Coenzyme Model Studies. I. Syntheses of 3-Carbamoylpyridinium Salts Containing Nucleic Acid Bases and Their Equilibria and Reaction Rates with Cyanide Ion in the Presence of Polyelectrolytes[1]

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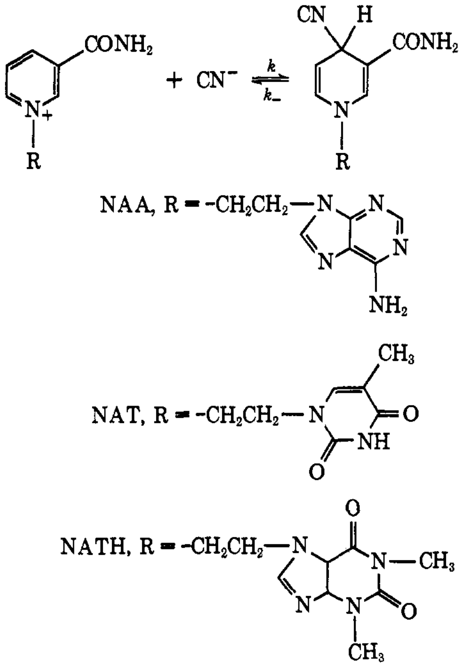
Abstract: The syntheses of analogs of nicotinamide adenine dinucleotide (NAD^+), namely, 3-carbamoylpyridinium compounds quaternized with ethylated adenine, thymine, and theophylline, were carried out. The rate and equilibrium constants (k and K) for addition of cyanide ions to the coenzyme model compounds were studied in the presence of polyelectrolytes and surfactants and in their absence. The k and K for *N*-2′-(9-ethyladeninyl)-3-carbamoylpyridinium chloride, for example, were 0.79 M^{-1} sec^{-1} and 173 M^{-1} at 25°, which are fairly close to those of β-NAD^+. The polymers examined were copolymers of diethyldiallylammonium chloride and sulfur dioxide (DECS), sodium poly(ethylenesulfonate) (NaPES), sodium poly(styrenesulfonate) (NaPSt), DNA, RNA, poly A, poly I, poly C, and poly U. The surfactant was cetyltrimethylammonium bromide (CTABr). All the electrolytes investigated decreased the second-order rate constant and the equilibrium constant of the cyanide addition reaction in the same proportion, indicating that the backward reaction was not influenced by the polyelectrolytes. The decrease was in the order, KCl < CTABr < DECS $\sim$ NaPES < NaPSt, suggesting the simultaneous contribution of hydrophobic and electrostatic interactions between the model compounds and the polymer "catalyst." The thermodynamic quantities of the cyanide addition reaction, *i.e.*, the free energy ($\Delta G^{\ddagger}$), enthalpy and entropy of activation, and the free energy (ΔG), enthalpy, and entropy of reaction, were derived. Both the $\Delta G^{\ddagger}$ and ΔG were increased by the additions of polyelectrolytes and simple electrolytes to the same extent while $\Delta G^{\ddagger}_{-}$, the free energy of activation of the backward reaction, was not affected at all. It is suggested that polyelectrolytes should not be regarded as "catalysts," contrary to the usually accepted view.

As is well known, nicotinamide adenine dinucleotide (NAD^+) with its reduced form (NADH) is a coenzyme in enzymic oxidations and reductions. In studies on the syntheses of analogs of NAD^+, their reactivities and physical properties are useful in the understanding of enzyme kinetics. There have been reported three types of model compounds, namely, (1) compounds having substituents other than a carbamoyl group at the 3 position of the pyridine ring, (2) 3-carbamoylpyridinium compounds quaternized with various groups without adenine base (*e.g.*, 1-methylnicotinamide), and (3) compounds in which purine and/or carbohydrate moieties are modified.[2]

In the present paper, the syntheses of model compounds of the second type, 3-carbamoylpyridinium salts quaternized with the groups containing neither phosphate nor carbohydrate residues but nucleic acid base only, will be described. These compounds will be used as substrates in equilibrium reactions in the presence of various types of polyelectrolytes, with the purpose of clarifying the mechanism of polymer "catalysis." In forthcoming papers, the functions of nucleic acid base in NAD^+ will be studied by using these model compounds.

The reactions studied were the formations of CN^- adducts of *N*-2′-(9-ethyladeninyl)-3-carbamoylpyridinium ions (NAA), *N*-2′-(1-ethylthyminyl)-3-carbamoylpyridinium ions (NAT), and *N*-β-(7-ethyltheophyllinyl)-3-carbamoylpyridinium ions (NATH) (eq 1).



