Proton Nuclear Magnetic Resonance Study on the Aromatic Amino Acid–Guanine Nucleotide System: Effect of Base Methylation on the Stacking Interaction with Tyrosine and Phenylalanine

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The stacking interactions of tyrosine methylester (TyrOMe)–guanosine-5′-monophosphate (GMP), TyrOMe–7-methylguanosine-5′-monophosphate (m7GMP), phenylalanine methylester (PheOMe)–GMP and PheOMe–m7GMP pairs in neutral buffer solution have been studied by proton nuclear magnetic resonance (^{1}H-NMR). The H8 proton signal of GMP showed no noticeable temperature dependence, while the signals of other protons showed usual dependences arising from the ring stacking interaction with aromatic amino acids. The results can be interpreted in terms of the intramolecular C–H···O hydrogen bonding and ring stacking. Complex formations in 1:1 molar ratio were deduced for all pairs from their Job plots. The association constant for each pair was obtained by analysis of the Scatchard plot. Further, the van't Hoff plot provided thermodynamic parameters of the complex structure. The analyses of these data suggested that albeit the N-quaternization of GMP strengthens the stacking interaction with aromatic amino acid, the bulky methyl group in m7GMP facilitates the dissociation from the amino acid with small environmental change. The possible conformations of GMP and m7GMP in the interaction states are discussed on the basis of the coupling constants.

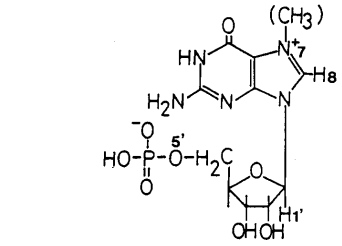
Keywords guanosine-5′-monophosphate; 7-methylguanosine-5′-monophosphate; tyrosine; phenylalanine; stacking interaction; association constant; thermodynamic parameter; ^{1}H-NMR

Introduction

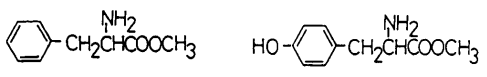
The study of interactions between amino acids and nucleotides is important for understanding the ability of a protein to recognize a specific base sequence of a nucleic acid. It is known that aromatic amino acids associate preferentially in a π–π stacking interaction with nucleic acid bases.[1] This interaction may be favorable for the specific recognition of nucleic acid base by amino acids, because π–π interaction, as well as hydrogen-bond pairing, can select the most suitable partners among various molecular combinations. However, this interaction force appears to be relatively weak, and consequently little clear-cut information is available concerning its importance in recognition,[2] compared with that of hydrogen-bonded pairing.

Recently we reported, based on X-ray crystallographic studies of nucleotide–aromatic amino acid complexes,[3-5] that marked reinforcement of the stacking interaction is caused by N-quaternization[6] of nucleic acid bases (especially purine base). This insight appears to be very important, because it means that the aromatic amino acids in a protein may be critical for the selective recognition of nucleic acid base sequences. Therefore, detailed investigation on the effect of nucleic acid base protonation upon the interaction with aromatic amino acids in the solution state would be useful.

As an extension of the foregoing proton nuclear magnetic resonance (^{1}H-NMR) study on the guanine–tryptophan system,[7] this paper deals with the guanosine-5′-monophosphate (GMP)–tyrosine (Tyr) and GMP–phenylalanine (Phe) interactions, and with the effect on their interactions of the N7-methylation of GMP (m7GMP). Chart 1 shows their chemical structures and the atomic numbering used.



GMP (m^7GMP)



PheOMe TyrOMe

Chart 1

Experimental

Materials m7GMP formate was synthesized by the methylation of GMP disodium salt according to the reported procedure.[7] All other materials used for this study were commercial products (reagent grade) and were used without further purification. Instead of Tyr and Phe, the methylester hydrochlorides (TyrOMe and PheOMe, respectively) were used for this study because of their higher solubilities in buffer solution.

^{1}H-NMR Measurements ^{1}H-NMR spectra were measured on a Varian XL-300 (300 MHz; Fourier-transform mode) spectrometer equipped with the temperature-control accessory (accuracy to ± 1 °C). Chemical shifts were measured from an internal standard, DSS (2,2-dimethyl-2-silapentane-5-sulfonate). The samples were lyophilized three times in 99.8% D_2O, and finally dissolved in deuterated 25 mM phosphate buffer (pD = 6.2—6.6). The sample concentrations were determined by measuring the dry weight and then adjusted to the desired values by dilution. The peak assignments were made by nuclear decoupling and by comparison of the spectra of related compounds.

