Kinetics of Hydrogen-Deuterium Exchange of 8-CH Groups in Adenosine 5′-Monophosphate and Guanosine 5′-Monophosphate by Laser Raman Spectroscopy

Sang Kyu Kim, Myung Soo Kim, and Se Won Suh†

Department of Chemistry, College of Natural Sciences, Seoul National University, Seoul 151, Korea
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The rate constants of the hydrogen-deuterium exchange of 8-CH groups in 5′-rAMP and 5′-GMP were measured by laser Raman spectroscopy. The Arrhenius activation energies calculated from the rate constants measured as a function of temperature were similar for both compounds. However, the effects of pD on exchange rate constants were different for the two compounds. Our kinetic data support the exchange reaction mechanism involving an ylide type intermediate.

Introduction

The hydrogen isotope exchange in purines, purine-nucleosides, and purine-nucleotides has been studied by several methods.[1-11] They include detritiation method,[8] proton magnetic resonance,[9] infrared,[10] and Raman spectroscopy.[11] Among these methods, the laser Raman spectroscopy can be conveniently used to measure the hydrogen-deuterium exchange of 8-CH groups in purines since only a small quantity of sample is required to record Raman spectra and the isotope exchange accompanies readily detectable changes in the Raman spectra.

The rate constants for hydrogen-deuterium exhange of 8-CH groups in 5′-rAMP and 5′-rGMP have been determined at a single pD by laser Raman spectroscopy.[3,4] In this paper, we have measured the rate constants at several pD's by the same method in order to investigate the effect of pD on exchange rate constants for 5′-rAMP and 5′-rGMP.

Experimental

1. *Sample Handling.* The nucleotides (Na salts; Sigma) were dissolved in D_2O (99.8% deuterium; Aldrich) to prepare 0.2 *M* 5′-rAMP and 0.1 *M* 5′-rGMP solutions. The pD of solution was adjusted at room temperature by addition of NaOD and DCl (Aldrich) using the relation pD = pH + 0.4. The pD values at higher temperatures are not significantly different from those measured at room temperature.[12] Test tubes containing D_2O solutions of each nucleotide were tightly sealed with rubber septa and they were incubated in the water bath maintained at constant temperature (within ±1°C).

Aliquots of the D_2O solutions were then withdrawn periodically from the test tubes using syringes with long needles and quickly introduced into Raman capillary cells through its open end. The other end has been sealed previously by using a flame. The open end of Raman capillary cells was then sealed air-tight with parafilm and the capillaries were stored in the refrigerator until they were used in recording Raman spectra. The exchange of 8-CH groups during the storage in the refrigerator and during the collection of Raman spectra at room temperature is negligible.[3,4]

2. *Raman Measurement.* Raman spectra were obtained at room temperature with a Japan Spectroscopic Company model R-300 laser Raman spectrometer using the 514.5 nm line of argon ion laser (Spectra Physics model 164-06) as an exciting source. Raman signals were detected with 90° geometry using a commercial photon counting system. The laser power was 260 mW, the spectral slit width was 6 cm^{-1}, the time constant was 1 sec., and the scan speed was 72 cm^{-1} min^{-1}.

3. *Data Handling.* The exchange rate constants can be determined by following intensity changes of Raman peaks which are sensitive to the isotope substitution. For 5′-rAMP the 1485 cm^{-1} peak is replaced by the 1462 cm^{-1} peak upon deuterium exchange at C-8. For 5′-rGMP the 1488 cm^{-1} peak is replaced by the 1460 cm^{-1} peak. The rate constants can be obtained either by the intensity decrease in the former lines or by the intensity increase in the latter lines. We use the former since they give more accurate rate constants.[3]

Raman lines unaffected by deuterium substitution are used as internal standards. They are the 721 cm^{-1} peak (adenine ring breathing mode[13]) for 5′-rAMP and the 978 cm^{-1} peak (phosphate group stretching vibration[4]) for 5′-rGMP. Raman intensities were determined by measuring the peak height above a base line tangent to the wings of the peak in question. This is justified because the bandwidths of peaks considered here are not affected by the deuterium exchange.

The deuterium exchange of 8-CH groups in 5′-rAMP and 5′-rGMP follows the pseudo-first-order kinetics[2-4,7] and the rate constants, k, can be calculated by plotting $\ell n[I(1485)/I(721)]$ vs. incubation time for 5′-rAMP or $\ell n[I(1488)/I(978)]$ vs. incubation time for 5′-rGMP. The Arrhenius activation energies, E_a, can be calculated by plotting $\ell n\, k$ vs. T^{-1} where k is the rate constant and T is the absolute temperature.

Results

Raman spectra of 5′-rGMP solution in D_2O are given as a function of incubation time at 80°C in Figure 1. Many spectral changes occur upon the hydrogen-deuterium exchange at C-8. Among these changes the replacement of the 1488 cm^{-1} peak by the 1460 cm^{-1} peak is most prominent. The two peaks have been assigned to 8-CH and 8-CD deformations,[4] respectively, and intensities of these peaks are proportional to the concentrations of 8-CH and 8-CD forms. Similar spectral changes are observed for 5′-rAMP.