(1)

It is now well established that interionic reactions between similarly charged ionic species can be accelerated by oppositely charged macroions or surfactant micelles, whereas those between ionic species of the opposite signs are decelerated. The reader should refer to recent review articles for previous work on this sub-

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[1] Presented at the 21st Symposium of Polymer Science, Osaka, Japan, Nov 1972; preliminary communication, N. Ise and T. Okubo, *Nature* (*London*), in press.

[2] (a) C. Woenckhaus and G. Pfleiderer, *Biochem. Z.*, **341**, 495 (1965); (*b*) N. J. Leonard, T. G. Scott, and P. C. Huang, *J. Amer. Chem. Soc.*, **89**, 7137 (1967); (c) S. Shifrin, "Molecular Association in Biology," B. Pullman, Ed., Academic Press, New York, N. Y., 1968, p 339; (d) T. G. Scott, R. D. Spencer, N. J. Leonard, and G. Weber, *J. Amer. Chem. Soc.*, **92**, 687 (1970).

ject.[3,4] However, it is not at all clear which of the elementary steps, forward or backward, is influenced by the polyelectrolytes. As continuation of our previous work[5-10] on polyelectrolyte "catalysis," the present reaction system was studied with the purpose of investigating the polyelectrolyte influence on the elementary processes of eq 1.

Experimental Section

Materials. The coenzyme model compounds, NAA, NAT, and NATH, were prepared by the Menschutkin reaction of nicotinamide with *N*-chloroethyladenine, thymine, and theophylline. The chloroethylated compounds of the bases were prepared by chlorination with thionyl chloride of hydroxyethylated compounds which were obtained from nucleic acid bases and ethylene carbonate in dimethylformamide. The hydroxyethylated and chloroethylated compounds of the nucleic acid bases, except 7-β-chloroethyltheophylline, were prepared by the methods reported by Takemoto, Imoto, and others,[11,12] and Pitha and Ts'o.[13] The 7β-chloroethyltheophylline was prepared as follows. 7β-Hydroxyethyltheophylline (11.2 g, 50 mmol) and thionyl chloride (100 ml, 1.3 mol) were heated for 1 hr at 80°. After the solution was cooled, unreacted thionyl chloride was evaporated under reduced pressure. Recrystallization of the residue from ethanol gave 7β-chloroethyltheophylline as colorless needles (90% in yield). *Anal.* Calcd for $C_9H_{11}N_4O_2Cl$. C, 44.5; H, 4.5; N, 23.1; Cl, 14.6; O, 13.2. Found: C, 44.2; H, 4.5; N, 23.1; Cl, 14.6; O, 13.4.

The quaternization reaction of 9-(2′-chloroethyl)adenine (4 g, 20 mmol) with nicotinamide (3 g, 24 mmol) was performed in 20 ml of dimethylformamide at 100° for 24 hr. The solvent was then eliminated by heating *in vacuo*. The residue was completely washed with ethanol, dissolved into water (10 ml), and further recrystallized with ethanol. By drying the precipitate *in vacuo*, NAA chloride was obtained as a hygroscopic white powder, yield 80%. *Anal.* Calcd for $C_{13}H_{14}N_7OCl$: C, 48.8, H, 4.4; N, 30.7; Cl, 11.1. Found: C, 48.4; H, 4.7; N, 30.2; Cl, 11.0.[14]

NAT chloride was prepared by heating 1.89 g (10 mmol) of 1-(2′-chloroethyl)thymine and 1.33 g (10 mmol) of nicotinamide in dimethylformamide (10 ml) at 120° for 24 hr. Upon cooling and with addition of sufficient ethanol, the solid product was washed. The product was twice recrystallized in water–ethanol solution, and a white highly hygroscopic powder was obtained, yield 80%. *Anal.* Calcd for $C_{13}H_{15}N_4O_3Cl$: C, 50.2; H, 4.8; N, 18.0; Cl, 11.4. Found: C, 49.6; H, 5.0; N, 17.9; Cl, 11.1.

NATH chloride was synthesized by heating 4.85 g (20 mmol) of 7-β-chloroethyltheophylline and 2.69 g (20 mmol) of nicotinamide in dimethylformamide (10 ml) at 130° for 24 hr in a nitrogen atmosphere. The solid product was washed thoroughly with ethanol and then twice recrystallized in water–ethanol, and a white highly hygroscopic powder was obtained, yield 70%. *Anal.* Calcd for $C_{15}H_{17}N_6O_3Cl$: C, 49.4; H, 4.7; N, 23.0; Cl, 9.7. Found: C, 48.8; H, 4.8; N, 22.8; Cl, 9.5.

