Kinetic and Thermodynamic Study of Syn−Anti Glycosyl Isomerization in Aqueous Solutions of AMP, ADP, and ATP by Ultrasonic Relaxation Methods

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Ultrasonic absorption coefficients in aqueous solutions of adenosine 5′-triphosphate (ATP) were measured at 25 °C as a function of concentration and pH in the frequency range from 0.8 to 220 MHz. An ultrasonic relaxational absorption with two relaxation frequencies was observed near pH 3. However, above pH 12, only a single relaxational absorption was detected at around 100 MHz, and this relaxation is the focus of this report. The relaxation frequency is independent of the concentration and pH at around pH 12, and the maximum absorption per wavelength increases linearly with concentration. From these results, the cause of the relaxation was attributed to a syn−anti glycosyl isomerization reaction of ATP. The rate of the rotational motion in ATP was found to be smaller than that for ADP and greater than those for AMP and adenosine. These results are discussed in relation to the charge distribution and the effect of the size of the phosphate group of the nucleotides. To examine the reaction mechanism further, ultrasonic absorption measurements were carried out for aqueous solutions of adenosine 5′-monophosphate (AMP), adenosine 5′-diphosphate (ADP), and ATP at 15, 20, and 25 °C above pH 12 from which activation enthalpies were determined. Furthermore, the detailed temperature dependence of the absorption in AMP was investigated at several concentrations in order to determine the standard Gibbs free energy, enthalpy, entropy, and volume changes of the reaction, revealing the thermodynamic properties of the syn−anti conformational isomerization process of the nucleotides.

Introduction

Roles of nucleotides and nucleosides in living systems are well-known, yet, there still remain many questions concerning their dynamical behavior in solution. This may be because even simple conformational changes in nucleotides and nucleosides proceed too rapidly to be analyzed by conventional tools. An understanding of the syn−anti conformational changes in these molecules is important, especially in designing their polymers. Although the conformational preferences of nucleotides in solution have been investigated by NMR spectroscopy,[1] a more direct observation of the kinetics of interconversion is desirable. Rhodes and Schimmel[2] proposed the existence of a relaxation associated with a syn−anti isomerization reaction of nucleosides and nucleotides in aqueous solution observed by ultrasonic methods. Our research group[3−5] has also studied this isomerization reaction by ultrasonic relaxation techniques. In our recent experimental study of aqueous solutions of adenosine 5′-monophosphate (AMP) and adenosine 5′-diphosphate (ADP),[5] it was shown that a rotational motion around the glycosyl bond is facilitated with increasing size of the phosphate group. This was concluded from the consideration that the attractive interaction between the base and the phosphate groups is weakened when the size of the charged phosphate group increases.

It is highly desirable to further clarify the mechanism of the syn−anti glycosyl rotational isomerization of adenine nucleotides. For this study, we have selected adenosine 5′-triphosphate (ATP) and the ultrasonic absorption coefficients have been measured as a function of both the concentration and pH. In addition, the temperature dependence of the ultrasonic absorptions in AMP, ADP, and ATP solutions has also been determined and leads to thermodynamic parameters, which provide information concerning the stability of nucleotides in water. The rate and thermodynamic properties in AMP solutions have been investigated in greater detail.

Experimental Section

Chemicals. Adenosine 5′-monophosphate sodium salt (AMP, more than 99.7% pure) and adenosine 5′-diphosphate sodium salt (ADP, more than 93.4% pure) were purchased from Wako Pure Chemicals Co. Ltd. as the purest grade. Adenosine 5′-triphosphate sodium salt (ATP, more than 99% pure, Grade I) was from Sigma. The compounds were used without further purification. Sodium hydroxide was also from Wako Pure Chemicals Co. Ltd. and a concentrated aqueous solution was used to adjust the solution pH to the desired values. Water used in the experiment was distilled and filtered through a MilliQ SP-TOC system from Japan Millipore Ltd. Sample solutions were prepared by weighing just before use.