Association Constants The association constant was obtained from Scatchard plots: the equilibrium equation for A and B molecules is defined by

$$A + B \rightleftarrows C$$

$$K = \frac{[C]}{[A][B]}$$

where C is the complex between A and B, and K the association constant

(in M^{-1}). For 1 : 1 complex formation, this K value takes the average value of the association constants of AB and BA complexes. Provided that $[A]_0$ (=[A]+[C]) and $[B]_0$ (=[B]+[C]) are the initial concentrations of A and B, this equation can be derived to the following Scatchard-type equation, according to the transformation described in ref. 7:

$$\frac{\Delta\sigma}{[B]_0} = -K(\Delta\sigma - \Delta\sigma_0)$$

when $[B]_0 \gg [A]_0$, $\Delta\sigma = \delta_0 - \delta$ and $\Delta\sigma_0 = \delta_0 - \delta_c$, where δ_0 and δ are the chemical shifts of a proton of A in the absence and presence of B, respectively, the δ_c is the chemical shift of the same proton of A completely complexed with B. The gradient of the $\Delta\sigma/[B]_0$ *vs.* $\Delta\sigma$ plot gives the association constant K. The 25—300 mM concentration range of TyrOMe or PheOMe (=B) was used with respect to 10 mM GMP or m7GMP (=A) for the present interaction study.[8]

Thermodynamic Parameters The thermodynamic parameters for the interaction can be obtained from a van't Hoff plot:

$$\ln K = -\frac{\Delta H}{RT} + \frac{\Delta S}{R}$$

$$\Delta G = -RT \ln K = \Delta H - T\Delta S$$

where ΔG, ΔH and ΔS are the changes of free energy, enthalpy and entropy accompanying the interaction, respectively, and R is the gas constant. For a constant value of ΔS, therefore, plots of ln K *versus* T^{-1} should be approximately linear with a slope of $-\Delta H/R$, provided that ΔH is independent of the temperature in the range of measurement.

Possible Sugar Puckerings of GMP and m7GMP The ribose puckering can be treated as a C2′-*endo*⇌C3′-*endo* equilibrium state. On this assumption, the population distribution of C3′-*endo* ribose puckering can be estimated from the widely accepted equation[9]:

$$\text{C3′-}endo\ (\%) = 100 \times \frac{J_{3'4'}}{J_{1'2'} + J_{3'4'}}$$

where the standard sums of 9.65 and 9.5 Hz for $J_{1',2'} + J_{3'4'}$ were used for GMP and m7GMP, respectively[10]; it was impossible to directly measure the $J_{3'4'}$ value, because of signal overlapping with other protons.

Results and Discussion

Temperature Dependences of GMP and m7GMP Chemical Shifts The temperature dependences of GMP and m7GMP proton chemical shift changes ($\Delta\sigma$) between the absence and presence of TyrOMe (0.075 M) are shown in Fig. 1; the H8 proton of m7GMP could not be monitored in the buffer solution because of the fast H–D exchange. Similar results have also been observed for PheOMe–GMP and m7GMP pairs. As is obvious from this figure, the H1′ and N-CH_3 protons of GMP and m7GMP experience noticeable upfield shifts in proportion to the decrease of temperature; the $-d\Delta\sigma/dT$ values are given in Table I. These changes, due to the ring current effects, imply the π–π stacking interaction of both molecules. On the other hand, the signal of the H8 proton of GMP showed an unusual temperature dependence. This can be interpreted as a result of the combination of (1) the breaking of a hydrogen bond in which the H8 proton participates and (2) the ring current effect of the aromatic amino acid. Factors (1) and (2) tend to increase and decrease the upfield shift of the H8 proton signal, respectively, as the temperature rises. The large $-d\delta/dT$ value of the GMP H8 proton in its isolated state reflects the predominant hydrogen bond formation at lower temperature. The C8–H8···O5′ intramolecular hydrogen bond seems most likely, judging from the possible conformations of GMP.[2] This unusual behavior of the H8 proton has also been observed in the self-stacking of GMP,[11] although such behavior was not observed in the GMP–tryptophan interaction.[7]

Stoichiometry of Nucleotide–Amino Acid Complex The stoichiometry of each complex pair was measured by plotting the ^1H chemical shifts of the nucleotide as a function of molar fraction{[nucleotide]/([nucleotide] + [amino acid])}, where the total concentration of the respective compounds is kept constant (Job plot). Figure 2 shows the Job plots for PheOMe–GMP and PheOMe–m7GMP pairs. The maximum of the Job plot occurs at 0.5 molar fraction for each pair, showing that a 1 : 1 complex is formed in each pair. Job plots for TyrOMe–GMP and –m7GMP (data are not shown) also indicated their complexes to have 1 : 1 molar ratios.

