Structural Effect of Nucleotides on Syn−Anti Glycosyl Isomerization Kinetics by Ultrasonic Relaxation Methods

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Above pH 12, a single ultrasonic relaxation absorption was observed at 100 MHz, while two relaxational frequencies were observed near pH 7 in 5′-GMP aqueous solutions. The single relaxation observed above pH 12 was attributed to a perturbation of the syn−anti rotational isomerization around the glycosyl bond based on the concentration and pH dependence of the relaxation frequency and the maximum absorption per wavelength. The kinetic and thermodynamic parameters were determined for the isomerization reaction. The results for the isomerization around the glycosyl bond in 5′-GMP are compared to those reported on related systems, and from such a comparison the effects of structural changes on the dynamics of syn−anti isomerization can be deduced.

Introduction

An understanding of the dynamic conformational changes in nucleotides is important in the design of oligonucleotides, such as ribozymes. To provide such understanding at the monomer level, we have carried out an elucidation of the ultrasonic relaxation behavior of nucleotides in the aqueous solutions,[1−5] since this method is almost uniquely suitable to obtain information for dynamic equilibrium. In a solution of nucleotides near neutral pH, there is a relaxation associated with an intermolecular proton transfer reaction at a frequency lower than 10 MHz, while there is a second relaxation superimposed at 100 MHz. The latter relaxation has been attributed to a perturbation of the syn−anti glycosidic equilibrium in nucleotides and nucleosides. Experimental evidence with adenine nucleotides has indicated that the phosphate side chain affects the rotational isomerization, probably due to subtle interaction between the base and the phosphate groups. To further elucidate the effects of the nature of the base, studies have been carried out with guanosine 5′-monophosphate (5′-GMP), enabling us to compare the results with those on adenosine, adenosine 5′-monophosphate (5′-AMP), adenosine 5′-diphosphate (5′-ADP), and adenosine 5′-triphosphate (5′-ATP).

Experimental Section

Chemicals. Guanosine 5′-monophosphate sodium salt was purchased from Sigma Co. Ltd. as the purest grade and was used without further purification. Solid NaOH (Wako Pure Chemicals Co. Ltd.) was used to prepare the stronger alkaline solutions. Water used in the experiment was distilled and filtered through a Milli-Q SP-TOC system from Japan Millipore Ltd. Sample solutions were prepared by weighing. The sound velocity of 5′-GMP was monitored for 2 days, every 2−3 h, indicating that no hydrolysis had taken place.

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Measurements. A resonator method was used to obtain the absorption coefficient, α, in the frequency range of 0.8−7 MHz at 25 °C. The temperature of the resonator cells with 3 and 5 MHz x-cut crystals was maintained by circulating water controlled to within ±0.01 °C using a Lauda RM-20B temperature controller. The absorption measurement was also carried out by a pulse method in the frequency range of 15−220 MHz using 5 and 20 MHz fundamental x-cut quartz crystals at 15, 20, 25, and 30 °C, and the cells for the apparatus were immersed in a water bath maintained within ±0.1 °C by Eyela Univ Ace NCB-2200. More details of the instruments and the procedure for the determination of the absorption coefficient were described elsewhere.[6,7] The sound velocity was measured by the resonator at around 3 MHz. The solution pH was obtained in the pulse cell with a glass electrode (HM-60S Toa Denpa) during the absorption measurement. Density was measured with a vibrating density meter (DMA 60/602 Anton Paar). All measurements were carried out in dry N_2 gas atmosphere to avoid CO_2 contamination of the solutions.

Results and Discussion

Representative ultrasonic absorption spectra are shown in Figures1 and 2, where absorption coefficients divided by the square of the frequency, α/f^2, are displayed as a function of the frequency, f. The frequency dependence of α/f^2 was analyzed by a conventional Debye-type relaxational equation as

$$(\alpha/f^2)f = \Sigma A_i f/[1 + (f/f_{ri})^2] + Bf \qquad (1)$$

where f_{ri} is the relaxation frequency, A_i is the amplitude of the ultrasonic relaxation, and B is the background absorption. The reason both sides of eq 1 are multiplied by the frequency is that α/f^2 is a monotonically decreasing function of the measurement frequency. The ultrasonic parameters, f_{ri}, A_i, and B, were