Measurements. A resonance method was utilized to obtain the absorption coefficient, α, in the frequency range of 0.8−7 MHz at 25 °C. The absorption was also measured by a pulse

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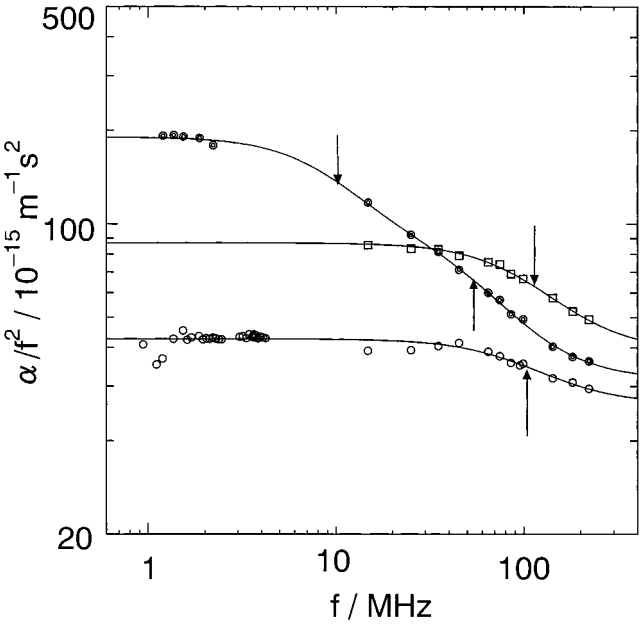

Figure 1. Representative ultrasonic absorption spectra in an aqueous solution of ATP at 25 °C: (◎) 0.140 mol dm^{-3} at pH 2.940 {$f_{r1} = 10 \pm 1$ MHz, $A_1 = (105 \pm 12) \times 10^{-15}$ m^{-1} s^2, $f_{r2} = 63 \pm 4$ MHz, $A_2 = (54 \pm 4) \times 10^{-15}$ m^{-1} s^2, and $B = (31.6 \pm 0.3) \times 10^{-15}$ m^{-1} s^2}; (□) 0.144 mol dm^{-3} at pH 12.298; (○) 0.0447 mol dm^{-3} at pH 12.362 {the ultrasonic parameters for these solutions are listed in Table 2}. The arrows show the position of the relaxation frequency.

method in the frequency range of 15−220 MHz using 5 and 20 MHz fundamental X-cut quartz crystals at 15, 20, and 25 °C. The reason the resonator is only used at 25 °C is that the parallelism of the two crystals in our resonators is settled out at 25 °C and even a few degrees of temperature change may cause an asymmetric alignment of the crystals, leading to incorrect absorption coefficients. 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 pH was measured with a glass electrode (HM-60S Toa Denpa). 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 into the solutions.

Results and Discussion

The absorption coefficients divided by the square of the frequency, α/f^2, as a function of the frequency are shown in Figure 1 for aqueous solutions of ATP at selected pH values and concentrations. The frequency dependence of α/f^2 was tested to see if it fits a conventional Debye-type relaxational equation as

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

where f_{ri} is the relaxation frequency, A_i is the amplitude of the ultrasonic relaxation, and B is the background absorption. The ultrasonic parameters, f_{ri}, A_i, and B, were determined by a nonlinear least-mean-squares method. The solid curves in Figure 1 describe the calculated values using the determined parameters. As seen, the spectra above pH 12 are well described by the single relaxational equation while those around pH 3 are fitted to the double relaxational one. From ultrasonic results for aqueous solutions of several nucleotides,[8−10] it is certain that there exists a large ultrasonic relaxational absorption below pH 9, which is associated with a proton-transfer reaction. The rate and thermodynamic constants have been determined, and the reaction mechanisms have been discussed. We have also observed a similar relaxational absorption for AMP and ADP in the frequency range less than 10 MHz and have assigned the relaxation to the same process.[5] Therefore, the relaxational absorption due to the proton transfer reaction and that observed at around 100 MHz are superimposed below pH 9 in ATP solutions. This is clearly seen in Figure 1. The relaxation below 10 MHz is associated with the proton transfer, and this disappears when the solution pH increases to around 12. The relaxation found at the higher frequency range in Figure 1 still remains at high pH although the relaxation frequency decreases at pH < 9.

