Structural Studies of the Interaction between Indole Derivatives and Biologically Important Aromatic Compounds. Part 19.[1] Effect of Base Methylation on the Ring-stacking Interaction between Tryptophan and Guanine Derivatives: a Nuclear Magnetic Resonance Investigation

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Stacking interactions between tryptophan methyl ester (TrpOMe) and guanine derivatives (base, nucleoside, and nucleotide of neutral and N-7-methylated guanines) in neutral aqueous solution have been studied by ^{1}H n.m.r. The 8-H protons of methylated guanine derivatives undergo a downfield shift in the presence of TrpOMe, while the other aromatic protons of guanine derivatives shift upfield due to the stacking interaction between the indole and guanine rings. This is interpretable in terms of ionic interactions between the electron positive 8-H and the π-electron-rich indole ring. Job plots show 1 : 1 complex formations in all guanine derivative–TrpOMe pairs. Association constants determined for the dependence of the ^{1}H chemical shifts (guanine derivative) as a function of TrpOMe concentration show that the stacking interaction with the indole ring is strengthened by methylation of N-7 of the guanine base. The measurements of thermodynamic parameters, however, show that methylation hinders the formation of a stable stacking structure with TrpOMe in the case of guanine nucleotide. These results imply that the methylated guanine nucleotide, compared with the unmethylated one, is more liable to dissociate from tryptophan with small environmental change, though both molecules easily associate with each other. A conformational difference between the unmethylated and methylated guanine derivatives in the interaction with TrpOMe is also discussed, based on the coupling constant and spin–lattice relaxation time measurements.

The mutual recognition between protein and nucleic acid is a specific and fundamental step in the expression of a cellular genome. The interaction between their component molecules plays a key role in specific recognition processes. Much work has suggested that different types of interaction are responsible for the binding between amino acids and nucleotides; they can principally be classified as hydrogen bonding, stacking, electrostatic, and hydrophobic interactions.[2] Among them, the former two interactions are especially important in the selective binding of amino acid to nucleotide, because the relative orientation between the interacting species is strictly fixed by these interactions.

It is well known that among the aromatic amino acids, tryptophan, with an indole ring, associates most preferentially in a π–π stacking interaction with nucleic acid bases,[2] because of the strong π-electron-donating character of the indole ring.[3] Although an interaction takes place between the indole ring and the nucleic acid base (especially purine base), and is dominated by various forces such as electrostatic, dipole–dipole and molecular orbital interactions,[4–6] it is largely affected by the side groups of tryptophan and the nucleotide. Therefore a comparative study of tryptophan–nucleic acid base, –nucleoside, and –nucleotide interactions illuminates the role of tryptophan residue in protein–nucleic acid interactions.

Recently, the biological implication of nucleic acid methylation has been discussed in the relation to the control of gene expression.[7] Previous *X*-ray diffraction studies showed that the methylation of adenine and guanine bases resulted in a more prominent stacking formation with aromatic amino acids.[6,8–10] Generally the stacking formation arises from interaction between the π-orbitals of two aromatic rings, particularly between the highest occupied molecular orbital (HOMO) of the donor ring (indole) and the lowest unoccupied one (LUMO) of the acceptor ring (nucleic base). The CNDO/2 molecular orbital calculations showed that the quaternization of the nucleic acid base nitrogen atom by protonation in acidic conditions or by methylation, lowers the LUMO energy of the base [for example, 0.1363 atomic unit (guanine) ⟶ −0.0903 a.u. (guaninium)], and consequently strengthens the interaction with the HOMO of the aromatic ring of the amino acid (−0.3996 a.u. for an indole ring). Therefore it is of interest to know to what extent the stacking interaction of tryptophan with nucleotide is affected by base methylation, and also the function of the aromatic amino acid in protein-methylated nucleic acid interaction.

This paper deals with the interaction between tryptophan and unmethylated and N-7-methylated guanine derivatives studied by ^{1}H n.m.r. The formulae of the compounds used are shown in Figure 1. This study will further provide some insights as to the interaction between the 'cap' structure of mRNA and the aromatic amino acids of cap binding protein, because the N-7-methylated guanine base constitutes part of the cap structure, m7GpppN(m), of most eukaryotic viral and cellular mRNAs,[11] and competitively inhibits binding of mRNA to cap binding protein.[12,13]

Experimental

Materials.—Instead of tryptophan, tryptophan methyl ester (TrpOMe) hydrochloride (Protein Research Foundation, Osaka) was used for this study because of its higher solubility in water at neutral pH. All other materials used for syntheses and spectroscopic measurements were commercial (reagent grade) and used without further purification.

