Effects of Lysine and Arginine Derivatives on the Base Stacking Association of 2′-Deoxyguanosine 5′-Monophosphate

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The effects of basic amino acid residues on the base stacking of nucleotides have been studied to clarify the nature of interaction between histones and DNA's. For this purpose, lysine and arginine derivatives were used as models for basic amino acid residues in histone. The base stacking association of 2′-deoxyguanosine 5′-monophosphate(5′-dGMP) in aqueous solution has been studied by the measurement of the concentration dependence of ^{1}H NMR chemical shifts of 5′-dGMP both in the absence and in the presence of the basic amino acid derivatives at different pH's. We clarified that the lysine favors the base stacking association of 5′-dGMP through the interaction with the phosphate group(s), while an excess of the arginine destabilizes the stacking aggregates through the interaction with the base moiety. We also clarified that the ionization of the phosphate group destabilizes the stacking association of 5′-dGMP both in the absence and in the presence of the basic amino acids.

To investigate the interaction of basic protein with nucleic acid, there has been studied many model systems consisting of basic oligo- or polypeptides and polynucleotides, nucleic acids, or its components by physicochemical methods.[1-9] The binding of histones to DNA has been clarified to occur mainly through an electrostatic linkage between basic amino acid residues such as lysine and arginine, and the DNA phosphate groups.[4,9] Wagner and Arav[1] have used equilibrium dialysis to study the binding of the various nucleotides to poly(L-lysine) and poly(L-arginine) at pH 7. Their results showed that there is some specificity in the interaction between the nucleotides and basic polypeptides. In particular, the binding constant of guanine nucleotide with poly(L-arginine) is much larger than that with poly(L-lysine). Rifkind and Eichhorn[2] have studied, using optical rotatory dispersion, the interaction of various ribonucleotides with poly(L-lysine) and poly(L-arginine) and shown that the guanosine nucleotide stacks more weakly in the presence of poly(L-arginine) than in the presence of poly(L-lysine).

Free nucleic acid bases and their derivatives are known to aggregate in aqueous solution. In these aggregations, as shown by ^{1}H NMR measurements, the planar bases are arranged in parallel with each other to take so-called stacking mode.[10-15] The stacking interaction has been extensively studied for nucleosides and nucleotides,[10-19] because the stacking contributes dominantly to the stability of the secondary structures of DNA and other polynucleotides.[12,13,19-21]

No attempt has so far been made to study the effect of basic amino acids on the base stacking interaction of nucleotides. It seems that the study of this effect would promote the understanding of a local interaction at lysine- or arginine-rich regions in nucleohistone. A study on the nucleotide having guanine base can also provide the information relevant to the specific interaction of the nucleotide with poly(L-arginine). In this work, we will first study the base stacking association of 2′-deoxyguanosine 5′-monophosphate(5′-dGMP) at acidic, neutral, and alkaline pH's by means of ^{1}H NMR, since no detailed study has so far been reported. The effect of phosphate ionization on a base stacking association will be discussed. On the basis of these results, we will study the effect of lysine and arginine derivatives, N_α-acetyl-L-lysine methyl ester(Ac–Lys–OMe) and N_α-acetyl-L-arginine methyl ester(Ac–Arg–OMe), at each pH. For the sake of comparison, 2′-deoxyadenosine 5′-monophosphate(5′-dAMP), 2′-deoxyadenosine (2′-dAdo), and 2′-deoxyguanosine(2′-dGuo) will be also studied.

Experimental

Materials. All samples were purchased from Sigma Chemical Co. Sodium salts of 5′-dGMP and 5′-dAMP were treated with Chelex-100(Bio-Rad Laboratories). The lysine and arginine derivatives, free acid of 5′-dGMP, and nucleosides (2′-dAdo and 2′-dGuo) were used without further purification. Deuterium oxide(D_2O) and perdeuterated dimethyl sulfoxide($(CD_3)_2SO$) used as solvents, were purchased from Merck Sharp and Dohme, Canada Ltd.

NMR Measurements. Fourier transformed ^{1}H NMR spectra were taken on a JEOL PS-100 NMR spectrometer equipped with a PFT-100 Fourier transform system and a JEOL EC-100 computer. The spectrometer was field-frequency locked on deuterium resonance of D_2O or $(CD_3)_2SO$ used as solvent. Digital resolution was 0.24 Hz. The methyl signal of 2-methyl-2-propanol was used as an internal reference for monitoring the chemical shifts. This signal is known to be shifted to upfield in the presence of high concentration of aromatic solutes.[22] This shift is, however, negligibly small in the experimental concentration range (up to 0.25 M for the nucleotides, 1 M = 1 mol dm^{-3}).