TABLE 1: Ultrasonic Parameters Associated with Syn−Anti Rotational Isomerization in Aqueous Solutions of AMP, ADP, and ATP

temp, °C	C_o, mol dm^{-3}	pH	f_r, MHz	10^{-15} s^2 m^{-1} A	10^{-15} s^2 m^{-1} B
			AMP Solution		
15	0.144	12.058	88 ± 5	20.4 ± 0.6	34.8 ± 0.3
20	0.144	11.964	93 ± 8	14.4 ± 0.6	30.4 ± 0.4
25	0.144	12.085	96 ± 5	16.0 ± 0.4	24.7 ± 0.2
			ADP Solution		
15	0.124	12.866	94 ± 3	45.5 ± 0.8	43.7 ± 0.5
20	0.124	12.707	113 ± 7	35.5 ± 0.8	35.7 ± 0.8
25	0.124	12.530	129 ± 9	29.3 ± 0.7	30.3 ± 0.9
			ATP Solution		
15	0.112	13.335	90 ± 4	64 ± 2	51.1 ± 0.8
20	0.112	13.151	99 ± 7	48.4 ± 0.2	45 ± 1
25	0.112	12.951	108 ± 4	37.5 ± 0.6	38.4 ± 0.5
25	0.144	12.591	116 ± 4	50.2 ± 0.7	41.6 ± 0.8
25	0.144	12.298	114 ± 3	47.9 ± 0.5	39.0 ± 0.6
25	0.144	13.205	111 ± 10	46 ± 2	45 ± 2
25	0.144	13.634	105 ± 4	36.1 ± 0.6	39.8 ± 0.5
25	0.106	12.694	111 ± 7	34.2 ± 0.9	38.2 ± 0.9
25	0.0745	12.561	115 ± 4	27.0 ± 0.3	30.2 ± 0.4
25	0.0447	12.362	106 ± 8	16.2 ± 0.5	26.4 ± 0.4

In this study, we focus on the ultrasonic relaxation observed above pH 12 as our aim is an examination of the syn−anti conformational interconversion in nucleotides. The absorption measurements have been performed as functions of the concentration and pH (see Table 1). The relaxation frequency is independent of the concentration, as observed for AMP and ADP.[5] Changes of pH around 12 produced no changes in the relaxation frequency. These results suggest that the source of the relaxation is due to a perturbation of an equilibrium associated with a unimolecular reaction, the probable mechanism being the syn−anti isomerization reaction (Anti ⇌ Syn) due to the rotation around the glycosyl bond in the nucleotides, since the relaxation frequency obeys a unimolecular process:

$$2\pi f_r = k_f + k_r \qquad (2)$$

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 ATP is shown in Table 2 along with those for AMP, ADP, and adenosine.[2,5] In the previous paper,[5] we suggested that the negative charges on the phosphate group reduce the attractive force between the phosphate group and the base in the nucleotides, leading to the relative rate of rotational isomerization as adenosine < AMP < ADP. This effect of the charge on the phosphate may cause a decrease of the relaxation frequency (around 63 MHz at pH 2.940 for ATP), as seen in Figure 1. However, the relaxation frequency for ATP obtained in this study at high pH is between that for AMP and that for ADP (Table 2). These results indicate that the rate of rotation around the glycosyl bond is not

TABLE 2: Values of Relaxation Frequency and Activation Parameter for Syn−Anti Rotational Isomerization in Aqueous Solutions of Adenosine, AMP, ADP, and ATP

solute	f_r,[a] MHz	$\Delta H^{\ddagger}$,[b] kJ mol^{-1}
adenosine	40	
AMP	97 ± 3	3.8 ± 0.7
ADP	142 ± 2	20 ± 1
ATP	112 ± 3	11 ± 1

[a] The relaxation frequency at 25 °C. [b] The activation enthalpy was calculated from the temperature dependence of the relaxation frequency listed in Table 1.