Syntheses of N-7-Methylated Guanine Derivatives.—m7GMP *formate.* m7GMP was synthesized by the direct methylation of disodium GMP with dimethyl sulphate.[14] To a solution of GMP·2Na (2.81 g) in water (200 ml), adjusted to pH 5 (acetic acid), was added dimethyl sulphate (4.7 ml), with stirring for 4 h at room temperature while maintaining the pH at 5 (by 0.5M-NaOH). The solution was then washed three times with ether

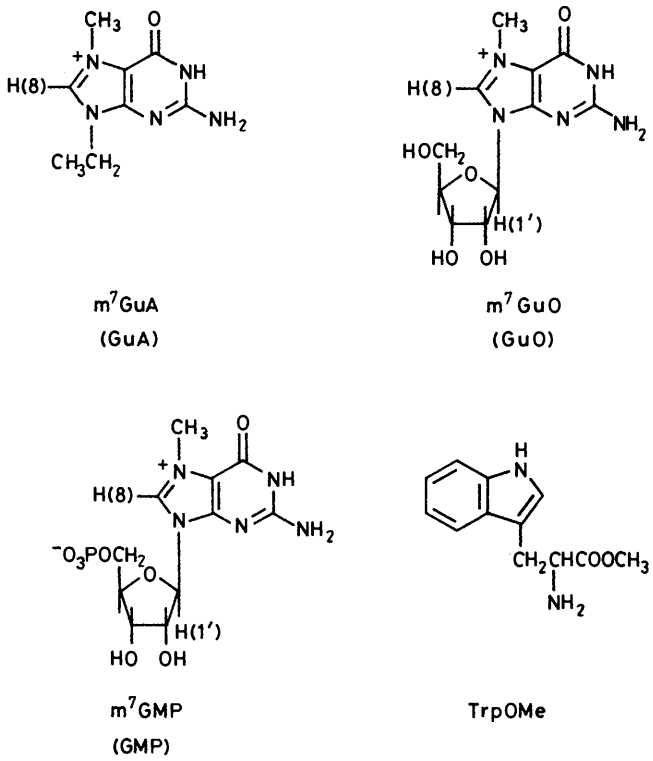

Figure 1. Formulae of compounds used. Formulae of GuA, GuO, and GMP shown in parentheses correspond to the ones without methyl group at N-7. The following abbreviations are employed: GuA, 9-ethylguanine; m7GuA, 7-methyl-9-ethylguanine; GuO, guanosine; m7GuO, 7-methylguanosine; GMP, guanosine 5′-monophosphate; m7GMP, 7-methylguanosine 5′-monophosphate; TrpOMe, L-tryptophan methyl ester

(900 ml) to remove unchanged dimethyl sulphate, adjusted to pH 8 (0.5M-NaOH), and applied to a column (2.5 × 18 cm) of anion exchange resin (Dowex 1 × 8, formate form). After washing the column with water, elution was performed with a linear gradient from 0 to 0.01M-HCOOH. The fractions containing the product were evaporated to dryness: mobility relative to AMP 0.65 in paper electrophoresis (50mM-phosphate buffer, pH 7.0); $\lambda_{max.}$ (25mM-phosphate buffer, pH 6.8) 258 nm, ε 10 300.

m7GuO *Iodide.* m7GuO was synthesized by the methylation of GuO with methyl iodide.[15] GuO·2H_2O (10.0 g) was dissolved in *NN*-dimethylacetamide (100 ml) containing CH_3I (10.0 g), stirred for 30 h at 30 °C, and then filtered through a thin layer of Celite (2 g). Ethanol (500 ml) and light petroleum (1 200 ml) were added to the filtrate to effect precipitation of an oil. The solid was collected and washed with acetone (300 ml) to give a crystalline product, R_F 0.32 (7:3 EtOH–H_2O), $\lambda_{max.}$ (25mM-phosphate buffer) 258 nm (ε 10 700).

m7GuA *Iodide.* 9-Ethylguanine was synthesized according to the procedure of Browne *et al.*[16] m7GuA was prepared from 9-ethylguanine and CH_3I as in the preparation of m7GuO, R_F 0.28 (7:3 EtOH–H_2O), $\lambda_{max.}$ (25mM-phosphate buffer) 283 nm (ε 7 400).

^{1}H *N.m.r. Measurements.*—^{1}H N.m.r. 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 with DSS (2,2-dimethyl-2-silapentane-5-sulphonate) as internal standard. The samples were lyophilized three times in 99.8% D_2O, and finally dissolved in deuteriated 25mM-phosphate buffer (pD 6.2—7.2). The sample concentrations were determined by dry weight and then adjusted to the desired values by dilution using a volumetrically variable pipette 4710 (Eppendorf). For the measurements of spin–lattice relaxation time (T_1) the samples were completely deoxygenated with a nitrogen gas purge. The assignments of the various resonances were made by nuclear decoupling as well as by comparison of the spectra from various derivatives.