All spectra were recorded at 30 °C. A JES VT-3 temperature controller was used for the stabilization, with accuracy of ±2 °C.

NMR samples were prepared in D_2O containing 5×10^{-3} M EDTA (ethylenediaminetetraacetic acid). An addition of EDTA did not affect ^{1}H chemical shifts. The pH of solution was adjusted to a desired value by an addition of very small amount of concentrated DCl and/or NaOD solution in D_2O. The pH value was measured, with an accuracy of ±0.01 pH unit, both before and after the NMR measurement with a TOKO TP-101 pH meter fitted with combination electrode of Type CE-103 which can be inserted to NMR sample tube. No correction was made for the deuterium isotope effect on pH value. Concentrations were determined by dry weight and then adjusted to the desired values by dilution using a volumetrically variable pipette 4710 (Eppendorf).

The data of chemical shifts were analyzed by using a Sharp MZ-80B personal computer: the observed variation of the chemical shift with concentration was fitted to an equation by

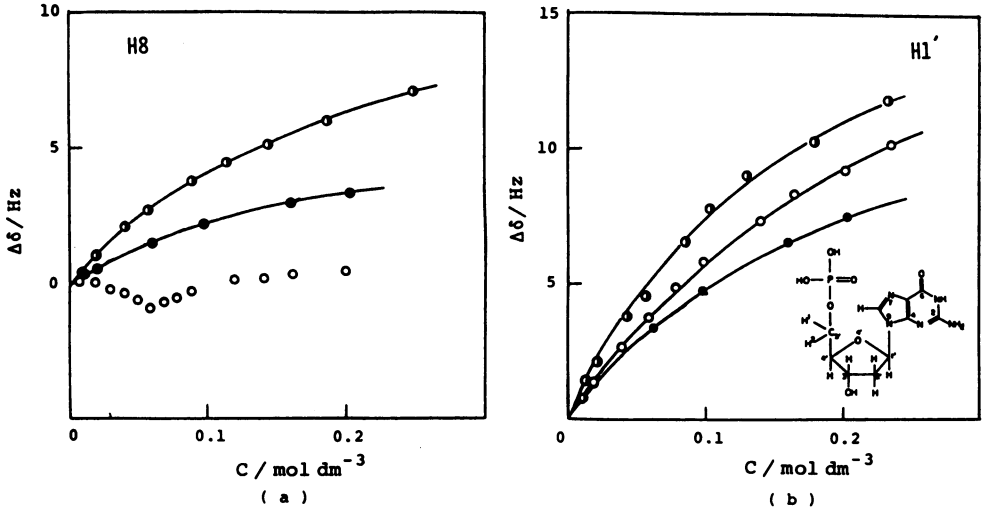

Fig. 1. Effect of pH on the base stacking association of 5′-dGMP. ^{1}H chemical shift differences $\triangle\delta$ are displayed for the H-8(a) and H-1′(b) as a function of 5′-dGMP concentration C at pH 4.0 (○), 6.6 (◑), and 9.0 (●), where $\triangle\delta$ is the chemical shift of a particular proton at each concentration referenced to that at an infinite dilution and a positive value represents an upfield shift.

using a Newton-Gauss nonlinear least-squares method.

Results

The Self-association of 5′-dGMP. We used the resonance of the base proton in position 8(H-8) and that of the ribose proton in position 1′(H-1′) (see Fig. 1), as a probe for studying the concentration dependence of intermolecular association of 5′-dGMP. The resonance of H-2 proton was further used in the experiment for 5′-dAMP. In Fig. 1 is shown the concentration dependence of the ^{1}H chemical shift differences ($\Delta\delta$) of the 5′-dGMP protons H-8(a) and H-1′(b) at pH 4.0, 6.6, and 9.0, where $\Delta\delta$ is the chemical shift of a particular proton at each concentration referenced to that at an infinite dilution and a positive value represents an upfield shift. These three pH's were chosen to correspond to monoanionic, pK, dianionic states of the phosphate group of 5′-dGMP. From the titration experiment of ^{31}P chemical shift of 5′-mononucleotides,[35] the phosphate group is mono and dianion at pH 4.0 and 9.0, respectively. The pH value of 6.6 is close to the pK value for the secondary ionization of the phosphate group. At pH 6.6 and 9.0, upfield shifts were observed with increasing concentration, indicating an increase of the fraction of the base stacking association.[10–15] The concentration dependence of these ^{1}H chemical shifts has been analyzed by applying the isodesmic model of indefinite non-cooperative stacking.[23] In this model, the relationship between the observed chemical shift difference ($\Delta\delta$) and total concentration (C) is given by