Adenine and thymine from Sigma were used without further purification as starting materials. Theophylline was also commercially available (Nakarai Chemical Co., Kyoto). Dimethylformamide, used as a solvent, was distilled under reduced pressure. Dioxane and ethanol were guaranteed reagents. Potassium cyanide of Merck G.R. Grade was used without further purification.

Sodium poly(styrenesulfonate) (NaPSt) was a gift from the Dow Chemical Co., Midland, Mich. The molecular weight was 6,300,000. Sodium poly(ethylenesulfonate) (NaPES), degree of polymerization 770, was kindly donated by the Hercules Powder Co., Wilmington, Del. Dilute solutions of these anionic polyelectrolytes were converted to the acid form by passing them through columns of cation- and anion-exchange resins. Completeness of the conversion was assured by using an atomic absorption spectrophotometer. The corresponding sodium salts were prepared by neutralization of aqueous solutions with sodium hydroxide. The copolymer of diethyldiallylammonium chloride and sulfur dioxide (DECS) is a strong basic polyelectrolyte prepared by Harada, *et al.*[15,16] The sodium salt of chicken blood DNA (NaDNA) was purchased from Calbiochem Corp., Los Angeles, Calif. RNA was purchased from Sigma Chemical Co., St. Louis, Mo. (Type XI), and used for measurements without further purifications. Poly A, poly U, poly C, and poly I were obtained from C. F. Boehringer & Soehnne GmbH, Mannheim. Deionized water was distilled under a nitrogen atmosphere for the preparation of the solutions of model compounds and polyelectrolytes.

Kinetic Measurements. The reaction was initiated by the mixing of a polyelectrolyte solution containing KCN with a substrate solution. The concentration of the substrate was determined by conductance measurements of chloride ion with silver nitrate. Kinetic measurements were carried out spectrophotometrically by using a Hitachi stopped-flow spectrophotometer (Model RSP-2) and a Hitachi spectrophotometer (Model EPS-3T) equipped with thermostated cell holder. The reaction was followed by observing the appearance of an absorption band near 340 nm, which is characteristic of 1,4 adducts of the pyridinium ions. In the absence of "catalysts," the substrate and KCN concentrations were 2×10^{-4} and ~0.004–0.02 *M*, respectively. In all cases an excess of cyanide ions was present, so that the pseudo-first-order rate behavior was observed. The first-order rate constants (k_1) were evaluated from the slopes of the log ($OD_\infty - OD_t$) against time plot. The second-order rate constants (k) were obtained from k_1 and the equation, $k = k_1/([CN^-] + K_-)$, where K_- is the dissociation equilibrium constant. In order to observe distinct "catalytic" influence, the CN^- concentration was set at a level as low as possible (in the present paper at 4×10^{-3} *M*) where the pseudo-first-order condition held. All reactions were conducted in aqueous alkaline solution (pH ~11).

Equilibrium Constants. The association constant (K) and the dissociation constant (K_-) were evaluated, using the method of Behme and Cordes,[17] from the slopes of the $(\epsilon - \epsilon_0)/N$ against ϵ plot, where ϵ_0 is the absorbance of the substrate, ϵ the absorbance of the equilibrium reaction mixture, and N the concentration of cyanide ions. Both the rate constant and the equilibrium constant were obtained at 25° unless otherwise specified. For the measurements of the temperature dependence, the experiments were done also at 20, 30, and 35°.

From duplicate or repeated runs, the reproducibility of the values of k and K was about 5% or less in the presence and absence of polyelectrolytes.

Results and Discussion

From the values of k and K, we can estimate k_- and K_- by using the relations $K = k/k_-$ and $K_- = k_-/k$. The results are tabulated in Table I. The equilibrium

Table I. Spectra, Rate Constants (k, k_-), and Association Constant (K) for CN^- Addition Reactions of NAA, NAT, and NATH at 25°[a]

Compd	ϵ_{max}[b]	k, M^{-1} sec^{-1}	k_-, sec^{-1}	K, M^{-1}
NAA	6320	0.79	0.0046	173
NAT	6470	0.234	0.0081	29.5
NATH	6200	1.18	0.0052	226

[a] All reactions were followed spectrophotometrically at 338 nm, [KOH] = 10^{-3} *M*, [KCN] = ~0.002–0.02 *M*, and [NAA] = [NAT] = [NATH] = 2×10^{-4} *M*. [b] ϵ_{max} denotes the molar extinction coefficient of the 1,4 adducts.

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(3) (a) H. Morawetz, *Accounts Chem. Res.*, **3**, 354 (1970); (b) N. Ise, *Advan. Polym. Sci.*, **7**, 536 (1970–1971).

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(16) Y. Negi, S. Harada, and O. Ishizuka, *J. Polym. Sci.*, **5**, 1951 (1967).