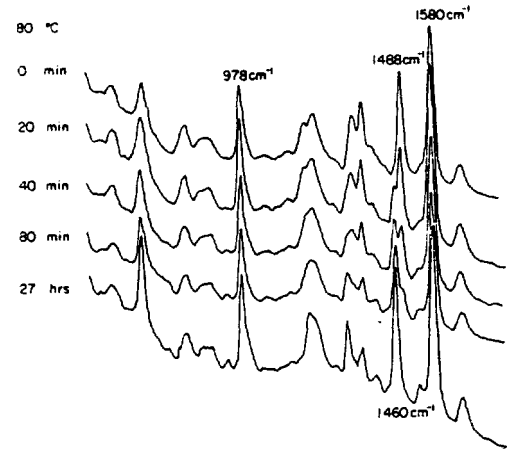

Figure 1. Raman spectra in the 500–1800 cm^{-1} region of 5′-rGMP in D_2O as a function of the incubation time at 80°C.

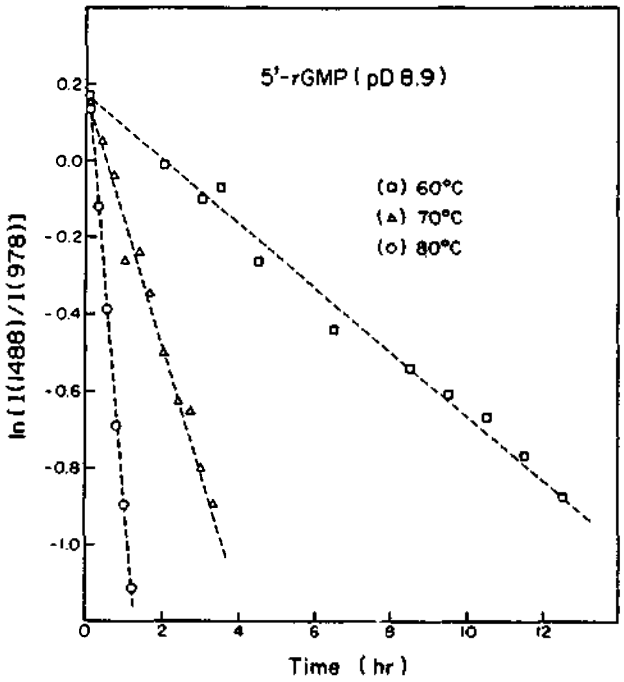

Figure 2. Plot of ℓn[I(1488)/I(978)] vs. the time of incubation for 5′-rGMP (pD 8.9).

The rate constants were determined at 60°C, 70°C, and 80°C for both 5′-rAMP and 5′-rGMP. The exchange becomes very slow below 60°C and too much time is required to achieve a measurable exchange. A typical example of the plot of ℓn[I(1488)/I(978)] vs. time of incubation is given in Figure 2 for 5′-rGMP.

In order to assess the effect of pD on exchange reaction, the rate constants were determined at several pD's; pD 6.4, 7.5, 9.3 for 5′-rAMP and pD 7.5, 8.9, 10.3 for 5′-rGMP. The pD values were limited in these ranges for two reasons. First, charged forms of both 5′-rAMP and 5′-rGMP in very acidic and very basic pD solutions are different from those in nearly neutral solutions. And thus Raman spectra are dramatically different.[14] Second, 5′-rGMP in acidic solution forms a gel or self-aggregate at room temperature. And Raman intensities are greatly affected by this process.[14,15]

The pseudo-first order rate constants and the Arrhenius activation energies obtained for 5′-rAMP and 5′-rGMP are listed in Table 1. The rate constants for 5′-rGMP are approximately three times larger than those for 5′-rAMP at the same pD. The Arrhenius activation energies for both compounds are similar.

TABLE 1: Rate Constants and Arrhenius Activation Energies for Deuterium Exchange of 8-CH Groups in 5′-rAMP and 5′-rGMP

k (hr^{-1})

	pD					
	5′-rAMP			5′-rGMP		
Temp(°C)	6.4	7.5	9.3	7.5	8.9	10.3*
60	0.036	0.031	0.031	0.088	0.081	0.17
70	0.10	0.089	0.089	0.23	0.32	0.71
80	0.30	0.28	0.21	0.67	1.02	1.40
E_a(kcal/mole)	24.3	25.2	22.2	23.2	28.4	24.1

*At this pD the result is less reliable than others due to an overlap of peaks caused by the simultaneous presence of keto and enolate forms of guanine base, both of which are undeuterated or deuterated at C-8 position.

However, the pD effects on rate constants are markedly different for the two nucleotides. The rate constants for 5′-rAMP remain essentially constant or show a slight decrease upon varying the pD from 6.4 to 9.3. The rate constants for 5′-rGMP show approximately two-fold increase upon raising the pD from 7.5 to 10.3.