$$(\Delta\delta/C)^{1/2} = (K/2\Delta\delta_D)^{1/2} \times (2\Delta\delta_D - \Delta\delta), \quad (1)$$

where $\Delta\delta = \delta_0 - \delta_{obsd}$, δ_0 and δ_{obsd} are the chemical shifts of the investigated proton at an infinite dilution and at each concentration, $\Delta\delta_D$ the dimerization shift, and K the association constant. The observed chemical shift displacements for both protons at pH 6.6 and 9.0 were consistent with this model.

At pH 4.0, the H-8 base proton resonance shifts little with increasing concentration, while the H-1′ ribose proton resonance shifts significantly to upfield. The results indicate that there are other interactions in addition to the base stacking association and the interaction sites are in the base moiety of 5′-dGMP. The unique shift of H-8 proton at pH 4.0 may be specific to the nucleotide having guanine base, since the H-8 base proton resonance of 5′-dAMP shifted to upfield with increasing concentration at pH 4.0.

In order to understand an interaction other than base stacking, we have also studied the concentration de-

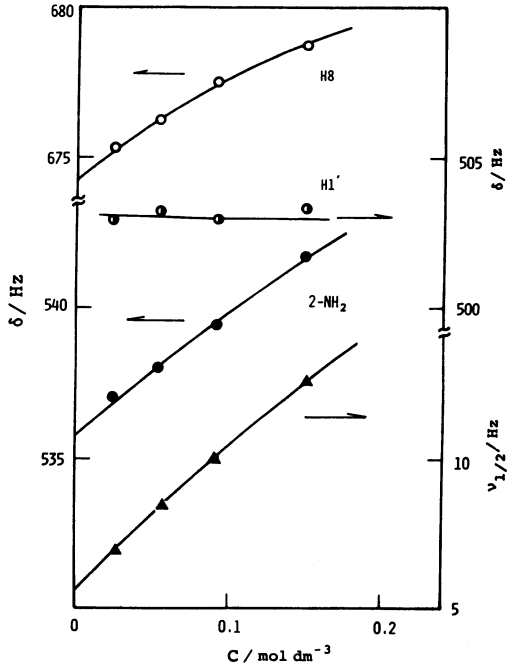

Fig. 2. ^{1}H chemical shifts δ from 2-methyl-2-propanol for the 5′-dGMP protons H-8 (○), H-1′ (◑), and 2-NH_2 (●) and the half-width $\nu_{1/2}$ of 2-NH_2 (▲) as a function of 5′-dGMP concentration C in $(CD_3)_2SO$.

TABLE 1. EFFECTS OF pH AND Ac–Lys–OMe ON THE ASSOCIATION CONSTANTS (K/M^{-1})[a] FOR THE BASE STACKING OF 5′-dGMP AND 5′-dAMP[b] ACCORDING TO THE ISODESMIC MODEL

pH	5′-dAMP			5′-dGMP		[Ac–Lys–OMe]/[5′-dGMP]=0.5		[Ac–Lys–OMe]/[5′dGMP]=1	
	H8	H2	H1′	H8	H1′	H8	H1′	H8	H1′
4.0	6.9	6.3	6.0	—[c]	1.5	6.2[d]	6.0[d]	7.1[d]	7.6[d]
6.6	4.6	4.2	4.5	2.7	3.0	3.4	3.5	4.3	4.4
9.0	3.7	3.3	3.2	1.9	2.0	2.9	3.2	2.7	3.0

a) Estimated errors for these association constants are 10—15%. b) Measurement for pH effect only. c) Not determined. d) For concentration below 0.06 M.

pendence of ^1H chemical shifts and line width of 5′-dGMP in $(CD_3)_2SO$ (Fig. 2). It is known that the base stacking association is negligibly small in this solvent.[24] The resonance position of H-1′ ribose proton is independent of concentration as expected. The resonance of 2-NH_2 shifts to downfield and its line width increases with increasing concentration, indicating the formation of hydrogen bond including 2-NH_2 group. Parallel to this variations, the resonance of H-8 proton shifts to downfield.

