) chloride and N,N'-dimethyl-4,4'-bipyridinium (methyl viologen: MV⁺⁺) dichloride were prepared by reacting methyl iodide with nicotinamide and 4,4'-bipyridine, respectively, followed by substitution of I⁻ into Cl⁻ and recrystallization from ethanol as described in Ref. 16. Sodium dodecylsulfate(SDS) was recrystallized from methanol, after washing with ether. Commercial reagent grade NAD⁺, adenine and indole were obtained from P-L. Biochemicals, BDH Biochemicals and Kanto Chemical Co., respectively, and used as received. Sample solutions were prepared with deionized distilled water. The pH's of solutions were adjusted with 0.15 M cacodylate buffer, if necessary.

Absorption spectra were recorded on a Beckman DU-8B UV-VIS spectrophotometer equipped with temperature controlled cell compartment. All spectral measurements for charge-transfer complex formation were carried out by maintaining the concentration of donor (C_{Di}), indole or adenine, constant at 2.5mM (in water) or 1.25 mM (in 0.1 M SDS). The concentration of acceptor (C_{Ai}), MV⁺⁺ or MNA, was varied in condition of C_{Ai} ≫ C_{Di}. Difference spectra of complex formation were taken with mixing tandem

double cells of light pathlength of 0.882 cm, which is partitioned into two equal compartments, and the data were analyzed as described in next paragraphs.

The values of the complex formation constant(K) and the molar absorptivities (ε) for the complex formation were obtained from the slope and the intercept of a plot of Abs./C_{Di}C_{Ai} versus Abs./C_{Di} according to equation (1), which satisfactorily describes the 1:1 complex formation in condition of C_{Ai} ≫ C_{Di}¹⁷. Abs. stands for the absorbance change

$$\frac{Abs.}{C_{Di}C_{Ai}} = K \cdot \epsilon \cdot l - \frac{Abs.}{C_{Di}} \cdot K \quad (1)$$

due to complex formation at a given wavelength in light pathlength of *l*.

The thermodynamic parameters of complex formation were evaluated from *K* values and the temperature dependencies of *K*'s using fundamental thermodynamic relationships, i.e., $\Delta G = -RT \ln K$, $\partial \ln K / \partial (1/T) = -\Delta H/R$ and $\Delta G = \Delta H - T\Delta S$.

Results and Discussion

Electron Accepting Properties of 1-Methyl Nicotinamide. Electron accepting properties of nicotinamide moiety of NAD⁺ was studied using MNA as a model compound for the moiety and the results were compared to those of MV⁺⁺. Indole was used as an electron donor. Though, mixtures of MNA and indole colored pale-yellow, absorption spectra of the mixtures run against water did not show separate charge-transfer band. However, the difference spectra of the mixtures taken against unmixed components displayed a diffused typical charge-transfer band peaked near 300 nm as shown in Figure 1. The spectra agreed well with reported charge-transfer difference for the complexes between MNA and indole derivatives,^{4,18} and model compounds of NAD⁺ and indole derivatives⁷. The only difference is the peak position which is ~300 nm in this study, rather than ~310nm reported for MNA and indole derivatives.

Series of difference spectra with varying concentration of MNA at desired temperature were taken. The absorbance values at 310, 320 and 330 nm were utilized to evaluate the complex formation constant (*K*) and molar absorptivities (ε) for the complex formation according to the equation (1). Figure 2 shows plots of data taken at 330 nm and at 20, 25, 30, 40 and 50°C. *K* values averaged from data taken at three wavelengths and ε values at each wavelength were summarized in Table 1.

The *K* value of 2.8 M of MNA with indole in water at 25°C is in fair agreement with that of MNA with N-acetyl tryptophan ethyl ester (3.1M)¹⁹, but slightly smaller than those of MNA with uncharged tryptophan derivatives reported (4.0–4.5 M)^{4,18}. This suggests that the size of side chain of indole ring has little effect on the charge-transfer interaction between pyridinium and indole rings, which is quite contrast to MV⁺⁺-indole derivatives^{16,20}. Such absence of steric influence seems to be reflected in the virtually same complex formation constant of MNA with tryptophan residues of α-lactalbumin, lysozyme, and N-acetyl tryptophan ethyl