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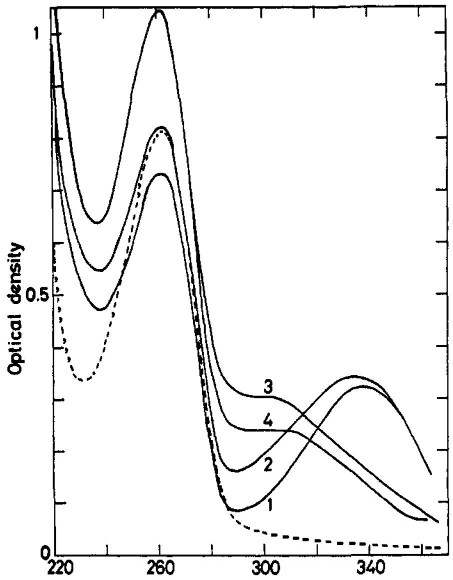

Figure 1. Ultraviolet spectra as a function of time for an aqueous solution containing 5.0×10^{-5} M NAA and 0.2 M KCN, [KOH] = 10^{-2} M: (1) after 5 min, (2) 90 min, (3) 1 day, (4) 50 days, broken curve; no KCN added.

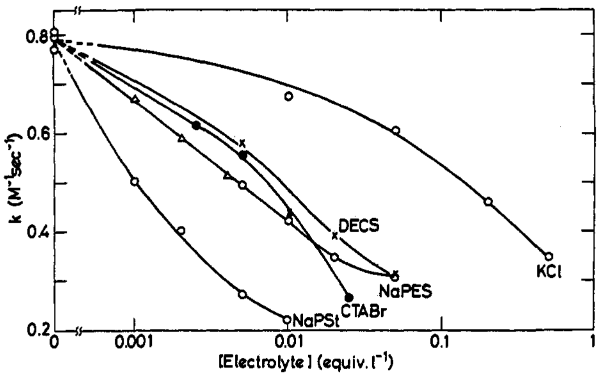

Figure 2. The polyelectrolyte effect on the k of the NAA–CN⁻ reaction at 25°: [NAA] = 1.98×10^{-4} M, [KCN] = 4.0×10^{-3} M, [KOH] = 1.0×10^{-3} M.

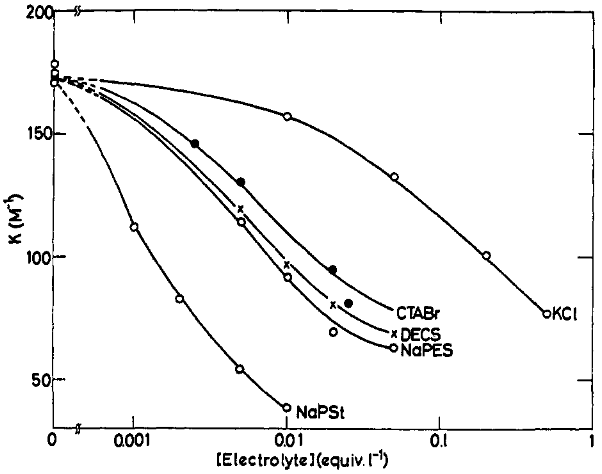

Figure 3. The polyelectrolyte effect on the K of the NAA–CN⁻ reaction at 25°: [NAA] = 1.98×10^{-4} M, [KCN] = 4.0×10^{-3} M, [KOH] = 1.0×10^{-3} M.

was reached within 30 min after mixing the model compounds with KCN under the experimental conditions examined. Both the rate and equilibrium of these reactions were found to be independent of the concentration of KOH in the range of 10^{-4}–10^{-2} M. The k and K values of nicotinamide adenine dinucleotide (β isomer) (β-NAD⁺) and nicotinamide adenine dinucleotide (α isomer) (α-NAD⁺) were reported by Lindquist and Cordes[18] to be 0.18 M^{-1} sec^{-1} and 164 M^{-1} and 0.075 M^{-1} sec^{-1} and 16.7 M^{-1}, respectively, at 25° and at ionic strength of 0.5. The k and K values of 3-carbamoylpyridinium chloride quaternized with alkyl groups (*e.g.*, methyl, ethyl, propyl) are much smaller compared with those of α-NAD⁺.[18] It is interesting to note that the k and K values of the present model compounds are fairly close to those of β-NAD⁺. This indicates that the nucleic acid bases enhance the susceptibility of the pyridinium ring to the CN⁻ attack,