In Table 1 are shown the effects of pH on the association constants for the stacking of 5′-dGMP and 5′-dAMP estimated by applying the isodesmic model. The association constants obtained from the chemical shift displacements of H-8 base protons agree well with those from the H-1′ ribose protons, indicating that the variations of ^1H chemical shifts of both protons reflect the degree of stacking association. The association constants for stacking of both nucleotides decrease with increasing pH, except for 5′-dGMP at pH 4.0.

For comparison, 2′-dAdo, lacking 5′-monophosphate group, has been studied. In Fig. 3 is shown the pH dependence of 2′-dAdo protons (H-8, H-2, and H-1′) at various concentration up to dissolvable limit 0.04 M. The chemical shifts of all protons do not change in the range of pH 5 to 9 and this constancy is kept over the concentration range studied here. The downfield shifts of the base proton resonances observed for the nucleotides with decreasing pH below 5 can be attributed to the protonation of the base.[11,25]

Effect of the Lysine on the Stacking of 5′-dGMP. In Fig. 4 is shown the concentration dependence of ^1H chemical shifts (H-8 and H-1′) of 5′-dGMP at pH 4.0, 6.6, and 9.0 in the 2 : 1 mixture of 5′-dGMP and Ac–Lys–OMe. At pH 6.6 and 9.0, upfield shifts are observed with increasing concentration, which is consistent with the isodesmic model. This indecates that the lysine does not disturb the indefinite non-cooperative stacking of the base of 5′-dGMP. Even at pH 4.0, upfield shifts are also observed for both protons, unlikely in the case of 5′-dGMP alone. This indicates that the lysine interacts with 5′-dGMP.

In Table 1 is also shown the effect of the lysine on the association constants of 5′-dGMP. At pH 6.6, the association constants obtained from the H-8 and H-1′ protons increase in the presence of the lysine and with an increase of its population. The lysine, therefore, participates in the base stacking association.

Effect of the Arginine on the Stacking of 5′-dGMP. In Fig. 5 is shown the concentration dependence of ^1H chemical shifts of 5′-dGMP at molar ratios [Ac–Arg–OMe]/[5′-dGMP]=0 (5′-dGMP alone), 0.5, 1.0, and 2.0 at pH 4.0. The resonance of the base proton H-8 shifts to upfield with concentration in the presence of the arginine, similar to that in the presence of the lysine. The magnitudes of shift differences ($\Delta\delta$) of the ribose proton H-1′ increase in the presence of the arginine at lower 5′-dGMP concentration ($\approx$0.08 M). The arginine interacts with 5′-dGMP and participates in increase the fraction of stacking aggregates, similar to the case of the lysine.

At 5′-dGMP concentration higher than 0.1 M, the magnitudes of shift differences of the H-8 and H-1′

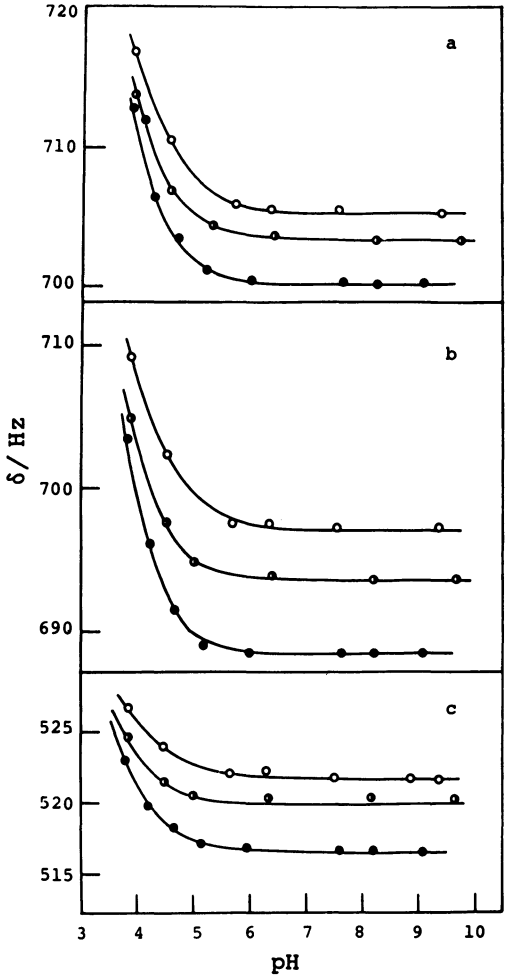

Fig. 3. ^1H chemical shifts δ *vs.* pH for protons H-8(a), H-2(b), and H-1′(c) of 2′-dAdo at 0.8×10^{-2} M (○), 2.2×10^{-2} M (◑), and 4×10^{-2} M (●).