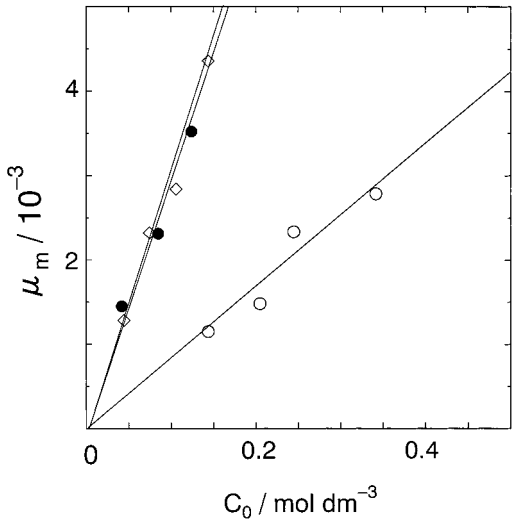

Figure 2. Plots of the maximum absorption per wavelength, μ_m, vs the analytical concentration, C_0, at 25 °C for pH > 12: (○) AMP; (●) ADP; (◇) ATP.

completely accounted for by the number of charges on the phosphate group. A second possible effect on rotational motion may be an increased hindrance to rotation with increasing steric bulk of the side chain, in opposition to the increasing charge−dipole interaction with increasing charge. In other words, for ATP solutions, the increasing negative charge and increasing steric bulk of the sugar phosphate side chain may have opposing effects on the f_r.

To obtain further support for assigning a unimolecular reaction to the observed relaxation, the concentration dependence of the maximum absorption per wavelength, $\mu_m = 0.5 A f_r c$ was examined:[11]

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

where ρ is the solution density, c is the sound velocity, K is the equilibrium constant, C_0 is the analytical concentration, and ΔV is the term associated with the standard volume change and the standard enthalpy of the reaction. The contributions of the sound velocity and density to μ_m are very small. Plots of μ_m vs C_0 are shown in Figure 2 along with those for AMP and ADP, and the linearity of the plots confirms that the relaxational absorption is due to a unimolecular reaction, i.e., the syn−anti isomerization.

To gain further insight into the relationship between the structure of the nucleotides and the syn−anti rotational motion, ultrasonic absorption measurement was carried out for solutions of AMP, ADP, and ATP at 15 and 20 °C, in addition to 25 °C. The measurements for ADP and ATP solutions were performed within no more than 7 h after the preparation of the sample solutions because of slow hydrolysis of the terminal phosphates in water.[12] These spectra can also be well analyzed according to the Debye-type single relaxational equation and the determined ultrasonic parameters are also listed in Table 1.

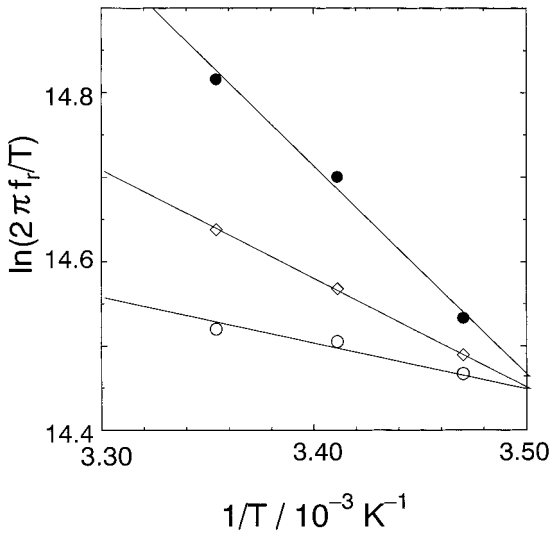

Figure 3. Temperature dependence of the relaxation frequency for pH > 12: (○) AMP; (●) ADP; (◇) ATP.