Table 1. Concentration dependence of chemical shift change for 8- and 1′-H (in Hz) of guanine derivatives at 30 °C[a]

Concentration		0.002M	0.006M	0.010M	0.014M	0.018M
m7GMP	N-CH_3	0.0	0.6	0.3	0.3	0.3
	1′-H	0.3	1.2	1.8	2.4	3.0
GMP	8-H	0.0	0.6	0.9	1.2	1.8
	1′-H	0.0	1.2	1.8	2.1	3.0
m7GuO	N-CH_3	0.0	−0.6	−0.9	−0.6	−0.9
	1′-H	0.0	0.9	1.5	2.4	2.4
GuO	8-H	0.0	0.6	0.6		
	1′-H	0.0	0.6	0.6		

[a] The listed values show the difference of each proton from that at infinite dilution. Error is ±0.3 Hz.

Association Constant.—For 1:1 complex formation by two interacting molecules A and B the apparent association constant K is described by equation (1):

$$K = [AB]/[A][B] \quad (1)$$

Provided that (1) A_0 and B_0 are the initial concentrations of A and B, (2) B_0 is always much larger than A_0, and (3) p is the fraction of A which is complexed, equation (1) can be rewritten as (2). The p value can be estimated from the chemical shifts of

$$K = \frac{pA_0}{(A_0 - pA_0)(B_0 - pA_0)} = \frac{p}{(1-p)(B_0 - pA_0)} \quad (2)$$

two interacting molecules in n.m.r. spectroscopy [equation (3)]

$$p = (\sigma - \sigma_0)/(\sigma_c - \sigma_0) \quad (3)$$

where σ_0 and σ are the chemical shifts of a proton of molecule A in the absence and presence of compound B, respectively, and σ_c is the chemical shift of the same proton of molecule A completely complexed with molecule B.

Equations (2) and (3) can be combined to give (4) (Scatchard equation) for [B] ≫ [A], where $\Delta\sigma = \sigma - \sigma_0$ and $\Delta\sigma_0 = \sigma_c - \sigma_0$.

$$\Delta\sigma/B_0 = -K(\Delta\sigma - \Delta\sigma_0) \quad (4)$$

As reported by Dimicoli *et al.*,[17] two binding modes can be considered in the 1:1 complex formation between A and B molecules [reaction (5) where two binding sites exist on molecule A with the same association constant k]. This treatment would be important in the stacking interaction, because complex BA is different from AB. Equation (4) can therefore be rewritten as (6). A plot of $\Delta\sigma/B_0$ against $\Delta\sigma$ should

$$BA \underset{k}{\rightleftharpoons} A + B \underset{k}{\rightleftharpoons} AB \quad (5)$$

$$\frac{\Delta\sigma}{B_0} = -2k\left(\Delta\sigma - \frac{\Delta\sigma_{AB} + \Delta\sigma_{BA}}{2}\right) \quad (6)$$