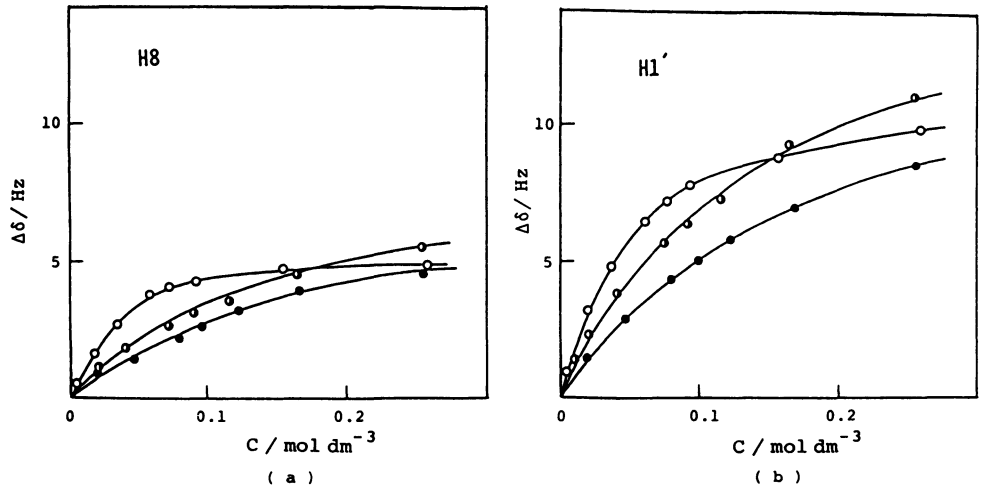

Fig. 4. Effect of pH on the self-association of 5′-dGMP at molar ratio [Ac–Lys–OMe]/[5′-dGMP]=0.5. ^{1}H chemical shift differences $\triangle\delta$ are displayed for the H-8(a) and H-1′(b) protons as a function of 5′-dGMP concentration C at pH 4.0 (○), 6.6 (◑), and 9.0 (●).

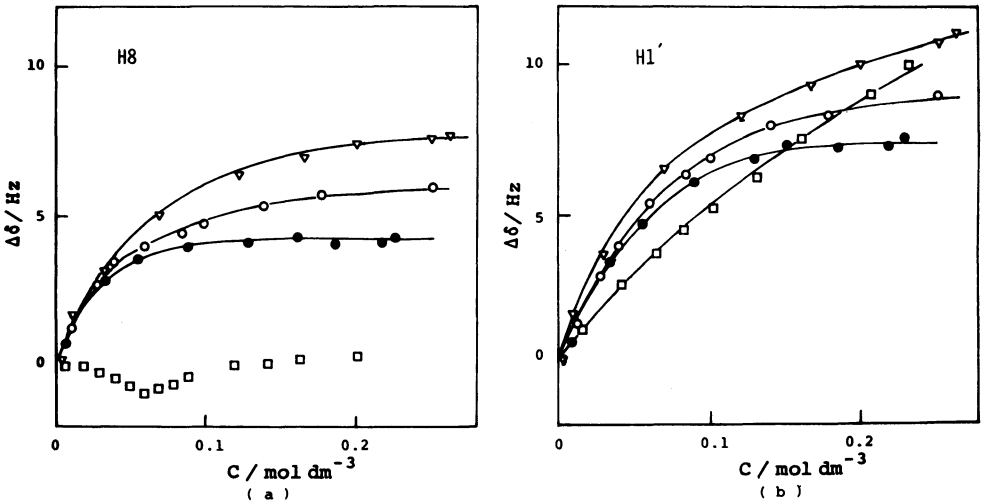

Fig. 5. Effect of the arginine on the self-association of 5′-dGMP at pH 4.0. ^{1}H chemical shift differences $\triangle\delta$ are displayed for the H-8(a) and H-1′(b) protons as a function of 5′-dGMP concentration C at molar ratios [Ac–Arg–OMe]/[5′-dGMP]=0(5′-dGMP alone) (□), 0.5 (▽), 1.0 (○), and 2.0 (●).