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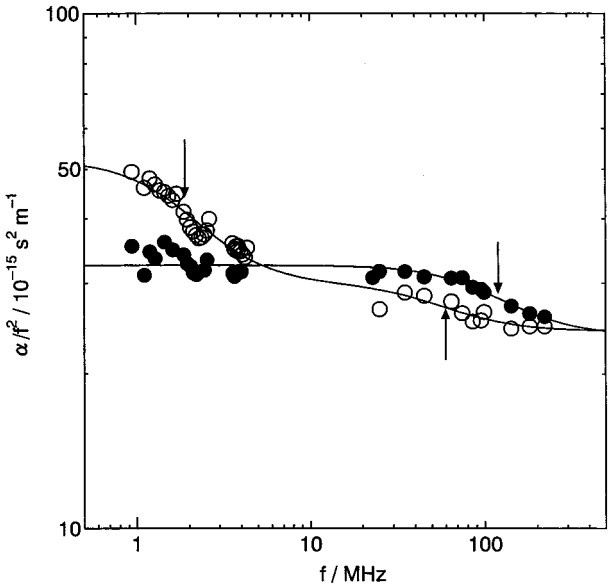

Figure 1. Representative ultrasonic spectra in aqueous solutions of 5′-GMP at 25 °C. The arrow shows the position of the relaxation frequency: (○) 0.100 mol dm $^{-3}$ and pH = 7.97; (●) 0.100 mol dm^{-3} and pH = 12.20

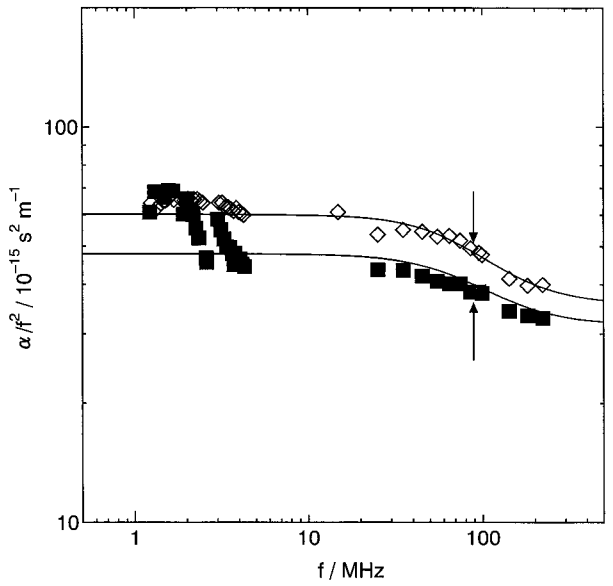

Figure 2. Representative ultrasonic spectra in aqueous solutions of 5′-GMP at 25 °C. (◇) 0.300 mol dm^{-3} and pH = 12.43; (■) 0.200 mol dm^{-3} and pH = 12.52.

determined by a nonlinear least mean squares method. The details of the data treatment are described elsewhere.[7,8] The spectra below pH 9 were not well fitted to a single relaxational equation ($i = 1$ in eq 1), but they were well described by the double relaxational one ($i = 2$ in eq 1) as is seen in Figure 1. On the other hand, the spectra above pH 12 were reasonably fitted to a single relaxational equation as indicated in Figure 2. The solid curves in Figures 1 and 2 show the calculated values using the obtained parameters. Such pH and concentration dependence of the ultrasonic absorption spectra has been also observed in 5′-AMP, 5′-ADP, and 5′-ATP solutions. From the present ultrasonic results and those for aqueous solutions of several nucleotides,[1−5,9] it is certain that the relaxation observed in the lower frequency range (less than 10 MHz) is associated with a proton-transfer reaction. Therefore, for solutions of 5′-GMP below pH 9, the relaxational absorption due to the proton transfer reaction and the absorption observed at around 100 MHz

TABLE 1: Ultrasonic Parameters for 5′-GMP Aqueous Solutions at 25 °C

C_0 mol dm^{-3}	pH	f_r MHz	$A\,10^{-15}$ s^2 m^{-1}	B	c m s^{-1}	ρ kg dm^{-3}
0.100	12.20	103 ± 6	9.1 ± 0.2	24.1 ± 0.2	1516	1.0184
0.151	12.74	90 ± 8	13.3 ± 0.7	29.6 ± 0.3	1552	
0.200	12.66	111 ± 16	14.4 ± 0.8	31.4 ± 0.8	1552	1.0458
0.200	12.52	92 ± 5	16.1 ± 0.5	31.7 ± 0.3	1555	1.0448
0.300	12.43	92 ± 6	24.4 ± 0.8	35.7 ± 0.4	1568	1.0734
0.350	12.40	92 ± 13	23 ± 2	39.5 ± 0.9		1.0746