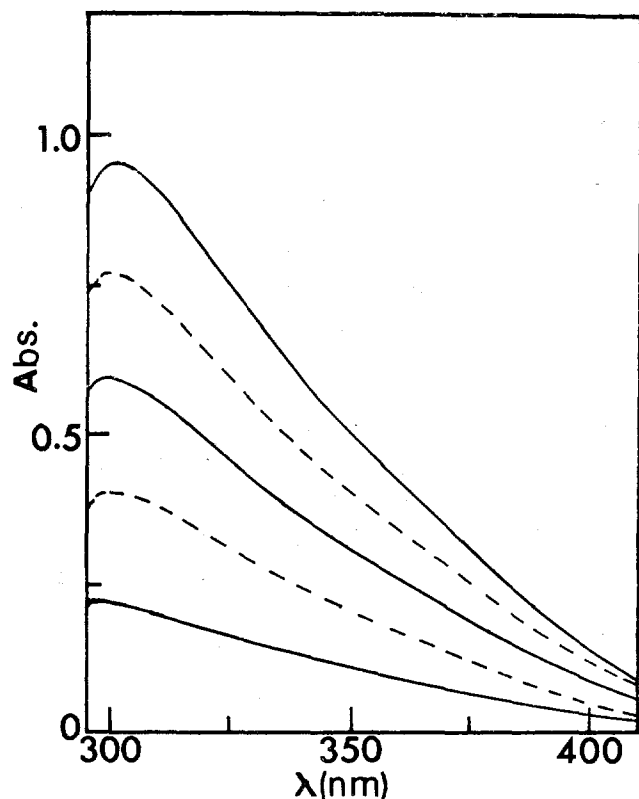


Figure 1. Difference absorption spectra of 1-methyl nicotinamide-indole mixtures in water at 25°C. MNA concentrations are 0.08, 0.06, 0.045, 0.03 and 0.015 M (from top to bottom). [Indole] is fixed at $2.5 \times 10^{-3} M$.

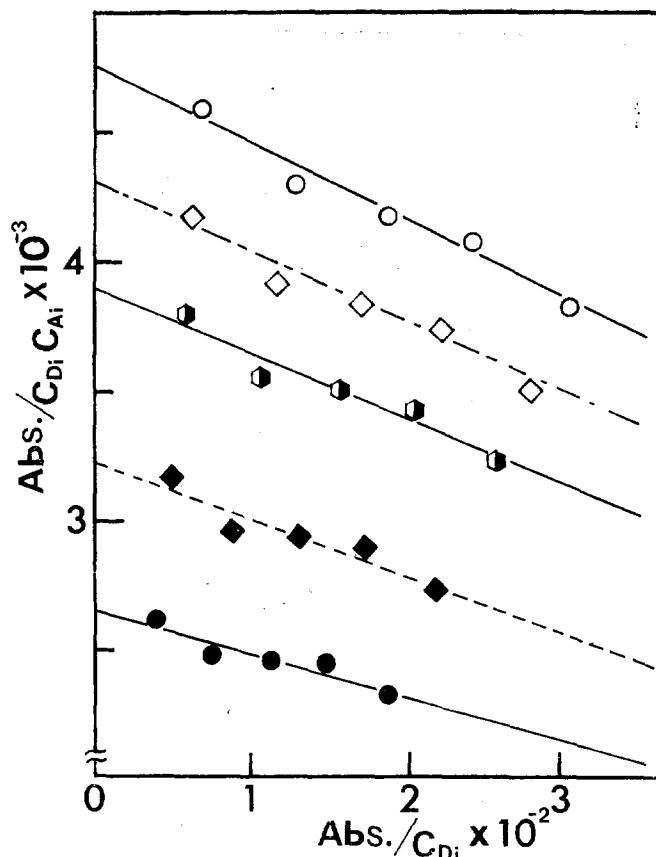


Figure 2. Plots of complex formation data of MNA-indole system in water according to equation (1). Absorbances were taken at 330 nm. Temperatures are 20, 25, 30, 40 and 50°C (from top to bottom).