If one of the two rate constants for the unimolecular reaction is greater, the temperature dependence of the relaxation frequency, f_r, can be described by the following equation using absolute rate theory:

$$2\pi f_r = (kT/h)\exp(-\Delta G_i^{\ddagger}/RT) \qquad (4)$$

where k is the Boltzmann constant, T is the absolute temperature, h is Planck's constant and $\Delta G_i^{\ddagger}$ is the activation free energy, which is expressed by the activation enthalpy, $\Delta H_i^{\ddagger}$, and entropy, $\Delta S_i^{\ddagger}$, as $\Delta G_i^{\ddagger} = \Delta H_i^{\ddagger} - T\Delta S_i^{\ddagger}$. Figure 3 shows that plots of $\ln(2pf_r/T)$ against $1/T$ are linear, indicating that one of the rate constants is considerably larger than the other. From the slopes, the activation enthalpy, $\Delta H_i^{\ddagger}$, was determined using the least-mean-squares method (see Table 2). In the solution of AMP, the temperature dependence of f_r is so small that the mean value at different concentrations has been taken (the results at 0.144 mol dm^{-3} are shown in Figure 3). However, determination of the activation free energy and the activation entropy may be too inaccurate. Even if the relaxation frequency in ADP is higher than that in ATP, the smaller activation enthalpy for ATP, when compared with that of ADP, perhaps indicates that the syn−anti rotational motion for the nucleotides is mostly under entropic rather than enthalpic control.

Further absorption experiments for AMP solutions have been carried out at concentrations of 0.205, 0.245, 0.300, and 0.342 mol dm^{-3}, in addition to those shown in Table 1, and at different temperatures, since the rate of hydrolysis in AMP is quite slow. If the syn conformer is more stable,[13] the relaxation frequency reflects the forward rate constant and the activation enthalpy obtained above is that for the forward process expressed as $\Delta H_f^{\ddagger}$. Therefore, it is reasonable to conclude that the equilibrium constant defined as K = [Syn]/[Anti] = k_f/k_b is greater than unity, and eq 3 is approximated as $\mu_m = \pi\rho c^2(\Delta V)^2K^{-1}\ C_0/2RT$. The contribution of density and sound velocity to the maximum absorption per wavelength is so small that the plots of μ_m vs C_0 give a straight line to yield $\mu_m = G\mathrm{Co}' = (13 \pm 5) \times 10^{-6}\mathrm{Co}'$ at 15 °C, $\mu_m = (9.7 \pm 0.7) \times 10^{-6}\mathrm{Co}'$ at 20 °C and $\mu_m = (8.5 \pm 0.1) \times 10^{-6}\mathrm{Co}'$ at 25 °C where G is the coefficient and Co′ is converted to the unit of mol m^{-3}. These coefficients, $G = \pi\rho c^2(\Delta V)^2K^{-1}/2RT$, provide the $(\Delta V)^2K^{-1}$ values at the measurement temperatures using the above approximate rela-

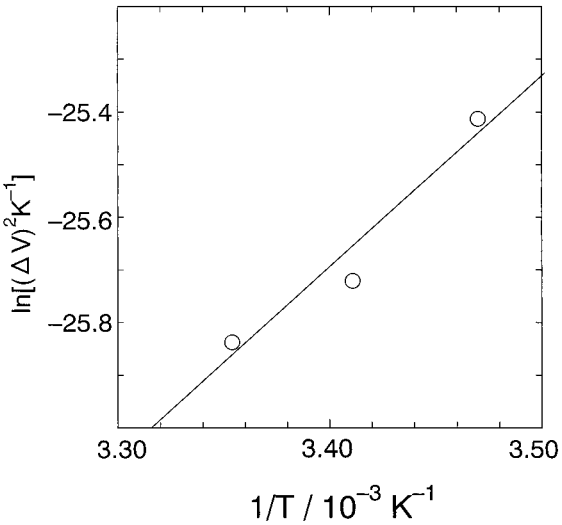

Figure 4. Plots of $\ln[(\Delta V)^2K^{-1}]$ vs $1/T$ for AMP solutions.

