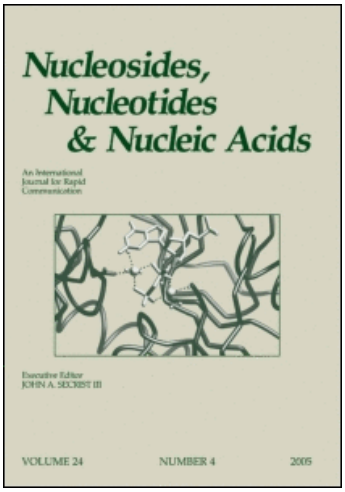

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Stacking Conformation of 9-[ω-(Thymin-1-yl)alkyl]adenine in Aqueous Solution

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ABSTRACT

An intramolecular stacking between adenine and thymine rings of 9-[ω-(thymin-1-yl)alkyl]adenine was investigated by means of NMR and UV spectroscopy. The stacking conformation was presumed on the basis of the temperature dependence on the chemical shifts of the adenine and thymine ring protons in the buffer solution at pD 7.0.

Key Words: Stacking; Adenine; Thymine; NMR; UV.

INTRODUCTION

Stacking is one of molecular aggregations among aromatic molecules that is caused by a stacking interaction, although the nature of the stacking interaction is still obscure.[1] The nucleic acid bases are stacked in most DNA and RNA by the help of twisted polymer chains of nucleic acids, and properly located each other through the stacking interaction. Sarai et al.[2] reported that the helical structure

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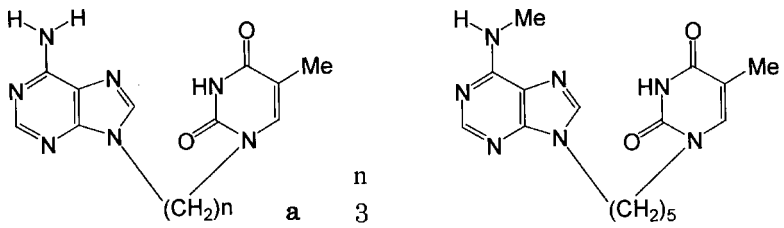

Chart 1.

of DNA was a consequence of the stacking interaction between the base pairs. The research regarding the stacking of the nucleic acid bases has been advanced from both viewpoints of the experimental[3] and the theoretical[4] approaches. One of the major problems is whether the nucleic acid bases form specific stacking conformations in aqueous solution.

While the literature contains numerous references to the hydrogen bond between adenine and thymine (or uracil),[5,6] the investigation of the stacking between adenine and thymine (or uracil) has not been extended beyond the intramolecular stacking of the dinucleotides[7] and a model compound.[8] Browne et al.[8] have reported the intramolecular stacking of 9-[3-(thymin-1-yl)propyl]adenine **(1a)** on the basis of the UV hypochromic effect. Although the stacking between adenine and thymine (or uracil) of dinucleotides has been investigated by means of NMR spectroscopy,[7] little attention has been paid to the stacking conformation of 9-[ω-(thymin-1-yl)alkyl]adenine **(1)**, which consists of two nucleic acid bases connected by an alkyl chain. When the NMR and UV spectra of dinucleotides and **1** are measured in very low concentrations, intermolecular associations of adenine-adenine and of thymine-thymine are expected to be negligible. Therefore, it becomes possible to study only the stacking between adenine and thymine.[7,8] We prepared **1a–g** and N^6-methyl-9-[5-(thymin-1-yl)pentyl]adenine **(2c)** (Chart 1), and investigated the stacking between the adenine and thymine rings by means of NMR and UV spectroscopy.

RESULTS AND DISCUSSION

The compounds **(1a–g)** and **(2c)** were prepared according to the method described before.[6] The UV absorption maxima of **1a-d** and **2c** in water at 25°C, 60°C, and 70°C are summarized in Table 1. A bathochomic shift was observed in the absorption maxima of **1a–d** with increasing the length in the alkyl chains or an increase in temperature. The molar absorptivities of **1a-d** also increased as the

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Table 1. Absorption maximum of **1a–d** and **2c** in water.[a]

	25°C		60°C		70°C	
	λ_{max}/nm	ε	λ_{max}/nm	ε	λ_{max}/nm	ε
1a	261.8	19600	262.5	19900	262.8	20200
1b	262.7	20500	263.1	20750	263.4	20850
1c	263.1	20800	263.3	20850	263.5	20900
1d	263.8	21050	264.0	21000	264.1	21000
2c	267.0	23500	267.4	23700	267.6	23850

[a]The spectra were measured at least three times and the average values of the absorption maxima are shown.

carbon numbers of the alkyl chains were increased. Furthermore, the data in Table 1 indicated a decrease of the molar absorptivity of **1a, 1b** and **2c** at 25°C compared with those at 70°C and 60°C, that is, a hypochromic effect of **1a, 1b** and **2c** clearly. However, the hypochromic effect of **1c** was very small and that of **1d** was not determined. Compound **2c** was remarkable for its hypochromic effect when compared with **1c,** probably because of a hydrophobic interaction of the N^6-methyl group.[10] From a consideration of this UV study, we suppose that stacking did occur between the adenine and thymine rings of **1**.