TABLE 1: Formation Constants and Molar Absorptivities of 1-Methyl Nicotinamide-Indole and Methyl Viologen-Adenine Complexes

Systems	T, °C	Water			0.1M SDS				
		K*	ε, M ⁻¹ cm ⁻¹			K*	ε, M ⁻¹ cm ⁻¹		
	310nm		320nm	330nm			310nm	320nm	330nm
MNA-Indole	20	3.0	2280	2110	1800	81	998	901	785
	25	2.8	2210	2020	1760	75	957	858	753
	30	2.5	2260	2100	1760	70	912	819	718
	40	2.1	2020	1990	1700	62	822	730	636
	50	1.7	2260	2070	1890	55	730	645	551
			ε			ε			
		K*	360nm	370nm	390nm	K*	360nm	370nm	390nm
MV ²⁺ -Adenine	25	2.1	311	303	204	12	193	178	112
	30	1.9	308	299	215	11	182	168	106
	40	1.7 ₃	277	280	195	9.9	152	141	91
	45	1.6 ₇	262	271	189	9.0	146	133	83

* Averaged from data at three wavelengths and in unit of M.

ester¹⁹. It is of interest to point out that the *K* values of 1-benzyl nicotinamide with indole and its derivatives (2.0–2.5 M)^{2,21} are smaller than those of MNA complexes, presumably, due to steric hinderance.

To examine the effect of hydrophobic microenvironment on MNA-indole interaction, the effect of SDS on the charge-transfer spectra was studied. The presence of SDS resulted in enhancement of charge-transfer absorption, but the shape and position of the absorption band remained unchanged.

Set of absorption spectra with varying concentration of NMA at various temperature were recorded and analyzed by the same methods as employed for those in water. The results are included in Table 1. As can be seen in Table 1, the SDS micelle enhanced the complex formation by about 30 times. This enhancement was greater than the enhancement observed in a self-assembling 1-lauryl nicotinamide micelle (6 times), and nearly the same as that observed in a polymer pendent nicotinamide, poly-1-(p-vinylbenzyl) nicotinamide⁵.

Recently, Murakami *et. al* reported larger enhancement of nicotinamide-indole interaction in a vesicle forming nicotinamide, $(\text{NA}^+)\text{C}_5\text{Ala}2\text{C}_{12}$, and attributed the different extent of charge-transfer interaction among organized acceptor systems to the reflection of extent of static organization of nicotinamide groups in each system²². However, greater enhancement of interaction in SDS micelle than in 1-lauryl nicotinamide micelle indicates that the hydrophobic nature of each system and the proximity of donor and acceptor in each system, rather than static organization of acceptors, are important factors deciding the extent of the charge-transfer interaction. Therefore, the greater association of nicotinamide and indole in SDS micelle, compared with that in 1-lauryl nicotinamide micelle, can be attributed to close contact of the acceptor and donor: Nicotinamide moiety of MNA can penetrate "Stern layer" of SDS micelle and would locate near indole which is incorporated in hydrophobic region of the micelle, whereas that of 1-lauryl nicotinamide forms "Stern layer" resulting less contact with indole.

Thermodynamic quantities of the complex formation were determined from K values and dependencies of K 's on temperature. Figure 3 shows $\ln K$ vs. $1/T$ plots of MNA-

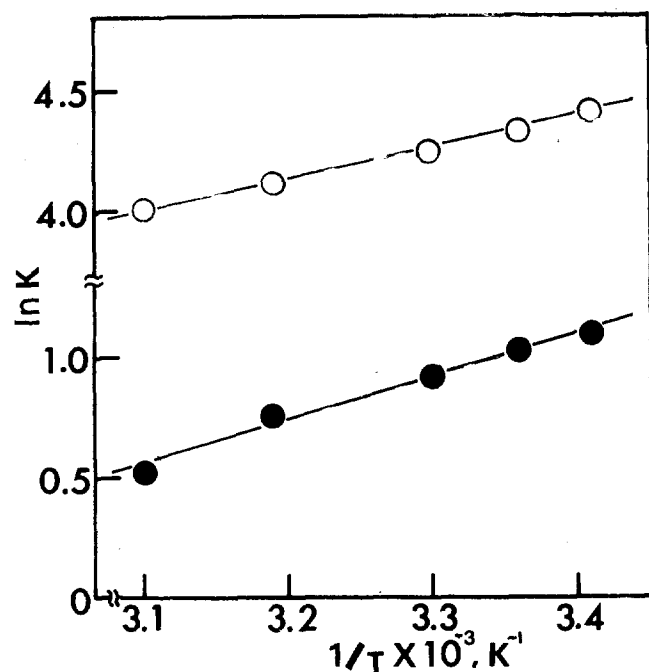


Figure 3. Plots of $\ln K$ vs. $1/T$ for data of MNA-indole system in water (●) and in 0.1M SDS (○).