The uv spectra of the products of the cyanide addition to NAA in the presence of excess KCN are given in Figure 1. The optical density at 338 nm slowly decreased with time, a new absorption peak appeared near 300 nm, and the absorption around ~260–270 nm was increased. The spectral shifts suggest possible rearrangement of the 1,4-cyanide adduct to the 1,6 and 1,2 adducts. The adduct formation was also checked by the nmr spectrum (Hitachi Perkin-Elmer R-20B). Because of high concentrations in the nmr measurements, direct quantitative comparison with the uv spectral change was rather difficult. However, the nmr spectrum clearly showed the 1,4-adduct formation and the rearrangement to the 1,2 adduct. Peaks at τ 3.3 (H_2), 4.08 multiplet containing two main peaks, J = 7.5 cps (H_6), and ~5.20–5.66 (H_4 and H_5) were observed shortly after mixing deuterated water solutions of NATH and KCN, in agreement with the observed values in deuterated dimethyl sulfoxide by Cordes, *et al.*,[18] and peaks of H_2, H_4, and H_5 became smaller with time. The 1,6-adduct formation was fairly difficult to check by this technique, and detailed study is in progress. However, it is believed on the basis of the earlier argument that the 1,6 adduct was formed also in the present case (see, for example, ref 18, in which the relevant references are given). No spectral changes were observed when cyanide ions were absent. These spectral changes were found for NAT and NATH, and also for other N-substituted 3-carbamoylpyridinium compounds, such as N-propyl-3-carbamoylpyridinium iodide.[18,19]

The salt effect on the rate and equilibrium constants will now be discussed. First, the effect of various synthetic polyelectrolytes must be examined. The electrolyte influences on the second-order rate constant and association equilibrium constant for the NAA–CN⁻ reaction are shown respectively in Figures 2 and 3. As is clearly seen, both k and K values are decreased by the addition of simple, poly-, and micelle electrolytes. In other words, the forward reaction was decelerated and the equilibrium was shifted to the reactant side. The deceleration effect of polyelectrolytes is much larger than that of simple electrolytes, which is ascribed to the huge electrostatic potential of the

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(19) K. Wallenfels and H. Diekmann, *Justus Liebigs Ann. Chem.*, **621**, 166 (1959).

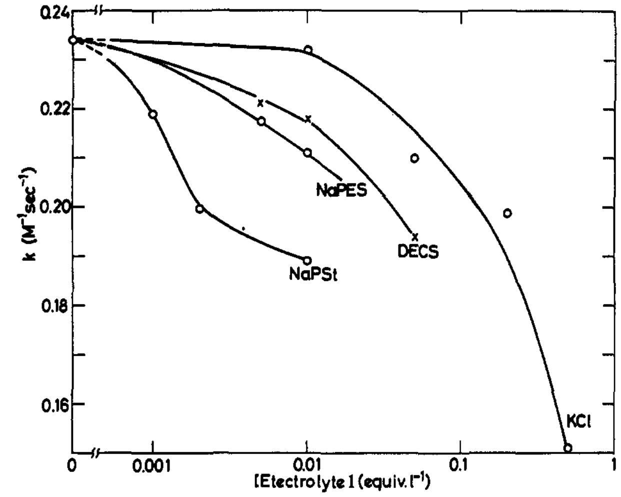

Figure 4. Polyelectrolyte effect on the k of the NAT-CN$^-$ reaction at 25°: [NAT] = 1.95×10^{-4} M, [KCN] = 4.0×10^{-3} M, [KOH] = 1.0×10^{-3} M.

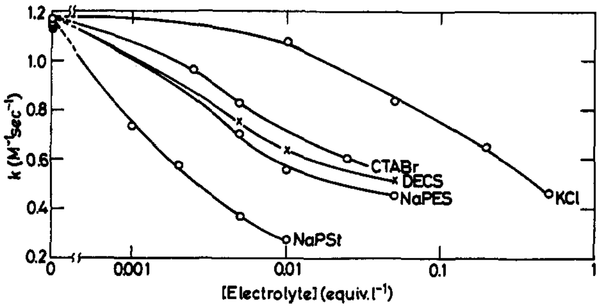

Figure 5. The polyelectrolyte effect on the k of the NATH-CN$^-$ reaction at 25°: [NATH] = 1.94×10^{-4} M, [KCN] = 4.0×10^{-3} M, [KOH] = 1.0×10^{-3} M.

macroions. By the addition of cationic or anionic polyelectrolytes, one of the two reactants, cyanide anions or substrate cations, is attracted to the vicinity of the macroion, whereas the other reactant is repelled. Thus, the reaction is decelerated by polyelectrolytes. This deceleration phenomenon has been already discussed quantitatively for the NH_4^+-OCN$^-$ reaction.[8] The deceleration effect of NaPSt was much higher than that of NaPES. This indicates that the affinity between the substrate and NaPSt is high because of the hydrophobic interactions besides the electrostatic ones. This feature has been also clearly demonstrated in the alkaline fading of triphenylmethane dyes.[20]

The effects of simple, poly-, and micelle electrolytes on the k of the cyanide addition of NAT and NATH are shown in Figures 4 and 5, respectively. The order of the deceleration by polyelectrolytes is similar to that of NAA. The following order of the deceleration is observed for the substrates: NAT < NAA < NATH. It should be noted that the difference between NAT and NAA is distinct whereas the one between NAA and NATH is not large. This reflects the hydrophobicity of the model compounds.