TABLE 2: Mean Relaxation Frequency and Activation Enthalpy Change for the Isomerization Reaction of Some Nucleotides at 25 °C

nucleotide	f_r MHz	$\Delta H_f^{\ddagger}$ kJ mol^{-1}	reference
adenosine	40		9
5′-GMP	97 ± 5	6.5 ± 0.7	this work
5′-AMP	97 ± 3	3.8 ± 0.7	5
5′-ADP	142 ± 2	20 ± 1	4
5′-ATP	112 ± 3	11 ± 1	4

are superimposed. As can be seen in Figures 1 and 2, the relaxation below 10 MHz disappears when the solution pH is raised to around 12.

The ultrasonic relaxation observed above pH 12 is the focus of this study, since the goal of the present study is an examination of the syn−anti conformational interconversion in nucleotides. The absorption measurements have been performed as functions of the concentration and pH at 25 °C and the ultrasonic parameters obtained are tabulated in Table 1. It has been found that the relaxation frequency is independent of the concentration of 5′-GMP and that slight changes of pH near 12 produce similar relaxation frequency. On the other hand, the amplitude of the ultrasonic relaxation, A, tends to increase with the concentration of nucleotide. These trends were also observed in solutions of adenosine, 5′-AMP, 5′-ADP, and 5′-ATP. The results with 5′-GMP solutions suggest that the source of the relaxation is a perturbation of an equilibrium associated with the syn−anti conformational isomerization reaction due to rotation around the glycosyl bond in 5′-GMP. The relationship between the relaxation frequency and the rate constant is given by $2\pi f_r = k_f + k_r$.

$$\text{anti conformation} \underset{k_r}{\overset{k_f}{\rightleftharpoons}} \text{syn conformation} \qquad (2)$$

where k_f and k_r are the unimolecular forward and reverse rate constants, respectively. The mean value of the relaxation frequency of aqueous solutions of 5′-GMP is shown in Table 2 along with those for 5′-AMP, 5′-ADP, 5′-ATP, and adenosine for comparison.[4,5,9,10]

Another important quantity that can be obtained from ultrasonic experiments is the maximum absorption per wavelength, $\mu_m = 0.5Af_rc$. This is related to the standard volume change and reactant concentrations.[11] For the reaction in eq 2, it leads to[12]

$$\mu_m = \pi\rho c^2(\Delta V)^2[K/(1+K)^2]C_0/2RT \qquad (3)$$

where ρ is the solution density, c is the sound velocity, K is the equilibrium constant defined as $K = k_f/k_r$, C_0 is the analytical concentration, and ΔV is the parameter associated with the standard volume change. Plots of μ_m vs $\rho c^2 C_0$ for 5′-GMP and 5′-AMP at 25 °C are shown in Figure 3, the linearity of which confirms that the source of the relaxation is associated with the syn−anti isomerization process.

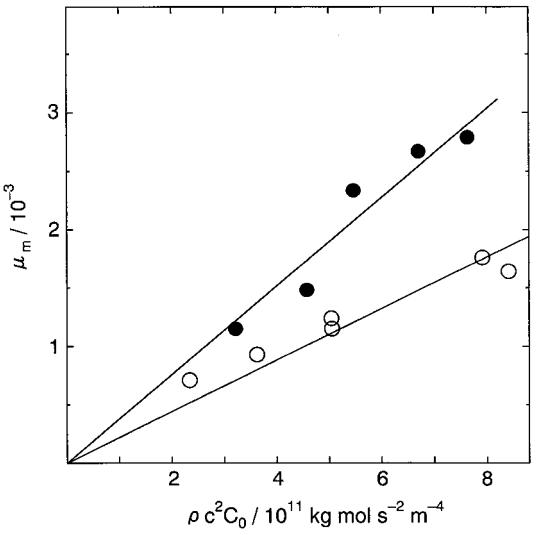

Figure 3. Concentration term dependence of the maximum absorption per wavelength, μ_m, for aqueous solution of 5′-GMP (○) and 5′-AMP (●) at 25 °C. The slope of the lines is denoted as $F = \pi(\Delta V)^2K^{-1}/2RT$.