Nuclear magnetic resonance (NMR) spectroscopy is a useful method for elucidating the stacking conformations. The concentration dependence on the chemical shifts of Ade-2, Ade-8, Thy-6, and Thy-Me of **1b** was investigated in buffer solution at pD 7.0[10] and in CD_3OD. The chemical shifts were little shifted in lower concentrations than 1.0 mM not only in CD_3OD but also in the buffer solution. This suggests that an intermolecular interaction was almost negligible when the measurement was carried out at lower concentrations than 1.0 mM.

Figure 1 shows the relationship between the carbon numbers of the alkyl chains and the chemical shifts of **1a–g** in the buffer solution and in CD_3OD at lower concentrations than 1.0 mM at 27°C. The chemical shifts, except for those of Ade-2 and Ade-8 of **1a**, were to higher fields along with a decrease of the carbon numbers of the alkyl chains in the buffer solution, but the chemical shifts were little dependent on the length of the alkyl chains in CD_3OD. It is conceivable that the stacking between adenine and thymine rings caused the shifts to higher field in the buffer solution but there was not stacking in CD_3OD.

Table 2 shows the temperature dependence on the chemical shifts of **1a–d** and **2c** at lower concentrations than 1.0 mM in the buffer solution. The chemical shift differences ($\Delta\delta = \delta(27°C) - \delta(80°C)$) of Ade-2 of **1a, 1b, 1c,** and **1d** were −0.033, −0.054, −0.034, and −0.031 ppm, respectively. The chemical shift differences of Ade-2 may result from the ring current effect of the thymine ring due to the intramolecular stacking. On the other hand, the chemical shifts of Ade-8 of **1a–d** were to higher fields along with an increase in temperature, suggesting that Ade-8 was located outside the stacking. The chemical shift differences of Thy-Me of **1a, 1b, 1c,** and **1d** were −0.058, −0.044, −0.020, and −0.010 ppm, respectively. The ring current of the adenine ring obviously affected the chemical shifts of Thy-Me. A contribution of Thy-6

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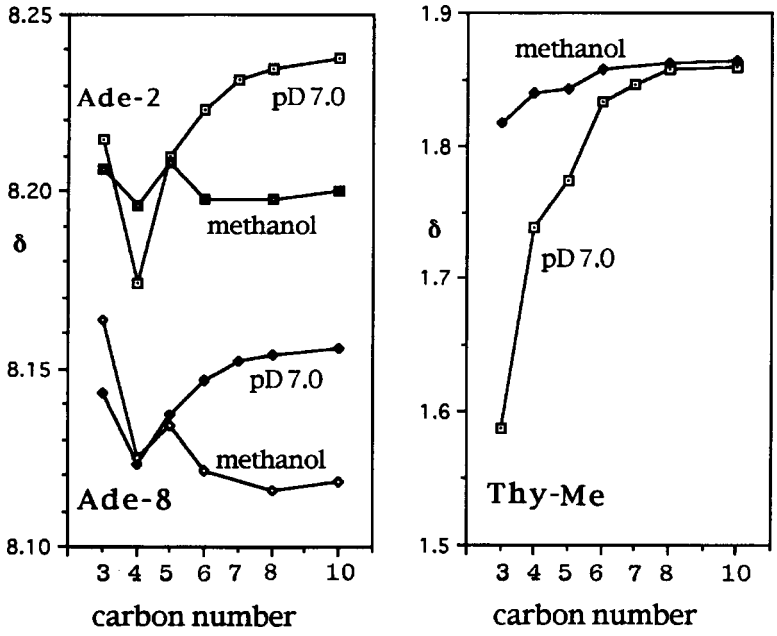

Figure 1. Relationship between the carbon numbers of the alkyl chains and the chemical shifts of Ade-2, Ade-8, and Thy-Me of **1a-g** (n = 3–8 and 10) in the buffer solution at pD7.0 and in methanol–d_4 at 27°C.

to the stacking was determined by a detailed comparison of Δδ of Thy-6 with Δδ of Thy-Me. Consequently it is conceivable that Thy-6 was located outside the stacking but in near distance from the stacking. In order to elucidate a contribution of the amino group of the adenine ring to the stacking, the temperature dependence on the chemical shifts of **2c** was studied. By a comparison of Δδ of N^6-Me group with Δδ of Ade-2, it is conceivable that the N^6-Me group of the adenine ring was also located outside the stacking but in near distance from the stacking. The results of the NMR studies seem to be consistent with the conclusion to be drawn from the UV data.