Next, the variations of k and K by polyelectrolytes are compared. Figure 6 clearly shows that the addition of NaPES or NaPSt decreased the k and K to the same extent, or $k/k_0 = K/K_0$ (0 denotes the absence of electrolyte). This implies that the backward rate constant, k_-, was not influenced at all. The same conclusion was obtained for other electrolytes. This is quite reasonable since the backward reaction is a decomposition process of an electrically neutral substance, which is hardly influenced by polyelectrolytes.

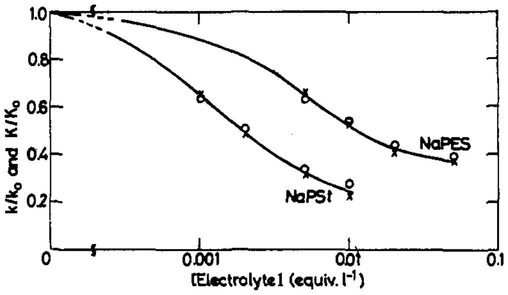

Figure 6. Comparison of the polyelectrolyte effects of NaPES and NaPSt on the k of the NAA-CN$^-$ reaction with those on the K at 25°: [NAA] = 1.98×10^{-4} M, [KCN] = 4.0×10^{-3} M, [KOH] = 1.0×10^{-3} M; (○) k/k_0; (×) K/K_0.

The k and K values for the NAA-CN$^-$ reaction were also determined at 20, 25, 30, and 35° and shown in Figures 7 and 8. The k value increased with temperature whereas K decreased. This reflects the fact that reaction 1 is exothermic. Table II gives the free energy ($\Delta G^{\ddagger}$), enthalpy ($\Delta H^{\ddagger}$), and entropy ($\Delta S^{\ddagger}$) of activation and also the free energy (ΔG), enthalpy (ΔH), and entropy (ΔS) of the NAA-CN$^-$ reaction. By polyelectrolyte addition, $\Delta G^{\ddagger}$ and ΔG were increased, which is in agreement with the observed changes (mentioned above) of k and K. It should be mentioned that the value of $\Delta G^{\ddagger}_-$ ($=\Delta G^{\ddagger} - \Delta G$) was always 20.6 kcal mol^{-1} with and without polyelectrolytes. *This result shows that for the reaction under consideration the backward reaction was not influenced at all, and it was the initial state (neither the activated nor the final state) that was influenced by polyelectrolyte addition.* From the thermodynamic parameters, we can state that polyelectrolytes should not be regarded as true catalysts. Thus the term "polyelectrolyte catalysis" [or "polymer catalysis" in general] used in the conventional fashion should be avoided. As Bender asserted in his recent

Table II. Thermodynamic Quantities for the NAA-CN$^-$ Reactions at 25°[a]

Thermodynamic quantity[b]	None	Polyelectrolytes DECS	NaPES	NaPSt
$\Delta G^{\ddagger}$	17.5	18.0	18.1	18.0
$\Delta H^{\ddagger}$	9.1	8.3	9.4	9.2
$\Delta S^{\ddagger}$	−28	−33	−29	−29
ΔG	−3.1	−2.6	−2.5	−2.6
ΔH	−9.8	−7.5	−7.7	−6.6
ΔS	−23	−16	−17	−14
$\Delta G^{\ddagger}_-$ ($=\Delta G^{\ddagger} - \Delta G$)	20.6	20.6	20.6	20.6

[a] [NAA] = 1.98×10^{-3} M, [KCN] = 4.0×10^{-3} M, [KOH] = 1.0×10^{-3} M, [DECS] = [NaPES] = 0.02 equiv l.$^{-1}$, [NaPSt] = 0.002 equiv l.$^{-1}$. [b] The units of free energies and enthalpies are in kcal mol^{-1} and the entropies in eu. The experimental uncertainty is believed to be ±0.1 for the free energies, ±0.5 for the enthalpies, and ±3 for the entropies.