indole system obtained in water and 0.1 M SDS. The ΔG , ΔH and ΔS values evaluated from these plots at 25°C were listed in Table 2. These quantities are in the range of the values for a typical charge-transfer complex formation, and agree well with the reported values of $\Delta G = -0.68$ Kcal/mole, $\Delta H = -3.5$ Kcal/mole and $\Delta S = -8.3$ e.u. for MNA and N-acetyl-L-tryptophan in water¹⁸. Table 2 also shows that the enhancement of charge-transfer interaction in a micelle results from increased entropy of complexation, not from enthalpy effect. This finding matches well with that of our previous study¹⁶ on MV^{++} -indole derivative systems (see also Table 2).

In comparing charge-transfer properties of MNA and MV^{++} to common electron donor, indole, several noticeable differences are evident. The difference in charge-transfer energy ($h\nu_{CT}$), ~ 300 nm (4.14 eV) for MNA vs. ~ 390 nm (3.18 eV) for MV^{++} complex, reflects the difference of electron affinity between the two acceptors: The $h\nu_{CT}$ is closely related to donor ionization potential (I_p) and electron affinity of acceptor (EA) by equation (2).

$$h\nu_{CT} = I_p - EA - W \quad (2)$$

W is a parameter related to the dissociation energy of the charge-transfer excited state. If one assumes that W is same for both complexes, EA of MNA is estimated to be 0.28 eV from EA of MV^{++} , 1.24 eV²³ and the $h\nu_{CT}$ values. The large difference in EA of MNA and MV^{++} matches well with the difference of half-wave potential of these cations in water, -0.71 V for MV^{++} ²⁴ versus -1.13 V (both vs. SCE) for MNA²⁵. This difference in electron affinity between two acceptors appears also in difference in K values.

The large ϵ values, despite of small K values, of MNA-indole complex formation compared to those of MV^{++} -indole complex are of special interest. This can be, in part, attributed to involvement of so-called "contact charge-transfer" in the former complex. However, the sensitivity of ϵ on temperature in the presence of SDS micelle, and apparent absence of linearity between ϵ and $1/K$ relationship predicted for contact charge-transfer indicate that other factors are also contributing to the large ϵ value of MNA-indole complex. One possibility is the strong dipole-dipole interaction between the two interacting species in the complex. This could arise from effective overlap of the donor and acceptor orbitals by parallel arrangement of the two species. Such parallel arrangement is not expected in MV^{++} -

TABLE 2: Parameters of the Charge-transfer Complex Formation Involving 1-Methyl Nicotinamide and Adenine at 25°C

Acceptor	Donor	Medium	K (M)	$\epsilon(\lambda, \text{nm})$ ($\text{M}^{-1} \text{cm}^{-1}$)	$-\Delta G$ (Kcal/mole)	$-\Delta H$ (Kcal/mole)	ΔS (e.u.)	Ref.
MNA	Indole	water	2.8	2210(310)	0.62	3.7	-10	this work
MNA	Indole	0.1M SDS	77	957(310)	2.56	2.4	+ 0.41	this work
MNA	Adenosine	water	1.4	180(325)	—	—	—	13
MNA	Adenosine	water	2.0	143(325)	—	—	—	27
MV^{++}	Indole	water	8.1	741(390)	1.22	2.8	- 5.5	16
MV^{++}	Indole	0.1M SDS	870	372(390)	4.08	1.5	+ 8.7	16
MV^{++}	Adenine	water	2.1	311(360)	0.44	1.9	- 5.0	this work
MV^{++}	Adenine	0.1M SDS	12	193(360)	1.47	2.3	- 2.8	this work

indole complex from geometric consideration of the acceptor. *Electron Donating Properties of Adenine.* Electron donating properties of adenine moiety of NAD^+ were studied from MV^{++} -adenine complex formation and the results were compared with those of indole. Much similar studies described in previous section for MNA-indole system were performed. The difference charge-transfer absorption spectra at various concentrations of MV^{++} were shown in Figure 4. The absorption maxima were ~ 360 nm (3.45eV) which is ~ 30 nm shorter than that of MV^{++} -indole. The actual charge-transfer energy would be slightly larger than this value, because the absorption from a complex could be partially masked by absorption from its components, and the maximum in a difference would appear at longer wavelength. The same argument could also be applied to MNA-indole spectra, but we felt that the apparent absorption maxima in difference spectra could be good approximation of charge-transfer energy for both systems.