TABLE I. Temperature Coefficients of GMP and m7GMP Proton Chemical Shift Changes in the Absence and Presence of TyrOMe or PheOMe[a]

Proton		$-d\delta/dT$ (Hz/°C)	Proton		$-d\Delta\sigma/dT$ (Hz/°C)
GMP	H8	0.42	+TyrOMe	H8	−1.47
	H1′	0.11		H1′	6.50
			+PheOMe	H8	−2.59
				H1′	8.60
m7GMP	N-CH_3	−0.02	+TyrOMe	N-CH_3	6.87
	H1′	0.03		H1′	6.07
			+PheOMe	N-CH_3	8.99
				H1′	8.49

a) The concentrations of nucleotide and aromatic amino acid were 0.01 and 0.075 M, respectively.

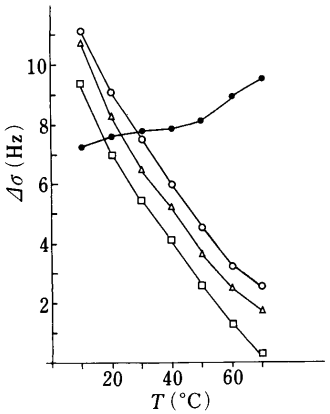


Fig. 1. Plots of $\Delta\sigma$ of GMP and m7GMP against Temperature

●—●, H_8 [GMP]; ○—○, N7-CH_3 [m7GMP]; △—△, H1′ [m7GMP]; □—□, H1′ [GMP].

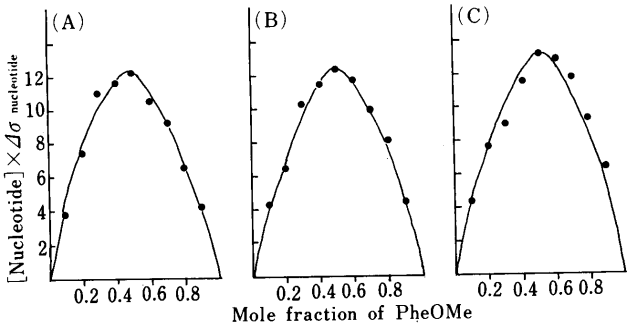


Fig. 2. Job Plots of Chemical Shifts Changes of GMP and m7GMP at 30 °C

(A) H1′ [m7GMP]; (B) N-CH_3[m7GMP]; (C) H1′ [GMP].

Association Constants between Nucleotide and Amino Acid The association constant (K) for a complex pair can be obtained by applying the Scatchard equation. Scatchard plots for the TyrOMe–m7GMP pair are exemplified in Fig. 3. The deviations of data from linearity were generally within experimental error (correlation coefficients >0.95); similar linearities were also observed for other pairs. The slope at 70 °C is about twice that at 50 °C. The Scatchard plot becomes somewhat imprecise at high temperature or at low concentration (25 or 50 mM) of TyrOMe because the data distribution falls within a limited range at high temperature, as is obvious in this figure, or because of the deviation from the assumption in deriving the Scatchard equation ([B]≫[A]). However, this result clearly shows that the complex at 50—70 °C is different from that at 10—50 °C, and the interaction mode in the former case causes $\delta_{H1'}$ to show a larger upfield shift than in the latter. The K and $\Delta\sigma_0$ at 30 °C, obtained by the least-squares fitting, are given in Table II; the H8 proton of GMP was not included because of its unusual temperature variation (see Fig. 1). These values appear to be reasonable, when they are compared with those determined by other methods; for example, the K value ($=2.486\,M^{-1}$) between Phe and guanosine obtained from a solubility experiment[12] is close to the present value of $2.0\,M^{-1}$ for the GMP–PheOMe pair.

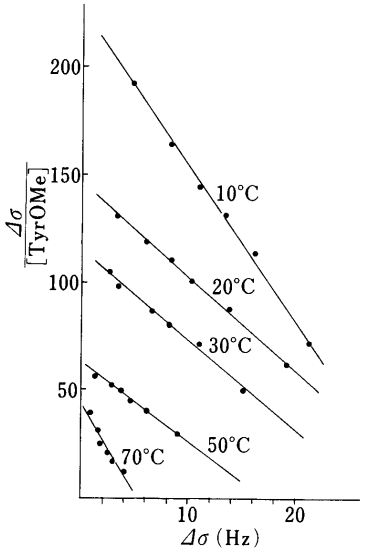


Fig. 3. H1′ Scatchard Plots for the m7GMP–TyrOMe Pair as a Function of Temperature

The concentration of TyrOMe was varied from 0.025 to 0.30 M in the presence of 0.01 M m7GMP.