Table 2. Effects of pH and Ac–Arg–OMe on the association constants (K/M^{-1})[a] for the base stacking of 5′-dGMP according to the isodesmic model

[Ac–Arg–OMe]/[5′-dGMP]	H-8			H-1′		
	pH 4.0	pH 6.6	pH 9.0	pH 4.0	pH 6.6	pH 9.0
0[b]	—[c]	2.7	1.9	1.5	3.0	2.0
0.5	8.4	5.0	4.3	8.6	5.5	3.8
1.0	9.5[d]	5.2	4.1[e]	9.1[d]	5.8	3.7[e]
2.0	9.3[g]	5.5[f]	4.6[f]	8.9[g]	6.0[f]	4.1[f]

a) Estimated errors for these association constants are 15—20%. b) 5′-dGMP alone. c) Not determined. d—g) Concentration ranges of 5′-dGMP applied to the isodesmic model: (d) 0.18 M, (e) 0.15 M, (f) 0.09 M, (g) 0.06 M.

protons decrease and the chemical shifts tend to finite values with increasing molar ratio of the arginine to 5′-dGMP. These differences can be regarded as significant, since the experimental errors are within 0.5 Hz. This result indicates that the increase of the fraction of stacking aggregates of 5′-dGMP with increasing concentration is depressed at higher molar ratio of the arginine.

Similarly, at pH 6.6 and 9.0, the magnitudes of shifts observed for both protons at higher 5′-dGMP concentration decrease with increasing molar ratio of the arginine to 5′-dGMP. The isodesmic model cannot be

applied to these ^1H chemical shift variations. This is different from the case of the lysine where the chemical shift variations of both protons at pH 6.6 and 9.0 obey the isodesmic model. These results indicate that the differences between the arginine and the lysine could not be explained by the difference in electrostatic interactions alone.

In Table 2 are shown the effects of the arginine on the stacking association constants at each pH and the concentration ranges where the isodesmic model is applicable. The applicable concentration ranges were determined from plots of $(\Delta\delta/C)^{1/2}$ against $\Delta\delta$ (Eq. 1). These plots give a straight line up to an upper limit of concentration which is applicable to the isodesmic model. When the arginine is added to the 5'-dGMP solution at the ratio of [Ac–Arg–OMe]/[5'-dGMP]=0.5, the association constant (K) increases, similar to the case of the lysine. The K values at [Ac–Arg–OMe]/[5'-dGMP]=1.0 in the concentration ranges where the isodesmic model is applicable are, however, the same as those at [Ac–Arg–OMe]/[5'-dGMP]=0.5 within an experimental error. The K values are not increased by further addition of the arginine. The 5'-dGMP concentration ranges applied at [Ac–Arg–OMe]/[5'-dGMP]=2 are more restricted (Table 2, footnote (f)—(g)). These results indicate that the increase of the fraction of stacking aggregates is depressed in the presence of an excess of the arginine at all pH studied here.

Discussion

Self-association of 5'-dGMP at pH 4.0. The unique variation of chemical shift of H-8 proton with concentration at pH 4.0 (Fig. 1) could be interpreted by the formation of planar interbase association, because a guanine base is unusual among nucleic acid bases in its ability to make a self-associated regular structure with higher stability.[15,26-32] Gelation of aqueous solution of guanosine 5'-monophosphate (5'-GMP) takes place in acidic pH.[26,27] The gel structure of 5'-GMP at acidic pH consists of helically arranged stacks of planar tetramer units formed by hydrogen bonding (N-1 and 2-NH_2 as donors, O-6 and N-7 as acceptors).[21] Since the electron density at N-7 of the guanine base is large at acidic pH,[25,33,34] the N-7 of 5'-dGMP becomes also a stronger proton acceptor at pH 4.0 as compared with that at higher pH. Although 5'-dGMP, unlike 5'-GMP, does not form an ordered self-structure with applicable stability, the weak planar interbase association of 5'-dGMP could occur in acidic solution.