K and ϵ values of MV^{++} -adenine complexes in water and in 0.1 M SDS solutions were calculated from difference spectra using equation (1) and included in Table 1. The thermodynamic parameters of the complex formation were also evaluated and summarized in Table 2. The formation constants of MV^{++} -adenine complex were considerably smaller than those of MV^{++} -indole complex. These decreased K values were accompanied by decreased absorptivity of the complex formation as well as increased charge-transfer energy. Such correlation among K , ϵ and $h\nu_{CT}$ is quite normal in many donor-acceptor complex formations. However, the values of MV^{++} -adenine complex formation are too small to exclude the possibility of less effective overlap of orbitals of the two interacting components in the complex than in MV^{++} -indole.

By taking the charge-transfer energy of MV^{++} -adenine complex as 3.45 eV, the ionization potential of adenine can be evaluated to be 8.28 eV from equation (2) using that of indole, 8.01 eV²⁶ and $h\nu_{CT}$ of MV^{++} -indole, 3.18 eV. As in MV^{++} -indole and MNA-indole complexes, the K values of MV^{++} -adenine complexation were enhanced by the presence of SDS micelle due to entropy effect. However, the enhancement (~ 6 times) was much smaller than those observed in indole complexes (see Tables 1 and 2). This result seems to reflect the weaker hydrophobic nature of adenine than indole.

Intramolecular Charge-Transfer Interaction in NAD^+ . From the results of previous sections, the intramolecular charge-transfer interaction in NAD^+ with nicotinamide as an acceptor and adenine as a donor is expected. The absorption spectra of NAD^+ and an equimolar mixture of MNA and adenine at pH 7 were compared in Figure 5. The spectrum of NAD^+ was consistent with the previously published spectrum for the compound^{10a}. The spectrum of MNA-adenine mixture was similar to that of NAD^+ . However, minor difference was evident, especially, in the absorbance of tail absorption region.

We are not the first to report such enhanced tail absorption in NAD^+ . Many workers (see Ref. 13 and references cited

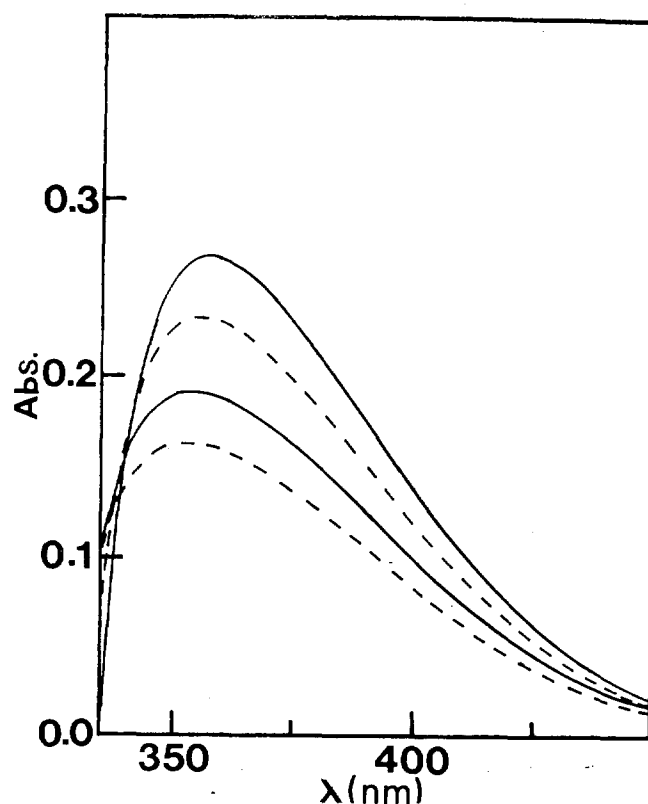


Figure 4. Difference absorption spectra of MV^{++} -adenine mixtures in water at 25°C. [MV^{++}] are 0.3, 0.24, 0.18 and 0.14M (from top to bottom). [Adenine] is fixed at 2.5×10^{-3} M.

therein.) have shown similar feature in UV spectra and circular dichroism of NAD^+ and its model compounds, and interpreted the result as an evidence of existence of intramolecular interaction between nicotinamide and adenine moieties. However, the characteristics of this interaction were not given in detail.