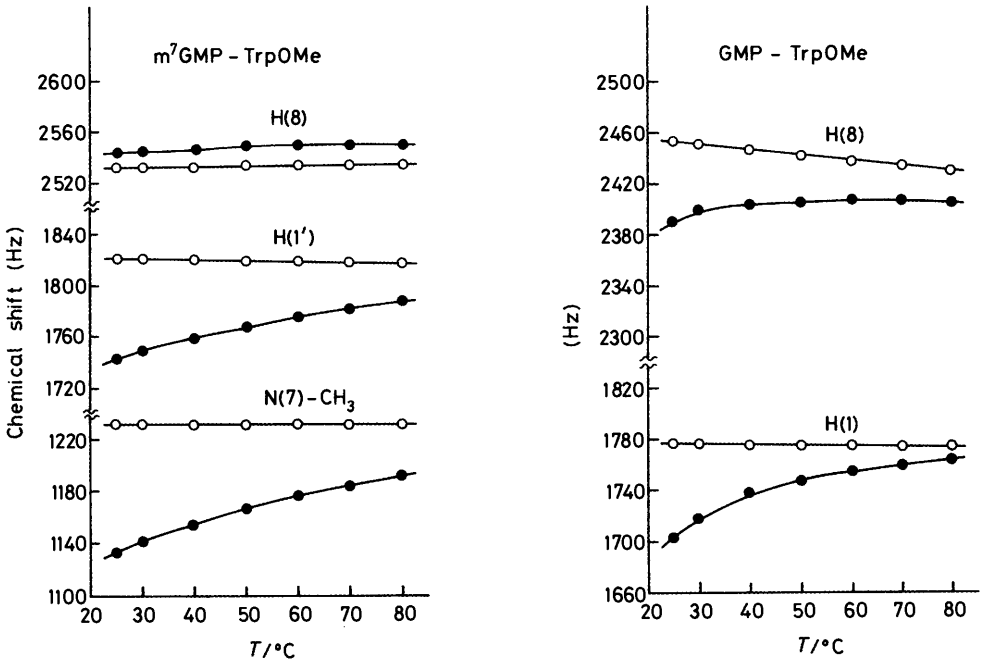

Figure 2. Variable-temperature plot of chemical shifts of m7GMP and GMP (0.01M) in the absence (○) and presence (●) of TrpOMe (0.25M)

give a straight line, and hence K could be obtained from the slope and $\Delta\sigma_0[=(\Delta\sigma_{AB} + \Delta\sigma_{BA})/2]$ from the intercept.

The chemical shift data were analysed during an NEC 9801 personal computer: the observed variation of the chemical shift with concentration or temperature was fitted to an equation using the least-squares method.

Results and Discussion

Concentration Dependence of Guanine Derivatives.—It is well known that the guanosine moiety is unusual among nucleic acid bases in its ability to self-associate in aqueous solution into highly stable, regular structures.[18] In order to study the interaction between the guanine derivative and tryptophan exactly, therefore, it is important to determine the concentration of guanine derivative which does not participate in self-association. The resonances of base (8-H for unmethylated guanines and 7-CH_3 for methylated derivatives) and sugar (1'-H) were used as probes for studying the concentration dependence. The difference in the chemical shift at each concentration (0.002—0.018M) referenced to that at infinite dilution is listed in Table 1; the exact differences could not be measured for m7GuA and GuA molecules because of their low solubilities ($\leq$0.005M), but their concentration dependence was much smaller than their nucleosides and nucleotides. All protons, except 7-CH_3 of m7GuO, shift slightly upfield when the concentration of guanine derivative is increased, indicating that stacking is the predominant mode of self-association. When these changes were compared with those observed at higher concentrations ($\geq$0.2M) at which the highly ordered association predominantly takes place,[18,19] however, it appears that most of each guanine derivative behaves as an unordered molecule. Therefore studies on the interaction with tryptophan were made at a relatively low and constant concentration: 0.01M for m7GMP, GMP, and m7GuO, 0.05M for GuO, and 0.001M for m7GuA and GuA. The corrections for self-association were based on the data of Table 1. On the other hand, the self-association of TrpOMe was apparently small relative to that of the guanine derivatives and concentrations up to 0.30M were used.

Temperature Dependence of Guanine Derivatives in the Presence of TrpOMe.—Plots of chemical shifts of guanine derivatives *versus* temperature in the absence and presence of TrpOMe are shown in Figure 2. This Figure indicates that the protons of guanine derivatives, except 8-H of 7-methylated ones, experience large upfield shifts in proportion to the decrease of temperature. Since chemical shifts in the absence of TrpOMe are almost constant, the changes could be interpretable as the ring current effect due to the stacking interaction between the guanine and indole rings. Furthermore, the fairly large shift changes, compared with those in self-association (see Table 1), imply that the majority of guanine derivatives experience a stacking interaction with TrpOMe ($\geq$95%). On the other hand, the 8-H 7-methylated guanine derivatives undergo a downfield shift in the presence of TrpOMe, and the shift changes are essentially independent of the temperature variation. It has been reported that a part of 8-H of GMP·2Na undergoes a downfield shift accompanying self-stacking formation, and the downfield shift is attributable to the electrostatic effects caused by a doubly ionized phosphate group in the vicinity of the 8-H.[20] Similarly, the present downfield shifts observed only for 7-methylated guanine derivatives may be interpretable as electrostatic effects, although the behaviour of the 8-H chemical shift is mainly dominated by the charge on N-7; since 7-methylation of guaine base increases the electron positivity of 8-H [−0.011 (in electronic units) for guanine and 0.075 for 7-methylguaninium, calculated by CNDO/2 method], it could be supposed that the proximity of the π-electron-rich indole ring causes an ionic interaction of 8-H with the indole ring, as a result of stacking formation. Consequently 8-H behaves like a 'salt' without temperature dependence. The TrpOMe concentration dependence of the 8-H shift shown in Figure 3 also suggests this interpretation.