TABLE II. Apparent Association Constant K and Extrapolated Upfield Chemical Shift $\Delta\sigma_0$ at 30 °C[a]

Pair	Position	K ($1\cdot M^{-1}$)	$\Delta\sigma_0$ (Hz)
m7GMP–PheOMe	N7-CH_3	3.6	40.0
	H1′	4.7	76.8
GMP–PheOMe	H1′	2.0	33.8
m7GMP–TyrOMe	N7-CH_3	4.1	32.2
	H1′	4.3	27.0
GMP–TyrOMe	H1′	3.3	32.8

a) The concentrations used were 0.01 M for m7GMP and GMP. The concentrations of PheOMe and TyrOMe were varied from 0.025 to 0.3 M. All data were computed by a linear least-squares fit, followed by error estimation. The mean error is 10% or less.

The K values for m7GMP are significantly larger than those of GMP, when the common H1′ proton for each pair is compared. A similar tendency has also been observed in the interaction with tryptophan.[7] This implies that the stacking interaction of GMP with aromatic amino acids is strengthened by the N7-methylation of the nucleic base. However, this difference between the K_s of GMP and m7GMP appears to be rather small (*ca.* 1.5—2 times larger for m7GMP, compared to GMP), if we take into account the drastic change of the interaction mode in the crystalline state, where the N-quaternization of the nucleic base causes a significant stacking interaction (including partial π–π charge-transfer in the ground state) with the aromatic amino acid, while the neutral base does not show such an interaction.[3,4] A more clearcut effect of the base quaternization in the interaction with aromatic amino acids, therefore, may be observed in a nonpolar solvent rather than in a polar aqueous one.

In the foregoing paper,[7] we reported the K values for tryptophan methylester (TrpOMe)–GMP and TrpOMe–m7GMP pairs, based on the H1′ proton, to be 15.8 and 21.8 M^{-1}, respectively. These values are much larger than the present ones. The present data show that there is no significant difference between Phe and Tyr molecules as regards interaction affinity with GMP or m7GMP. Consequently, the affinity of aromatic amino acids for these nucleotides is in the order of Trp≫Tyr≧Phe. This conclusion is somewhat different from the widely accepted one, that the stacking ability of aromatic amino acids with nucleic bases is in accordance with the π-donating ability (Trp>Tyr>Phe). The phenol oxygen atom of the Tyr side chain may not be so important for the interaction with nucleic base in the buffer solution, although nearly the same $\Delta\sigma_0$ values of the N7-CH_3 and H1′ protons in TyrOMe–m7GMP pair, compared with those in PheOMe–m7GMP pair, suggests that this phenol oxygen atom participates in keeping the aromatic ring at the center of the guanine base.

Thermodynamic Parameters for Complex Structure van't Hoff plots of $\ln K$ *versus* T^{-1} for the TyrOMe–m7GMP pair are exemplified in Fig. 4; similar profiles were also obtained for other pairs. These nonlinear profiles imply that ΔH and/or ΔS are not constants over the temperature range employed, and there may be several kinds of interactions. The van't Hoff profiles for all interaction pairs, as exemplified in Fig. 4, could be divided into two linear parts (10—50 °C and 50—70 °C), suggesting that the complex structure below 50 °C is different from that above 50 °C. The ΔH values above 50 °C are all in the

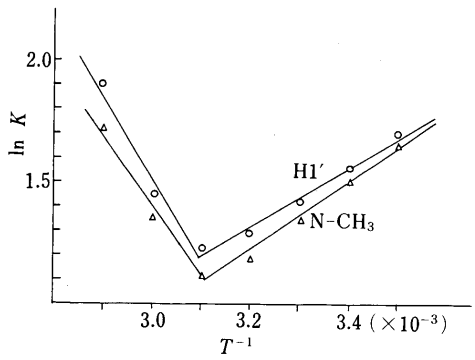


Fig. 4. van't Hoff Plots for the m7GMP–TyrOMe Pair

Table III. Thermodynamic Parameters for m7GMP–PheOMe, GMP–PheOMe, m7GMP–TyrOMe and GMP–TyrOMe Complex Structures (10—50 °C)[a]