At high concentration of MNA and adenine, the mixture also displayed enhanced tail absorption as in NAD^+ . However, the absorbance of MNA-adenine mixtures was much smaller than that of NAD^+ at equivalent concentration, and concentration dependencies of the mixture and NAD^+ were different (see inset of Figure 5). The linearity between absorbance and NAD^+ concentration, and absence of such linearity in MNA-adenine mixture confirm intramolecular interaction in NAD^+ .

The apparent molar absorptivity of NAD^+ at 330 nm at 25°C was evaluated to be $52 \text{ M}^{-1}\text{cm}^{-1}$. If one assumes the amount of complexed (folded) NAD^+ as 40%^{12,13}, the ϵ value of folded form of NAD^+ at 330 nm is estimated to be $130 \text{ M}^{-1}\text{cm}^{-1}$. This estimation seems to be reasonable as judged from reported ϵ values of MNA-adenosine complex shown in Table 2. We also have attempted to determine K and ϵ values of MNA-adenine complex, independently, but failed to obtain them with reasonable accuracy. The difficulty arises from small K and ϵ values of the complex, and the large discrepancy in these values for MNA-adenosine complex in Table 2 points the fact.

In 0.1 M SDS solutions, ϵ of NAD^+ at 330 nm was slightly

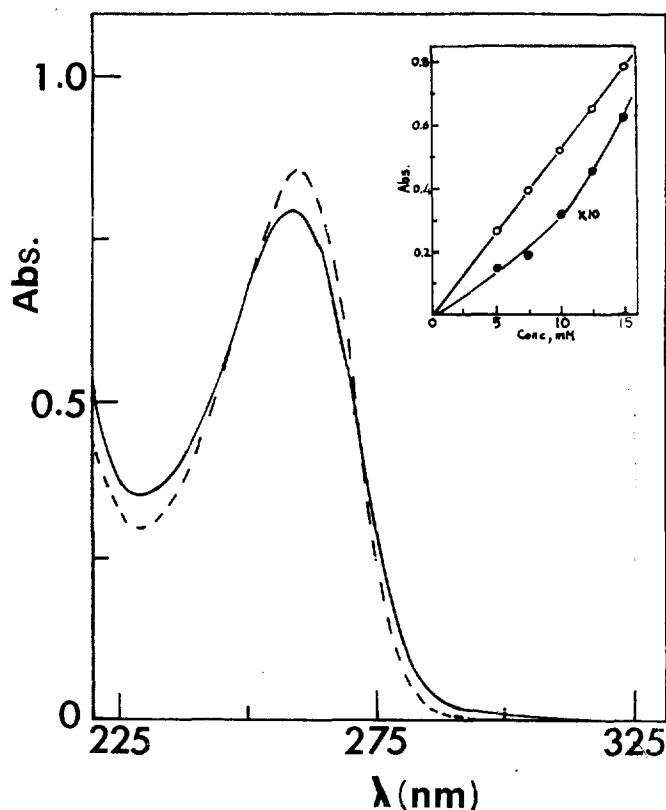


Figure 5. Absorption spectra of $5 \times 10^{-3} \text{M}$ NAD^+ (—) and an equimolar ($5 \times 10^{-3} \text{M}$) mixture of MNA and adenine (.....) at pH 7 and at 25°C . Inset shows variations of absorbances at 330 nm with concentrations of NAD^+ (○) and MNA-adenine (●).

increased to $56 \text{ M}^{-1} \text{cm}^{-1}$, and the linearity between absorbance and NAD^+ concentration was still observed. This implies that the intramolecular interaction in NAD^+ is only slightly enhanced in a micellar medium.

It was expected that the interaction between nicotinamide and adenine is sensitive to pH due to the presence of amino group in adenine moiety. Effects of pH on absorbance of NAD^+ and MNA-adenine mixture at 330 nm were presented in Figure 6. This figure reveals that the intramolecular complexing in NAD^+ as well as intermolecular interaction between MNA and adenine decreases with decreasing pH near pH 4. This pH range accords well with pK of amino group of adenine in NAD^+ , which was reported to be 3.76^{11a}. A similar pH effect was also shown in NMR studies on NAD^+ . Thus, the reduction in charge-transfer interaction in NAD^+ at low pH can be attributed to electrostatic repulsion between positively charged nicotinamide and adenine moieties. The closeness of pH dependencies of MNA-adenine mixture and NAD^+ can be regarded as an indication that the amino group of adenine moiety in NAD^+ is not directly involved in the intramolecular complex formation.