On the basis of the data in Table 2, it seems reasonable to assume that a stacking conformation of **1** was the structure shown in Chart 2. Interestingly the stacking conformation is similar to the stacking structures in DNA.[11] Therefore, it may be concluded from the results of this investigation that the adenine and thymine rings of **1** formed a specific stacking conformation similar to the stacking in DNA in aqueous solution, although the alkyl chains between adenine and thymine rings may play an important role to the stacking conformation.

Experimental Section

The melting points were determined on a Yanagimoto micro melting-point apparatus and are uncorrected. The elemental analyses were performed in the

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Table 2. Temperature dependence on the chemical shifts of **1a–d** and **2c** in the buffer solution at pD7.0.[a]

Temp. (°C)	**1a**/chemical shift (δ)				**1b**/chemical shift (δ)				**1c**/chemical shift (δ)				**1d**/chemical shift (δ)				**2c**/chemical shift (δ)				
	A-2	A-8	T-6	T-Me	A-2	A-8	T-6	T-Me	A-2	A-8	T-6	T-Me	A-2	A-8	T-6	T-Me	A-2	A-8	A-NMe	T-6	T-Me
27	8.215	8.143	7.089	1.587	8.174	8.123	7.206	1.738	8.210	8.137	7.254	1.774	8.223	8.147	7.366	1.834	8.214	8.067	3.120	7.213	1.747
35	8.222	8.142	7.090	1.597	8.184	8.124	7.211	1.745	8.217	8.134	7.252	1.776	8.227	8.145	7.362	1.836	8.222	8.065	3.122	7.214	1.750
40	8.226	8.140	7.089	1.601	8.191	8.125	7.214	1.752	8.220	8.132	7.252	1.777	8.231	8.143	7.358	1.836	8.226	8.064	3.124	7.214	1.752
50	8.233	8.138	7.090	1.611	8.202	8.124	7.218	1.760	8.228	8.129	7.250	1.783	8.237	8.140	7.354	1.838	8.233	8.060	3.128	7.213	1.759
60	8.239	8.136	7.092	1.622	8.211	8.123	7.222	1.768	8.233	8.125	7.248	1.785	8.244	8.138	7.350	1.840	8.240	8.057	3.133	7.214	1.764
70	8.244	8.133	7.095	1.634	8.220	8.122	7.227	1.776	8.239	8.122	7.247	1.790	8.248	8.135	7.343	1.841	8.246	8.053	3.136	7.214	1.772
80	8.248	8.129	7.098	1.645	8.228	8.120	7.229	1.782	8.244	8.118	7.244	1.794	8.254	8.130	7.338	1.844	8.251	8.049	3.139	7.215	1.778
Δδ[b]	−0.033	0.014	−0.009	−0.058	−0.054	0.003	−0.023	−0.044	−0.034	0.009	0.010	−0.020	−0.031	0.017	0.028	−0.010	−0.037	0.018	−0.019	−0.002	−0.031

[a]The concentrations were less than 1.0 mM.
[b]$\Delta\delta = \delta(27°C) - \delta(80°C)$.

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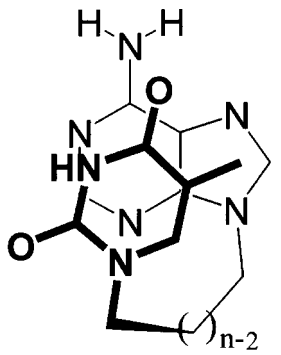

A Stacking Conformation of 1

Chart 2.

Analytical Center of Kyoto University. The ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were obtained with a JEOL GSX400 spectrometer. The chemical shifts (δ-values) were measured in parts per million down-field from sodium 3-(trimethylsilyl)propionate-2,2,3,3-d_4 in the buffer solution at pD 7.0, and from tetramethylsilane in CD_3OD and DMSO-d_6 as internal references. The ^{1}H NMR spectra were obtained from accumulation of 40-2200 free induction decays after each 45° pulse (5.7 ms) repeated every 5.73 s and were observed over a spectral width of 6002.4 Hz, corresponding to 32768 data points for acquisition time of 2.73 s. The electronic absorption spectra were recorded on a Shimadzu UV-2450 spectrometer equipped with a temperature controlled cell holder (Shimadzu TCC-240A).