Stoicheiometry of Guanine Derivative–Tryptophan Complex.—The stoicheiometry of each complex pair was obtained by plotting the ^1H chemical shift of the guanine derivative as a function of molar fraction {[guanine derivative]/([guanine derivative] + [TrpOMe]}, the total concentration of the respective compounds being constant (Job plot).[21] Figure 4

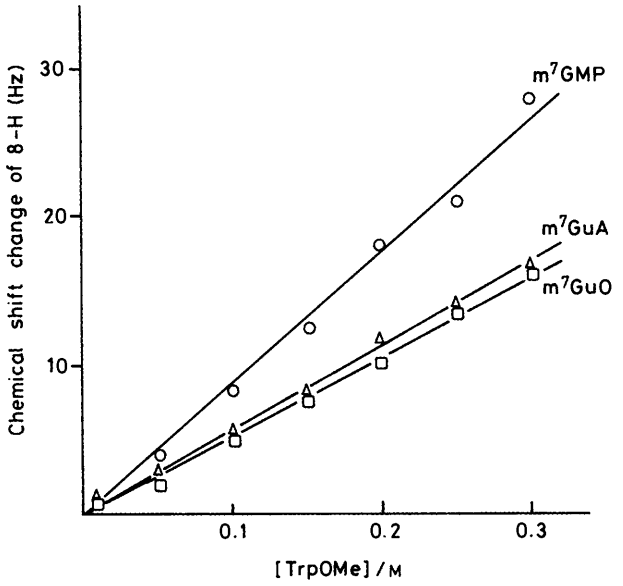

Figure 3. Effect of TrpOMe concentration on the downfield shifts of 8-H of m7GMP (○), m7GuO (□), and m7GuA (△) at 30 °C. Concentrations of 0.01M were used for m7GMP and m7GuO and 0.005M for m7GuA. In the case of m7GuA, the changes are half the observed ones in order to make a comparison with the other values

shows Job plots for m7GMP–, GMP–, m7GuO–, and GuO–TrpOMe pairs. The maximum of the Job plot occurs at 0.5 molar fraction of each guanine derivative–TrpOMe pair. A 1:1 complex is therefore formed in these pairs. Job plots for m7GuA– and GuA–TrpOMe could not be obtained exactly because of their low solubilities. However, these complex pairs also appear to have 1:1 stoicheiometry, judging from the fact that the $\Delta\sigma \times$ concentration values of m7GuA and GuA were largest when the molar fractions of the respective pairs were *ca.* 0.5. *X*-Ray analysis of a related complex has also suggested formation of a 1:1 complex between guanine base and tryptophan.[8]

Association Constant between Guanine Derivative and TrpOMe.—The association constant K and $\Delta\sigma_0$ for each pair was obtained using equation (4). Figure 5 shows Scatchard plots for m7GMP– and GMP–TrpOMe. The deviations of the data for linearity were generally within experimental error, indicating molecular association with 1:1 stoicheiometry. Similar linearities were observed for other pairs. The K and $\Delta\sigma_0$ values obtained by least-squares treatment (correlation coefficients >0.95) are given in Table 2, where 8-H shifts of 7-methylated guanine derivatives could not be used because of their abnormal behaviour in the presence of TrpOMe. The apparent association constants, 17.7 and 15.8 l mol^{-1}, observed for 8- and 1′-H of GMP, correspond to the microscopic k values of 8.9 and 7.9 l mol^{-1}, which are obtained from equation (6). These constants are in good agreement with those published by Wagner *et al.*,[22] 9.0 and 7.6 l mol^{-1} for GMP–tryptamine at 37 °C and pD 7.9, indicating the validity of the data in Table 2. These show that the apparent association constant of 7-methylated guanine derivatives are significantly larger than those of unmethylated ones when their 1′-H or CH_2 protons are compared with each other, and the differences in the concentration used are taken into account. These results clearly support our hypotheses, *i.e.* the stacking interaction with the indole ring is strengthened by 7-methylation of the guanine base. Although the difference between the association constants of the methylated and unmethylated guanine derivatives in solution would be rather small (*ca.* 1.5 times larger for the methylated guanine derivatives than for the unmethylated ones), this difference appears to influence significantly the interaction mode in the crystalline state; preliminary *X*-ray