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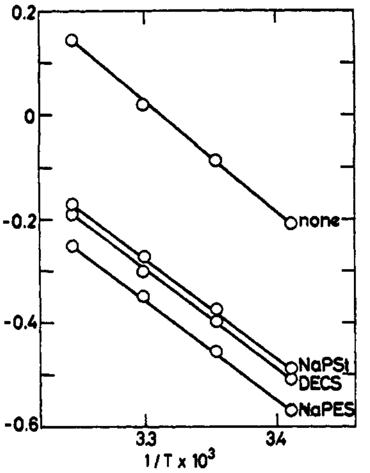

Figure 7. Temperature dependence of the k of the NAA–CN$^-$ reaction: [NAA] = 1.98×10^{-4} M, [KCN] = 4.0×10^{-3} M, [KOH] = 1.0×10^{-3} M, [DECS] = [NaPES] = 0.02 equiv l.$^{-1}$, [NaPSt] = 0.002 equiv l.$^{-1}$.

book,[21] the observed change of the reaction rate in the presence of electrolytes is not due to their catalytic action, but due to "field catalysis."

It is of interest to mention the micellar influence on elementary processes. It has been reported[22,23] that the rate constants of the forward and backward processes of Meisenheimer complex formation were affected in a different proportion by ionic and nonionic surfactants. For example, CTABr accelerated the forward reaction by 36-fold, whereas it decelerated the backward reaction by a factor of 2.8. An anionic surfactant, sodium lauryl sulfate, decelerated the forward step by a factor of 43 and had practically no influence on the backward reaction. A similar observation was independently reported by Cordes and his associates[24,25] that addition of cyanide ion to *N*-hexadecyl-3-carbamoylpyridinium ion was enhanced by a factor of 10^3 by CTABr, and the association equilibrium constant was increased about (2.5×10^4)-fold. This means that the backward reaction was retarded by a factor of 25. These observations are apparently in contradiction with our results mentioned above, but are quite reasonable if we take into consideration that the polyelectrolytes used in the present work are rather hydrophilic, whereas the surfactants contain a strongly hydrophobic moiety, so that the hydrophobic interactions between the micelles and the substrates would cause different influence on the elementary steps than those observed by us. Furthermore, our substrates appear to be less hydrophobic than those used by Fendler[22,23] and Cordes,[24,25] as is seen from the observation (Figures 2, 3, and 5) that CTABr decreased both k and K. In any case, we have to note that the forward and backward reactions were influenced in different ways. Thus, micelles are not catalysts, either.

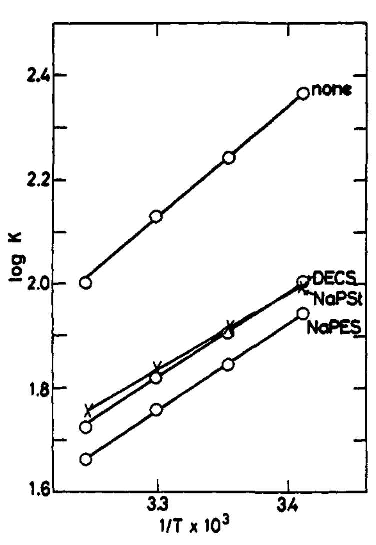

Figure 8. Temperature dependence of the K of the NAA–CN$^-$ reaction: [NAA] = 1.98×10^{-4} M, [KCN] = 4.0×10^{-3} M, [KOH] = 1.0×10^{-3} M, [DECS] = [NaPES] = 0.02 equiv l.$^{-1}$, [NaPSt] = 0.002 equiv l.$^{-1}$.

As is clear in Table II, both ΔH and ΔS sharply increased with polyelectrolytes, whereas $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ did not change so profoundly.

Next, we will discuss the catalytic influences of polynucleotides in the NAA–CN$^-$, NATH–CN$^-$, and NAT–CN$^-$ reactions. The results are compiled in Table III.

Table III. Influences of Polynucleotides on the CN$^-$ Addition Reactions of NAA, NAT, and NATH at 25°[a]

	None	DNA	RNA	Poly A	Poly I	Poly C	Poly U
NAA							
k^b	0.79	0.66	0.74	0.67	0.56	0.68	0.64
K^c	173	149	158	143	112	147	134
NAT							
k	0.23	0.23	0.23	0.20	0.22	0.23	0.20
K	29.5	29.3	29.7	28.4	28.8	29.0	28.1
NATH							
k	1.15	1.00	1.00	0.93	0.78	0.96	
K	225	182	194	175	114	182	

[a] [Polynucleotide] = 1.0×10^{-3} equiv l.$^{-1}$, [KCN] = 4×10^{-3} M, [NAA] = [NAT] = [NATH] = 2×10^{-4} M, [KOH] = 1×10^{-3} M. [b] In M^{-1} sec^{-1}. [c] In M^{-1}.