Results with 5′-AMP, 5′-ADP, and 5′-ATP solutions[4,5] suggest that the rate of rotation around the glycosyl bond is related to the number of charges on the phosphate group and to the hindrance to rotation with increasing steric bulk of the side chain, in opposition to the increasing charge−dipole interaction with increasing charge. The latter effect for 5′-GMP is the same as for 5′-AMP, and the relaxation frequency at 25 °C is almost the same as for 5′-AMP. According to Rich et al.,[13] the syn form in nucleotides is more stable than the anti form. Therefore, the relaxation frequency is expected to reflect the forward rate constant, k_f. To compare the results further with those for 5′-AMP, the ultrasonic absorption measurement was also carried out at 15, 20, and 30 °C, in addition to 25 °C.

As the forward rate constant is larger, the temperature dependence of the relaxation frequency, f_r, can be described by the following equation using absolute rate theory $2\pi f_r = (kT/h)\exp(-\Delta G_f^{\ddagger}/RT)$, where k is the Boltzmann constant, T is the absolute temperature, h is Planck's constant, and $\Delta G_f^{\ddagger}$ is the change of activation free energy which is related to the activation enthalpy change, $\Delta H_f^{\ddagger}$, and entropy change, $\Delta S_f^{\ddagger}$, as $\Delta G_f^{\ddagger} = \Delta H_f^{\ddagger} - T\Delta S_f^{\ddagger}$. Figure 4 shows plots of $\ln(2pf_r/T)$ against $1/T$ where the mean relaxation frequencies at individual concentrations are used. From the slopes, the changes of activation enthalpy, $\Delta H_f^{\ddagger}$, were determined using the least mean squares method (see Table 2). The free energy and entropy changes of activation cannot be determined because the reaction is proceeding in solution and the transmission coefficient in the rate theory is arbitrary. It is noteworthy that the activation enthalpy change for 5′-GMP is greater than for 5′-AMP. It is reasonable to assume that the equilibrium constant, K, is greater than unity because $k_f \gg k_r$. Then, eq 3 is approximated as $\mu_m = \pi\rho c^2(\Delta V)^2K^{-1}C_0/2RT$. The plots of μ_m vs ρc^2C_0 at constant temperature provide values of $F = \pi(\Delta V)^2K^{-1}/2RT$ (Figure 3). For 5′-GMP solutions, we have obtained $F = (3.2 \pm 0.4) \times 10^{-15}$ (kg mol s^{-2} $m^{-4})^{-1}$ at 15 °C, $F = (2.8 \pm 0.4) \times 10^{-15}$ (kg mol s^{-2} $m^{-4})^{-1}$ at 20 °C, $F = (2.2 \pm 0.4) \times 10^{-15}$ (kg mol s^{-2} $m^{-4})^{-1}$ at 25 °C, and $F = (1.7 \pm 0.4) \times 10^{-15}$ (kg mol s^{-2} $m^{-4})^{-1}$ at 30 °C. To a good approximation, the parameter F is only dependent on the temperature. From the relationship for F, the slope of the plots of $\ln(FT)$ vs $1/T$ gives the standard enthalpy change of the isomerization reaction, since $K = \exp(-(\Delta H - T\Delta S)/RT)$, where ΔS is the standard entropy change of the reaction. Figure 5 presents the above plots, and the

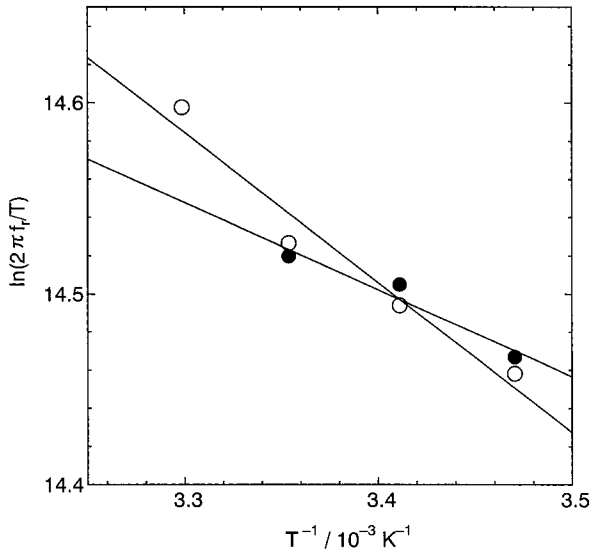

Figure 4. Plots of $\ln(2pf_r/T)$ vs $1/T$ for 5′-GMP (○) and 5′-AMP (●) to determine the activation enthalpy change of the reaction $\Delta H^{\ddagger}$.