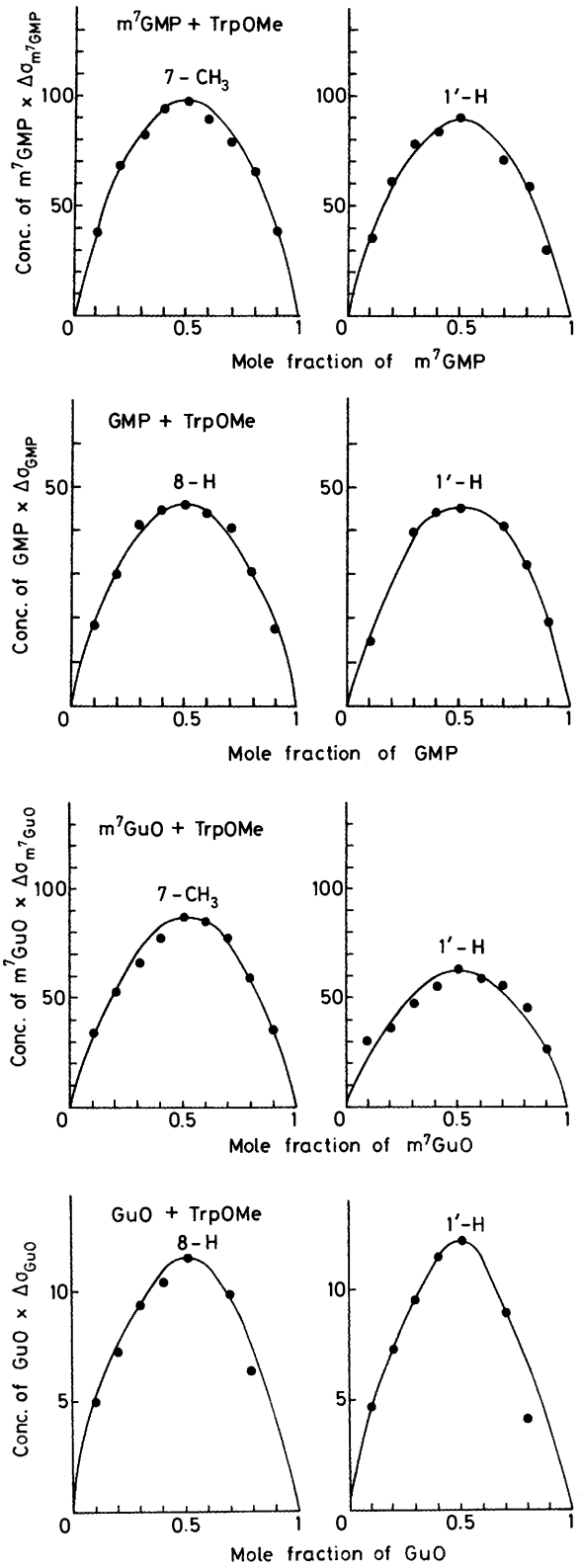

Figure 4. Job plots of chemical shifts (upfield) of guanine derivative protons at 30 °C

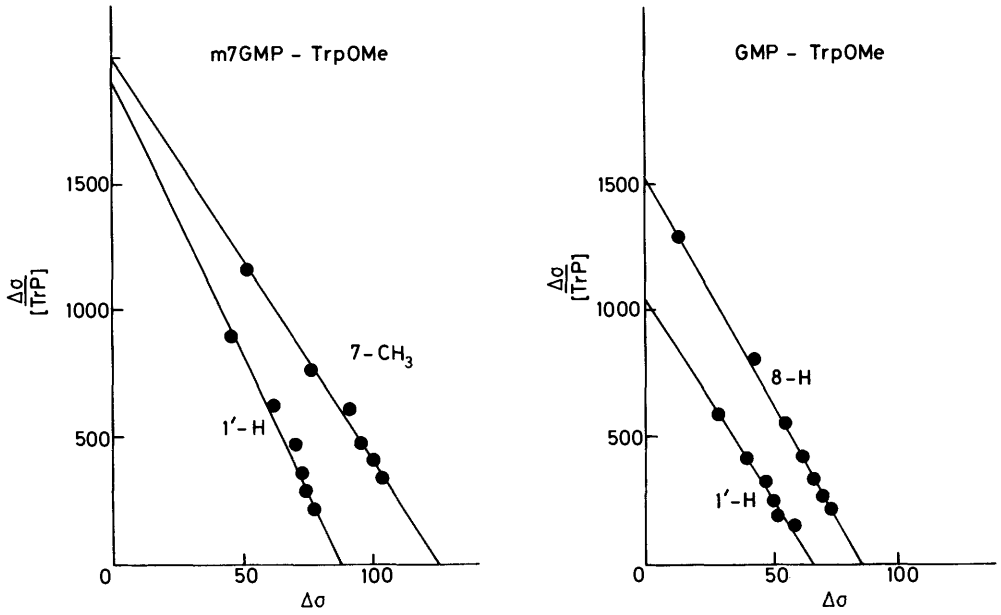

Figure 5. Scatchard plots of m7GMP–TrpOMe and GMP–TrpOMe pairs. The concentration of TrpOMe was varied from 0.05 to 0.30M in the presence of 0.01M-m7GMP or GMP at 30 °C

Table 2. Apparent association constants K and extrapolated upfield chemical shift changes ($\Delta\sigma_0$) for guanine derivative–TrpOMe complexes at 30 °C[a]