All polymers decreased both k and K, and their effectiveness was in the order RNA < DNA ~ poly C < poly A < poly U < poly I for the NAA–CN$^-$ reaction; RNA < DNA ~ poly C ~ poly A < poly I for the NATH–CN$^-$ reaction; and RNA < DNA < poly C ~ poly I < poly A ~ poly U for the NAT–CN$^-$ reaction, respectively. It is clear that the effects of DNA or RNA, which is composed of four kinds of bases, are not so large compared with the homonucleotides. If the hydrogen-bonding interactions between the substrate and polynucleotide are most important, poly C or poly U and poly A or poly I should be most effective in the retardation of the NAA–CN$^-$ and NAT–CN$^-$ reactions, respectively. This, however, is not the case. The observed data indicate that the polynucleotides having purine bases are effective for substrates having purine and those having pyrimidine are not specific. As was above mentioned, the hydrophobicity of purine

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is higher than that of pyrimidine. In other words, the hydrophobic interactions between the substrate and the polynucleotide in the present reaction system are predominant and the hydrogen-bonding interactions are of the secondary importance. This feature was earlier proposed by Ts'o, *et al.*,[26] from the thermodynamic measurements of various nucleic acid bases and nucleosides in aqueous solution. They showed that the strength of the interactions of the free bases and nucleosides could be arranged in the series: purine–purine > purine–pyrimidine > pyrimidine–pyrimidine.

It would be useful to sum up the present investigation as follows. Model compounds of coenzyme I, nicotinamide derivatives containing nucleic acid bases, were synthesized. In the CN^- addition reaction of these compounds, polyelectrolytes were found to decelerate the reaction, as was the case for interionic reactions between oppositely charged ionic species. Taking advantage of the equilibrium nature of the CN^- adduct formation, we were able to find polyelectrolyte influence on the forward and backward reaction rates; the forward rate constant was decreased by the polyelectrolyte addition whereas the backward rate constant was not influenced at all. Thus, it is clear that the polyelectrolytes cannot be regarded as catalysts, if we follow the earlier definition by Ostwald.[27] It was pointed out that, like the polyelectrolytes, micelles affect the two elementary processes in a different proportion. The "catalytic" influences by polyelectrolytes and micelles are mainly due to the electrostatic interactions. The hydrophobic interactions are also important, especially so in micelle-containing systems.

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The Structure of Dihydroisohistrionicotoxin, a Unique Unsaturated Alkaloid and Anticholinergic Agent

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Abstract: Dihydroisohistrionicotoxin, (2-*pro-R*,6*S*,7-*pro-S*,8a*S*)-7-(*cis*-1-buten-3-ynyl)-8-hydroxy-2-(3,4-pentadienyl)-1-azaspiro[5.5]undecane, crystallizes as the hydrochloride in space group $P2_12_12_1$ with $a = 11.438 \pm 0.005$ Å, $b = 14.598 \pm 0.004$ Å, and $c = 11.379 \pm 0.004$ Å. The material, derived from the Colombian frog *Dendrobates histrionicus*, is a potent and selective inhibitor of cholinergic mechanisms. Its configuration is compared with the established configurations of other cholinergic agonists and antagonists. Structural and conformational parameters have been determined for the allene and vinylacetylene substituents in the solid state.

Dihydroisohistrionicotoxin, a venom isolated from the skin of the brightly colored Colombian frog *Dendrobates histrionicus*, has an unique chemical constitution (I) consisting of a spiro alkaloid moiety and two unsaturated side chains, one containing an allene moiety and the other a vinylacetylene moiety.[1] This compound and its congener, histrionicotoxin (II), appear to be the first examples of acetylenic and allenic bonds occurring in natural products from the animal kingdom.[1] The molecular formulas of both congeners had been elucidated by crystal structure analyses using X-ray diffraction;[1] however, the only crystal of I available for the initial experiment was disordered. Although the molecular formula could be established, the data could not be refined to a reasonable agreement factor. Since that time, more material has become available, new crystals have been grown, and the structure determination has been repeated. The agreement factor between the observed and calculated structure factors is now 4.8%.

The histrionicotoxins, of which I and II are examples, are a third class of alkaloids isolated from the skin secretions of tropical American frogs. Batrachotoxin (III), from the Colombian *Phyllobates aurotania*, is a steroidal alkaloid and the most toxic nonprotein substance known.[2-4] Pumiliotoxin C (IV), from the Panamian *Dendrobates pumilio*, is a *cis*-decahydroquinoline.[5] Although these toxins occur in frogs which all belong to the family *Dendrobatidae*, their molecular formulas are quite diverse.

Histrionicotoxins have specific affinities as inhibitors of cholinergic receptor mechanisms in the neuromuscular system. In these molecules there are geometrical features which are comparable to portions of acetylcholine and other cholinergic materials. These

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