Discussion

According to the mechanism proposed by Elvidge *et al.*,[2,7] the exchange reaction proceeds by way of an ylide type intermediate and at high pH it involves an attack by hydroxide ions on neutral substrates in detritiation process. This mechanism is consistent with the exchange rate-pH profiles for adenine, adenosine,[2] 5′-rAMP,[7] guanine, and guanosine[2] obtained by the detritiation method.

According to the pH profile for 8-CH exchange rate constants in 5′-rAMP obtained by the detritiation method,[7] the exchange rate constant is nearly constant in the pH range of 6–9. Our Raman result for 5′-rAMP is in good agreement with the reported pH profile and thus supports the exchange reaction mechanism proposed by Elvidge *et al.*[2,7]

The pH profile for 8-CH exchange rate constants in 5′-rGMP has not been reported. The rate-pH profile for guanosine obtained by the detritiation method[2] is nearly constant in the pH 3–7 range and increases sharply in the pH 8–10 range and becomes constant again in the pH 10–12 region. At least in the pD 7.5–10.3 range, our Raman result for 5′-rGMP is in qualitative agreement with the reported pH profile for guanosine. Therefore, the present kinetic data obtained by laser Raman spectroscopic method support the exchange mechanism propsed by Elvidge *et al.*[2]

The exchange rate-pH profiles were determined at 85°C by the detritiation method.[2,7] Our Raman measurements cover the temperature range of 60–80°C. The agreement between the two results suggests that the isotope exchange at C-8 of 5′-rAMP and 5′-rGMP proceeds by the same mechanism at least in the temperature range of 60–85°C.

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Synthesis and Reaction of Biheterocyclic Thiazolo[3,2-a]pyrimidinium-betaines

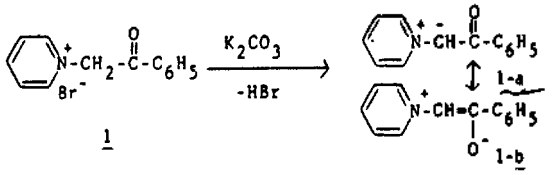
Kyung Ho Yoo and Sang Woo Park†

Division of Chemistry, Korea Advanced Institute of Science and Technology, P.O. Box 131 Dongdaemun, Seoul 131, Korea (Received May 11, 1985)

Various new kinds of biheterocyclic betaines were prepared by the reaction of 3-substituted-6, 7-dihydro-5H-thiazolo[3,2-a] pyrimidine with electrophiles such as isothiocyanates, isocyanates in aprotic solvents, respectively. The biheterocyclic betaines containing methyl group at 3-position of thiazole ring were obtained particularly in good yields at room temperature. These betaines were also reacted with alkyl halide to give quarternary ammonium salts. It was found that these betaines are dissociated in polar organic solvents depending on temperature. And new biheterocyclic compounds via ring transformation were prepared by the reaction of 8-phenyl (thiocarbamoyl)-3-phenyl-6, 7-dihydro-5H-thiazolo[3, 2-a]pyrimidinium-betaine with α-halo kester α-halo ester and γ-halo keto ester.

Introduction

The first compound N-ylid structure may be assigned was prepared in 1933 by Krollpferiffer and Müller[1] by treating an aqueous solution of N-(2-ethyl-mercapto-5-ethyl-phenacyl)-pyridinium bromide with sodium hydroxide. Two years later, Kröhnke[2] isolated a crystalline product by elimination of hydrogen bromide from phenacylpyridinium bromide and he assigned to it an "enol-betaine" structure 1-b.



The betaines with N-ylid are highly reactive organic compounds. Owing to the presence of the carbanion they appear to be strong nucleophiles and react therefore with a large variety of organic compounds; in most cases a new c-c bond is formed in such reactions, which are very important for organic synthesis.

Some thiazolo[3,2-a]pyrimidines reported[3-6] earlier have shown fairly good antibacterial activity and a variety of pharmaceutical activities, including an anti-inflammatory effect. And it was known that certain 8-substituted-6,7-dihydro-5H-thiazolo[3, 2-a]pyrimidinium salts had useful pesticidal and acaricidal properties[7]. The N-bridged thiazolo compounds, isolated as the hydrobromides or hydrochlorides, were prepared by reacting the cyclic thioureas with α-halo ketone or α-halo ester by following the general procedure reported previously[5, 8-10].

It was known[11-13] that 8-substituted-3-phenyl-6,7-dihydro-5H-thiazolo[3,2-a] pyrimidinium-betaines with pyrimidinium—ylid structure are obtained from reaction of 3-pheny-6,7-dihydro-5H-thiazolo [3,2-a] pyrimidine with phenyl isothiocyanate, phenyl isocyanate or carbon disulfide. And it was also reported[14, 15] that the reaction of phenacyl bromide with the bicyclic zwitterionic thiazole, which result from a three-component reaction of cyclic thioureas, mercury bis(phenylacetylide), and phenyl isothiocyanate, does not yield the bromide; instead ring opening and renewed cyclization af-