Guanine derivative	Position	K/l mol^{-1}	$\Delta\sigma_0$/Hz
m7GMP	7-CH_3	15.9	125.7
	1′-H	21.8	87.5
GMP	8-H	17.7	85.7
	1′-H	15.8	65.9
m7GuO	7-CH_3	5.9	131.9
	1′-H	8.0	70.5
GuO	8-H	5.0	79.3
	1′-H	7.0	62.1
m7GuA	7-CH_3	10.9	133.8
	CH_2	14.3	113.4
GuA	8-H	3.9	201.9
	CH_2	4.8	177.4

[a] The concentrations used were 0.01M for m7GMP, GMP, and m7GuO, 0.005M for GuO, and 0.001M for m7GuA and GuA. The concentrations of TrpOMe was varied from 0.05 to 0.30M. All data were computed by a linear least-squares fit followed by an error estimation. The mean error is 10% or less; for the data for GuA and m7GuA, the error is up to 20%.

Table 3. Thermodynamic data for complexes of TrpOMe and guanine derivatives[a]

Compound	$-\Delta H$/ kcal mol^{-1}	$-\Delta S$/ cal mol^{-1} K^{-1}	$-\Delta G^b$/ kcal mol^{-1}
m7GMP	9.1	24.6	1.8
GMP	12.0	34.7	1.6
m7GuO	11.4	31.6	1.9
GuO	10.7	31.4	1.3
m7GuA	9.0	24.0	1.9
GuA	5.7	15.7	1.1

[a] The mean error is 15% or less; for the data concerning m7GuA and GuA, the error is 25 and 30%, respectively. [b] The values correspond to those at 25 °C.

results have shown prominent stacking formations in the crystal structures of indole–7-methylated guanine pairs,[8,9] while such an interaction has not yet been reported in the case of unmethylated guanine derivatives. A comparison of the association constants of 8-H or 7-CH_3 indicated that the affinity of guanine derivatives to tryptophan decreases in the order m7GMP > m7GuA > m7GuO for methylated guanines and GMP > GuO > GuA for unmethylated ones. The existence of a phosphate group results in a stacking interaction with tryptophan whether or not 7-methylation has occurred. This is probably due to electrostatic and/or hydrogen-bond interactions between the tryptophan side chain and the phosphate group. On the other hand, 7-methylation would hinder the tryptophan–nucleoside interaction, considering that m7GuA > m7GuO for 7-methylated compounds and GuO > GuA for unmethylated ones. A 7-methyl group, because of its bulk, may reduce the contribution of factors such as the hydrogen bonds formed between tryptophan and the guanosine sugar moiety.

The $\Delta\sigma_0$ values, which are the chemical shift changes in the completely complexed state, reflect the stacking mode of both molecules: the shifts are dependent on the distances from the centre of the indole ring as shown by the theoretical calculations.[2,3] Although it is impossible to figure out the whole stacking mode only from the values listed in Table 2, the larger values of 7-CH_3 protons than those of 1′-H or CH_2 in the same pairs imply that the former groups are located closer to the centre of the indole ring. On the other hand, the relatively larger $\Delta\sigma_0$ values of guanine base than for the nucleosides and nucleotides, on the whole, reflect the easily accessibility of the base to the indole ring.

Thermodynamic Parameters for Stacking Interactions.—The thermodynamic parameters for the stacking interactions can be obtained by equations (7) and (8), where ΔG, ΔH, and ΔS are

$$\Delta G = -RT\ln K = \triangle H - T\Delta S \quad (7)$$

$$\ln K = -\Delta H/RT + \Delta S/R \quad (8)$$

Table 4. Coupling constants[a] sugar puckering populations and spin–lattice relaxation time (T_1)[b] of m7GMP and GMP at 30 °C

Compound	$J_{1'2'}$/Hz	Ribose C-3′-*endo* (%)	$(T_1)_8$/s	$(T_1)_{1'}$/s	$(T_1)_8:(T_1)_{1'}$
0.01M-m7GMP	3.9	60	27.3	3.3	8.3
+0.01M-TrpOMe	3.6	63	16.4	2.7	6.0
+0.25M-TrpOMe	3.5	64	5.3	2.0	2.7
0.01M-GMP	5.8	40	1.3	3.0	0.4
+0.01M-TrpOMe	5.7	41	0.7	2.0	0.4
+0.25M-TrpOMe	5.7	41	0.5	1.7	0.3

[a] Estimated error is ±0.3 Hz. [b] Measured with the pulse Fourier transform mode by applying a 180°—τ—90° pulse sequence. Estimated error is 0.6 s.