tionship. The slope of the plots of $\ln((\Delta V)^2K^{-1})$ vs $1/T$ in Figure 4 provides the standard enthalpy change of the reaction, ΔH, because $K = \exp(-\Delta G/RT)$ and $\Delta G = \Delta H - T\Delta S$ where ΔG is the standard Gibbs free energy change and ΔS is the standard entropy change of the reaction. The ΔH so determined is 30 ± 5 kJ mol^{-1}, and consequently, the activation energy for the reverse process is calculated to be 34 ± 5 kJ mol^{-1} for AMP. It is not possible to estimate directly ΔV and ΔS from the above relationship because the intercept of the above plots provides the sum as $-\Delta S/R + \ln(\Delta V)^2$. As the standard enthalpy change has a positive value, the standard entropy change should be positive in order to satisfy the condition $K > 1$. Using the approximate equation for the maximum absorption per wavelength, an appropriate value of ΔS has been assumed in order to obtain a straight line in the plots of G (the coefficient of the μ_m and Co′ relation) vs $[\exp((\Delta H - T\Delta S)/RT)]/T$, and this calculation was repeated until the best straight line resulted. When $\Delta S = 125$ J K^{-1} mol^{-1}, a good straight line with a zero intercept was obtained (the standard deviation was smallest) and the value of ΔV was calculated to be $(8 \pm 1) \times 10^{-6}$ m^3 mol^{-1}. As the estimated ΔS has a considerable error (less than 8%), the propagation of the error causes the differences of the Gibbs free energy and the equilibrium constants. However, when $\Delta S = 125$ J K^{-1} mol^{-1} and $\Delta H = 30$ kJ mol^{-1} are used, we calculate $\Delta G = -6.0$ kJ mol^{-1} and $K = 12$ at 15 °C, $\Delta G = -6.6$ kJ mol^{-1} and $K = 15$ at 20 °C, and $\Delta G = -7.3$ kJ mol^{-1} and $K = 19$ at 25 °C. These results indicate that the rotational isomerization of AMP is controlled by the entropy term, which gives the negative free energy change and stabilizes the syn-conformer. With increasing temperature, the equilibrium constant increases, favoring one conformer, probably the syn form. It is now also concluded that the reverse rate constant (conversion from syn- to anti-) is estimated to be at least 10 times smaller than the forward rate constants (from anti- to syn-).

Although only the absolute value of ΔV can be obtained, it also includes the standard enthalpy term, ΔH, as $\Delta V = \Delta V' - \alpha_p \Delta H/\rho C_p$, where $\Delta V'$ is the standard volume change of the reaction, α_p is the thermal expansion coefficient, and C_p is the specific heat at a constant pressure. For the rotational motion in the molecule, the enthalpy term might contribute more to the ΔV value.[14] Using the values of α_p, ρ, and C_p for solvent water, the term $a_p \Delta H/\rho C_p$ is estimated to be 0.2×10^{-6} m^3 mol^{-1}, which is too small when compared with the obtained ΔV value. This means that the standard volume change for the rotational motion of AMP is quite large and the rotational motion may be accompanied by solvent water molecules reorganized in the hydration of the solutes. To our knowledge, this experimental study is the first one reporting these thermodynamic functions for the syn−anti conformational interconversion in nucleotides. It will be of interest to carry out similar experimental studies for other purine and pyrimidine nucleotides.

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