The effect of hydrogen bonding including 2-NH_2 on the chemical shift of H-8 proton, where the 2-NH_2 proton is the hydrogen bond donor for N-7 in planar interbase aggregate,[27-32] has been studied for 5'-dGMP in $(CD_3)_2SO$ (Fig. 2). With increasing the fraction of the interbase association by hydrogen bonding, the H-8 resonance shifts to downfield. This indicates that the formation of interbase hydrogen bonds including N-7(acceptor for 2-NH_2 proton) deshields the H-8 proton.

In aqueous solution, the hydrogen bonding becomes weak by simultaneous hydration of proton donor and acceptor groups, but the planar interbase association may be strong, since 5'-GMP forms gel even in aqueous solution.

The above results indicate that at pH 4.0, the deshielding of H-8 proton by planar interbase association can offset the upfield shift due to the base stacking association. There is, therefore, interbase association in addition to the base stacking association in the solution of 5'-dGMP at pH 4.0.

Effect of pH on the Base Stacking Association of Nucleotides. At the region of pH 6.6 to 9.0, the effect of pH is on the deprotonation of the phosphate group of nucleotides.[11] The association constants (K) for stacking of both 5'-dGMP and 5'-dAMP decrease with increasing pH (Table 1). These are possibly due to the increase of electrostatic repulsion between negatively charged phosphate groups in stacked aggregates. Scheller *et al.*[36] indicated that the tendency of self-stacking decreases in a series of adenosine$\gg AMP^{2-} > ADP^{3-} \approx ATP^{4-}$. The deprotonation of the phosphate group causes a decrease of stacking affinity by an increase of the intrinsic intermolecular electrostatic repulsion.

This interpretation is confirmed by comparison of 5'-dAMP and 2'-dAdo lacking 5'-monophosphate: the association constant (K) of 5'-dAMP decreases with increasing pH (Table 1), while 2'-dAdo does not induce the change of chemical shifts of all protons studied (H-8, H-2, and H-1') from pH 5 to 9 and this constancy is kept over the concentration range in this experiment (Fig. 3), that is, constancy of K value in this range of pH. The chemical shift of 2'-dGuo also did not change in the pH range up to dissolvable limit of concentration 0.01 M (not illustrated). The effect of charge of phosphate group is, thus, significant on the base stacking association.

Effects of Lysine and Arginine on the Self-association of 5'-dGMP. In the presence of the lysine or arginine derivative, monotonous upfield shifts are observed for H-8 and H-1' protons of 5'-dGMP even at pH 4.0 (Figs. 4 and 5), indicating that the base stacking association is easier to take place than the planar interbase association. The binding of lysine or arginine with 5'-dGMP results in the depression of the planar interbase association. Possibly, the interactions between these basic amino acids and 5'-dGMP include coulombic attraction and/or hydrogen bonding between the protonated ε-amino group ($pK \simeq 10$) of the lysine or the guanidinium group ($pK \simeq 11$) of the arginine and monoanionic phosphate group (s) in the stacked 5'-dGMP aggregates.

When the lysine is added to the 5'-dGMP solution at pH 6.6, the affinity of base stacking increases (Table 1). This increment is due to the neutralization of the negatively charged phosphate group by the positively charged ε-amino group of the lysine which reduces the intermolecular electrostatic repulsion between phosphate groups in the stacked 5'-dGMP. At pH 9.0, the association constant does not increase by an addition of the lysine. Since the degree of protonation ($pK \simeq 10$) of the lysine at pH 9.0 is low compared to that at lower pH, the addition of the lysine has a less effect on the stacking association of 5'-dGMP.

When an excess of the arginine is added to the 5'-dGMP solution at all pH studied, the increase of stacking

fraction with increasing concentration is depressed (Fig. 5 and Table 2). It is known that a guanidinium group of arginine can associate through hydrogen bonding with a guanine base.[37,38] An excess of the arginine can interact with the guanine base as well as the phosphate group of 5′-dGMP. On the other hand, the guanidinium cation is known to reduce a hydrophobic interaction.[39] This, leads to a reduction of fraction of the stacking aggregates, since the hydrophobic interaction contributes to the stabilization of stacking aggregates.[14,40] The hydrophobic interaction is more effective in the multiple stacked bases. We conclude, therefore, that the depression of stacking association is due to the interaction between the guanidinium group of the arginine and the guanine base of 5′-dGMP. From this interpretation, one could explain the fact that the stacking of guanine nucleotide occurs much less in the presence of poly(L-arginine) than in the presence of poly(L-lysine).[2]

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