Stacking pair	$-\Delta H$ (kcal·mol^{-1})	$-\Delta S$ (cal·mol^{-1}·K^{-1})	$-\Delta G$ (kcal·mol^{-1})[b]
m7GMP–PheOMe	3.0	6.8	0.94
m7GMP–TyrOMe	2.8	6.7	0.77
GMP–PheOMe	9.6	30.1	0.48
GMP–TyrOMe	5.8	16.9	0.68

a) The mean error is 15% or less. b) The values are those at 30 °C.

positive range (1—8 kcal/mol). This indicates that the complex structures above 50 °C are energetically unstable, compared with the unstacked and isolated ones, and are not highly likely to be formed under physiological conditions. Therefore, the thermodynamic parameters for complex structures below 50 °C, obtained from the least-squares fit of the van't Hoff plots, are listed in Table III, where the data for the m7GMP–TyrOMe and m7GMP–PheOMe pairs are averaged values obtained from H1′ and N-CH_3 protons. When these data are considered in relation to the complex structures, the values of $-\Delta H$, $-\Delta S$ and $-\Delta G$ could be closely related to the difference between the energies of stacked and unstacked structures, the order of complex structure and the ease of the stacking formation, respectively. The present values, along with the K values listed in Table II, suggest that albeit N7-methylation of GMP strengthens the stacking interaction with aromatic amino acids (K and $-\Delta G$), the existence of a bulky methyl group disturbs the energetic stability of the complex structure ($-\Delta H$ and $-\Delta S$). In other words, m7GMP, compared with GMP, is more liable to dissociate from Tyr or Phe in response to a small environmental change, though both molecules readily associate with each other. The same conclusion has also been reached for the interaction with tryptophan.[7] Further, a comparison of its thermodynamic parameters with the present ones allows us to conclude that the stability of complex structure is in the order of

$$\text{Trp} \gg \text{Phe} \geqq \text{Tyr}$$

Possible GMP and m7GMP Conformations The stability of complex structure is largely dependent on the conformations of the interacting molecules. The ribose puckerings of GMP and m7GMP, as well as the orientations about the glycosyl and *exocyclic* C4′–C5′ bonds, appear to significantly affect the interaction modes. Therefore, it is of interest to investigate the possible conformations of GMP and m7GMP in the interacting state with TyrOMe or PheOMe.

The possible sugar puckerings (%), estimated from the $J_{1'2'}$ coupling constant, are given in Table IV. As is clear from this table, m7GMP prefers to take a C3′-*endo* ribose puckering rather than a C2′-*endo* one, while GMP shows a reverse preference. Neither the ribose puckering of m7GMP nor that of GMP is significantly affected by the interactions with aromatic amino acids. Judging from these data, N7-methylation of GMP appears to cause a significant shift in the ribose pucker toward the C3′-*endo* form, and this is in accordance with the conclusion of Kim and Sarma.[10] On the other hand, the nucleotide conformation is further characterized by the orientations about the glycosyl and C4′–C5′ bonds. As was already stated, the unusual temperature dependence of the GMP H8 proton could be interpreted in terms of participation in the C8–H8···O5′ hydrogen bond. The formation of this intramolecular hydrogen bond leads GMP to take *anti* and *gauche*, *gauche* orientations about the glycosyl and C4′–C5′ bonds, respectively. Similarly, the *anti–gauche*, *gauche* preference of m7GMP has also been suggested from many spectroscopic[7,10] and X-ray crystallographic[4,5] studies. Therefore, the difference between the ribose puckerings of m7GMP and GMP, in addition to the N7-methylation, affects the association constant and thermodynamic parameters for the interaction with aromatic amino acids.

Table IV. Coupling Constants and Sugar Puckering Populations at 30 °C[a]

Compound	$J_{1'2'}$ (Hz)	Ribose C3′-*endo* (%)	Compound	$J_{1'2'}$ (Hz)	Ribose C3′-*endo* (%)
m7GMP	3.8	60	GMP	6.3	35
+0.025 M PheOMe	3.9	59	+0.025 M PheOMe	5.8	40
+0.1 M PheOMe	3.5	63	+0.1 M PheOMe	6.2	36
+0.3 M PheOMe	3.5	63	+0.3 M PheOMe	6.1	37
+0.025 M TyrOMe	3.8	60	+0.025 M TyrOMe	5.9	39
+0.1 M TyrOMe	3.8	60	+0.1 M TyrOMe	5.9	39
+0.3 M TyrOMe	3.8	60	+0.3 M TyrOMe	5.7	41

a) The standard error is ±0.3 Hz.

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