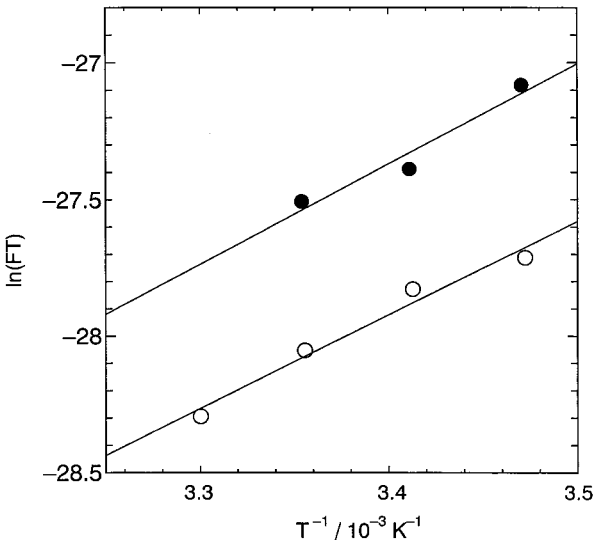

Figure 5. Plots of $\ln(FT)$ vs $1/T$ for 5′-GMP (○) and 5′-AMP (●) solutions to determine the enthalpy change of the reaction ΔH.

TABLE 3: Thermodynamic Parameters for Syn−Anti Isomerization of 5′-AMP and 5′-GMP[a]

	$\Delta H_f^{\ddagger}$ kJ mol^{-1}	$\Delta H_b^{\ddagger}$ J mol^{-1} K^{-1}	ΔH 10^{-6} m^3 mol^{-1}	ΔS	ΔV
5′-AMP	4 ± 0.7	34 ± 5	30 ± 5	125	∼8
5′-GMP	6 ± 0.7	34 ± 1	28 ± 0.2	122	∼9

[a] Standard Gibbs free energy change, ΔG, is obtainable from the relationship, $\Delta G = \Delta H - T\Delta S$.

estimated ΔH value is tabulated in Table 3 along with that for 5′-AMP. The ΔV and ΔS values cannot be determined analytically. However, once a value for ΔS is estimated, a value for ΔG is obtained from the relationship $\Delta G = \Delta H - T\Delta S$ and the value of K can be calculated. Assuming values for ΔS, plots of F vs $[K/(1 + K)^2]/T$ were recalculated until the smallest standard deviation was obtained. For $\Delta S = 122$ J mol^{-1} T^{-1}, the smallest standard deviation resulted, giving values of ΔG from −6.7 to −8.5 kJ mol^{-1} and values of K from 16 to 29 using the ΔH and ΔS values in Table 3. It should be noted that a small change in ΔS (5%) leads to a large change in the K values (approximately 50%) because of the relationship $K = \exp(-\Delta G/RT)$. Therefore, an estimation of the temperature

dependence of the reverse rate constant, k_r, from $K = k_f/k_r$ may not be adequate. The order of magnitude of the reverse rate constant is approximately 3×10^7 s^{-1}. It is, however, certain that the syn−anti conformational isomerization is an entropy-driven process.

The parameter, ΔV, has been then obtainable using eq 3, the result of which is listed in Table 3. Precisely, the term of the standard enthalpy change should be included in ΔV as $\Delta V = \Delta V' - \alpha_p \Delta H/\rho C_p$, where $\Delta V'$ is the standard volume change of the reaction, Δ_p is the thermal expansion coefficient, and C_p is the specific heat at a constant pressure. In our previous report,[5] we have underestimated the term $\alpha_p \Delta H/\rho C_p$ using the value for liquid water. When $\Delta H \doteqdot 30$ kJ mol^{-1}, it should be about 2×10^{-6} m^3 mol^{-1}. This means the volume changes in Table 2 are over- or underestimates of the actual standard volume change of the reaction, $\Delta V'$. However, the determination of the concentration and temperature dependence of the above term, $\alpha_p \Delta H/\Delta C_p$, is not possible at this stage experimentally, and the contribution of this term may be in the experimental error because the equilibrium constant, K, has a considerably large error. Therefore, the estimation of the error for ΔV is meaningless.

In conclusion, it has been found that the ultrasonic relaxation observed in aqueous solutions of 5′-GMP at around pH 12 is due to the interconversion of the syn−anti conformations around the glycosyl bond. The activation enthalpy change for the process from the anti form to the syn form is greater for 5′-GMP than for 5′-AMP. The greater stability of the syn conformation in aqueous solutions of nucleotides is believed to be controlled by entropy rather than enthalpy. Further experiments with pyrimidine nucleotides might provide clearer insights into the mechanism of interaction between the base and sugar in nucleotides.

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