9-[ω-(Thymin-1-yl)alkyl]adenine. Into a solution of adenine (1 mmol) in N,N-dimethylformamide (30 mL), 1-(ω-bromoalkyl)thymine[9] (1 mmol) and K_2CO_3 (1 mmol) were added. The mixture was stirred at room temperature for 15 h. The resulting mixture was evaporated to give a residue, which was submitted to chromatography over silica gel. By elution with a mixture of ethyl acetate and methanol, **1a** (12% yield), **1b** (15% yield), or **1c** (20% yield) was obtained. The preparation of **1d–g** had been reported.[6] Under similar conditions **2c** was prepared in 22% yield by the treatment of 1-(5-bromopentyl)thymine with N^6-methyladenine instead of adenine.

9-[3-(Thymin-1-yl)propyl]adenine (1a). Mp > 300°C (lit.[8] 303–305°C dec.); ^{1}H NMR (DMSO-d_6) δ = 11.25 (s, 1H, T-NH), 8.15 (s, 1H, A-2or8), 8.14 (s, 1H, A-2or8), 7.50 (s, 1H, T-6), 7.20 (s, 2H, NH2), 4.17 (t, 2H, J = 7 Hz), 3.68 (t, 2H, J = 7 Hz), 2.16 (quintet, 2H, J = 7 Hz), 1.73 (s, 3H); ^{13}C NMR (DMSO-d_6) δ = 164.19, 155.86, 152.27, 150.88, 149.46, 141.16, 140.65, 118.67, 108.50, 44.87, 40.41, 28.72, 11.80.

9-[4-(Thymin-1-yl)butyl]adenine (1b). Mp > 300°C; ^{1}H NMR (DMSO-d_6) δ = 11.21 (s, 1H, T-NH), 8.14 (s, 1H, A-2or8), 8.13 (s, 1H, A-2or8), 7.49 (s, 1H, T-6), 7.18 (s, 2H, NH2), 4.17 (t, 2H, J = 7 Hz), 3.65 (t, 2H, J = 7 Hz), 1.79 (broad

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quintet, 2H, J = 7 Hz), 1.73 (s, 3H), 1.54 (broad quintet, 2H, J = 7 Hz); ^{13}C NMR (DMSO-d_6) δ = 164.17, 155.86, 152.27, 150.82, 149.44, 141.25, 140.76, 118.65, 108.40, 46.50, 42.33, 26.29, 25.52, 11.81. Found: C, 51.31; H, 5.56; N, 29.96%. Calcd for $C_{14}H_{17}N_7O_2 \cdot 2/3H_2O$: C, 51.36; H, 5.64; N, 29.95%.

9-[5-(Thymin-1-yl)pentyl]adenine (1c). Mp 240–244°C; ^{1}H NMR (DMSO-d_6) δ = 11.20 (s, 1H, T-NH), 8.13 (s, 1H, A-2or8), 8.12 (s, 1H, A-2or8), 7.49 (s, 1H, T-6), 7.19 (s, 2H, NH2), 4.14 (t, 2H, J = 7 Hz), 3.58 (t, 2H, J = 7 Hz), 1.83 (quintet, 2H, J = 7 Hz), 1.74 (s, 3H), 1.60 (quintet, 2H, J = 7 Hz), 1.22 (quintet, 2H, J = 7 Hz); ^{13}C NMR (DMSO-d_6) δ = 164.17, 155.84, 152.25, 150.76, 149.46, 141.28, 140.71, 118.64, 108.30, 46.82, 42.56, 28.89, 27.78, 22.77, 11.79. Found: C, 54.28; H, 5.87; N, 29.30%. Calcd for $C_{15}H_{19}N_7O_2 \cdot 1/5\ H_2O$: C, 54.11; H, 5.87; N, 29.45%.

N^6-Methyl-9-[5-(thymin-1-yl)pentyl]adenine (2c). Mp 218–220°C; ^{1}H NMR (DMSO-d_6) δ = 11.19 (s, 1H, T-NH), 8.22 (s, 1H, A-2or8), 8.11 (s, 1H, A-2or8), 7.62 (s, 1H, A-NH), 7.47 (s, 1H, T-6), 4.15 (t, 2H, J = 7 Hz), 3.59 (t, 2H, J = 7 Hz), 2.97 (s, 3H, A-NMe), 1.83 (quintet, 2H, J = 7 Hz), 1.74 (s, 3H), 1.60 (quintet, 2H, J = 7 Hz), 1.22 (quintet, 2H, J = 7 Hz); ^{13}C NMR (DMSO-d_6) δ = 164.17, 154.93, 152.29, 150.77, 148.51, 141.27, 140.38, 119.09, 108.30, 46.83, 42.56, 28.93, 27.78, 26.91, 22.77, 11.81. Found: C, 55.96; H, 6.23; N, 28.81%. Calcd for $C_{16}H_{21}N_7O_2$: C, 55.96; H, 6.16; N, 28.55%.

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