So far, we have shown that the charge-transfer abilities of nicotinamide and adenine moieties of NAD^+ are much weaker than MV^{++} and indole, respectively. However, it was also demonstrated that there is considerable intramolecular interaction in NAD^+ . From the evaluated I_p of adenine (8.28 eV), EA of nicotinamide (0.28 eV), and W of equation (2), 3.59 eV, calculated from MV^{++} -indole complex using

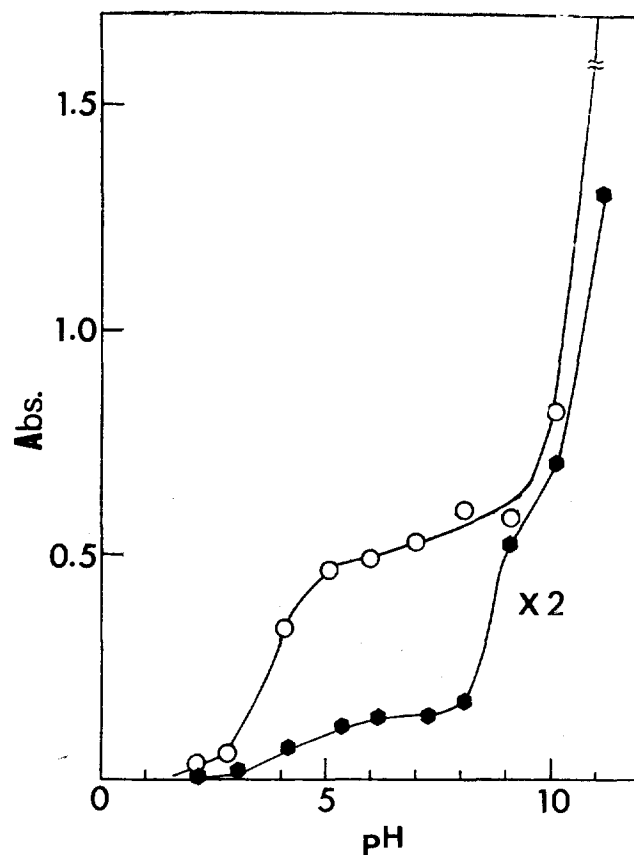


Figure 6. Changes in absorbances at 340 nm of $1.5 \times 10^{-2} \text{M}$ NAD^+ (○) and 0.2 M MNA- $2.5 \times 10^{-3} \text{M}$ adenine mixture (●) with pH at 25°C .

equation (2), the $h\nu_{CT}$ of nicotinamide-adenine complex was estimated to be 4.41 eV, *i.e.*, 280 nm. This charge-transfer energy is too high to yield separated charge-transfer band due to strong absorption from its components peaked near 260 nm. This feature, together with small K and ϵ values, could be responsible for the uncertainty in extent, and even the presence, of the complex for long time.

We have also demonstrated that indole is a better electron donor and more hydrophobic than adenine is. Therefore, it is expected that the nicotinamide-adenine interaction in NAD^+ could be replaced by nicotinamide-indole interaction, when the two groups come close. Such replacement would be more favorable in hydrophobic environment, and can explain the observation of open form of NAD^+ in enzyme bound states¹⁵. In this case, the role of adenine moiety of NAD^+ may be simply to provide a suitable environment for interaction of the coenzyme with enzymes as suggested^{9a,b}.

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Vibrational Assignment of S_8 from Normal Coordinate Analysis

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Normal modes of crystalline orthorhombic sulfur belonging to the space group D_{2h} -Fddd, have been evaluated by taking the lowest temperature phase in the solid. Normal modes are obtained by the valence force field with modified force constants and a quantitative description of the mode is adjusted by the potential energy distribution. Since the full crystal system of orthorhombic sulfur is so large, we intended to calculate the normal modes simply by constructing the imaginary box made by the infinite mass boundary. And the Raman experiment is done by using the more powerful Ar-Kr gas laser with lowering the temperature to $\sim 10^\circ\text{K}$.

Introduction

Crystalline orthorhombic sulfur has been shown to contain sixteen molecules in an unit cell, which belongs to the space

group D_{2h} -Fddd and each sulfur molecule finds itself at a site of only C_2 symmetry, where the symmetry of the isolated molecule is $D_{4d}^{1,2}$. This means that the degenerate modes of the isolated molecule may be split and that all vibrational