the free energy, enthalpy, and entropy of interaction, respectively, and R is the gas constant. For a constant value of ΔS, therefore, plots of $\ln K$ *versus* T^{-1} (van't Hoff plot) should be approximately linear with a slope of $-\Delta H/R$, provided that ΔH is independent of the measured temperature range. These thermodynamic parameters, obtained from least-squares fits of van't Hoff plots, are listed in Table 3, where the data are the averaged values obtained from the respective protons. The values of ΔH, ΔS, and ΔG are closely related to the magnitude of the force of ring stacking formation, the order of the stacking structure, and the difference in energy between the unstacked and stacked states, respectively. Among them, ΔH and ΔS values show the following tendency with respect to the structural stability of TrpOMe–guanine derivatives: GMP > m7GMP; m7GuO = GuO; m7GuA > GuA. This would imply that though 7-methylation stabilizes the stacking structure between the indole and guanine rings energetically, this effect makes the interaction rather unstable in the case of the nucleoside and nucleotide. Further, the data show the following tendency for structural stability: m7GuO > m7GMP > m7GuA for methylated guanines and GMP > GuO > GuA for unmethylated guanines. These orders are not necessarily proportional to the K values; for example, the large K and small ΔH values of m7GMP–TrpOMe, compared with those for GMP–TrpOMe, would imply that m7GMP is more liable to dissociate from tryptophan with a small environmental change, though both molecules easily associate with each other.

Conformations of GMP *and* m7GMP *in the Stacking Interaction with Tryptophan.*—The measurement of apparent association constants and thermodynamic parameters provides an interesting insight: albeit 7-methylation of guanine base strengthens the stacking interaction with tryptophan, the existence of a methyl group disturbs the tight binding in GMP–tryptophan. This means that the interactions between the sugar and phosphate groups of GMP and the side-chain of tryptophan are unco-operative with the stacking interaction strengthened by methylation. Therefore, it is interesting to investigate to what extent the conformation of GMP is affected by methylation in its interaction with tryptophan.

The ΔS value is a useful parameter to elucidate, particularly in this system, as it indicates whether there are marked conformational or solvation effects occurring on complexing. The small value of $-\Delta S$ for m7GMP–TrpOMe, compared with that for GMP–TrpOMe, indicate that m7GMP assumes an unfavourable conformation by interaction with TrpOMe. Since the predominant conformations of GMP and m7GMP in aqueous solution have already been reported,[24,25] the conformations of these molecules in the presence of TrpOMe were analysed according to the usual method. Unfortunately the extensive overlapping of aliphatic protons in GMP and m7GMP with those of the TrpOMe side-chain made conformational analysis around the exocyclic C-4′–C-5′ bond impossible. The $J_{1'2'}$ and the spin–lattice relaxation times (T_1) of 8- and 1′-H protons are listed in Table 4.

The puckering of the ribose ring can be assessed by assuming a C-2′-*endo*(^2E) $\rightleftharpoons$ C-3′-*endo*(^3E) equilibrium. In order to estimate the population distribution of the ^3E puckering, the magnitudes of $J_{1'2'}$ and $J_{3'4'}$, and the sum $(J_{1'2'} + J_{3'4'})$ are important{$^3E(\%) = 100 \times [J_{3'4'}/J_{1'2'} + J_{3'4'})]$},[26] because the former two coupling constants are sensitive to sugar puckering, and the summation has a constant value. According to the method of Kim *et al.*[25] the population of ^3E puckering was calculated using $J_{1'2'} + J_{3'4'} = 9.65$ Hz. These results are also given in Table 4. As already stated,[25] 7-methylation of GMP causes a shift in ribose pucker toward the ^3E conformation. It is worth noting that TrpOMe causes a modest increase in the ^3E population in m7GMP, while no significant effect is observed in GMP. From the thermodynamic parameters, it seems reasonable to consider 7-methylation, as well as the interaction witn tryptophan, increases the energetically unstable ^3E conformer of GMP.

In nucleosides and nucleotides, stable conformations about the glycosyl bond are found in both the *anti* and *syn* ranges. Measurement of T_1 for 8- and 1′-H allows the conformation about the glycosyl bond to be estimated:[27] the ratio $(T_1)_8:(T_1)_{1'}$ is larger for the *syn* than the *anti* conformation. The $(T_1)_8$ value of m7GMP showed an abnormally large value. This is due to the 'salt'-like character of 8-H as already mentioned. However, the ratio $(T_1)_8:(T_1)_{1'}$ decreases with increasing concentration of TrpOMe, implying an increase in *anti* conformation as a result of the stacking interaction with tryptophan. On the other hand, the effect of TrpOMe on the glycosyl bond is meagre for GMP.

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