Recognition of Nucleic Acid Base by Tryptophan-Containing Peptides: Spectroscopic Study on Interaction of *N*-AcetylTrp-(Gly)$_m$-Asp-(Gly)$_n$-TrpNHCH$_3$ ($m=0$—2, $n=0$—2) with Guanine Base[1)]

Yayoi KAFUKU, Junko OHNISHI, Mitsunobu DOI, Masatoshi INOUE and Toshimasa ISHIDA*

Department of Physical Chemistry, Osaka University of Pharmaceutical Sciences, 2-10-65 Kawai, Matsubara, Osaka 580, Japan. Received June 15, 1992

As part of the designs of tryptophan-containing peptides which possess specific binding ability for each nucleic acid base, a series of *N*-acetylTrp-(Gly)$_m$-Asp-(Gly)$_n$-TrpNHCH$_3$ ($m=0$—2, $n=0$—2) were chemically synthesized, and their abilities to form complexes with a guanine base were examined by the fluorescence and ^{1}H-NMR methods. Fluorescence titration suggested the most preferential stacking interaction of the Trp-Gly-Asp-Trp sequence with the base. Analysis of low-field shifts of guanine N2-amino protons showed the hydrogen bond pairing with the Asp carboxyl side chain and its favorable formation for the -Gly-Asp-Trp peptide sequence. On the other hand, the largest up-field shift of guanine H8 proton was observed for Trp-Gly-Asp-Trp peptide, although its shifting degree caused by the stacking interaction with the Trp indole ring was not so significant. Thus, both spectroscopic methods indicated the Trp-Gly-Asp-Trp sequence to be most suitable for the guanine base recognition, which is constituted with the intimate cooperation of the hydrogen bond formation between the Asp carboxyl and guanine NH$_2$ groups and the stacking interaction of the base with two neighboring Trp indole rings. This sequence preference would also be possible in acidic circumstances where the guanine N7 atom is protonated. A tentative interaction model is proposed based on these spectroscopic results.

Keywords tryptophan-containing peptide; guanine base; hydrogen bond; stacking interaction; fluorescence; ^{1}H-NMR

Introduction

Selective recognition of a specific nucleotide or nucleic acid sequence by the enzyme is essential to a variety of biological functions. As an important interaction pattern that makes such specific and precise molecular recognition possible, it could involve the coupling of the hydrogen bond formation between the polar atoms and the stacking interaction between the aromatic rings. For example, the guanine base recognition by RNase T$_1$,[2)] elongation factor-Tu[3)] or c-H-*ras* oncogene p21[4)] protein is commonly carried out by this pattern, *i.e.*, the guanine base is fixed by multiple hydrogen bonds with polar amino acids and is sandwiched between two hydrophobic amino acids.

It is of special interest from a stereochemical point of view to investigate what kind of sequence of functional amino acids is necessary for the selective recognition of a specific nucleic acid base. This is also valuable for realizing the precise recognition mechanism between protein and nucleic acid in an enzymimic model system. As part of systematic studies on the peptide designs showing high selectivity for a target nucleic acid base, the interaction of a series of tryptophan-containing dipeptide[5)] and tripeptides[1)] with four kinds of nucleic acid bases was spectroscopically investigated. As a result, it was elucidated that the binding ability to the nucleic acid base is dependent on the sequence of the functional amino acids and the Trp-Asp-Trp fragment has a relatively high selectivity for the guanine base where both the hydrogen bond and stacking interactions function simultaneously. On the other hand, the model consideration suggested that this Trp-Asp-Trp tripeptide is too short to form hydrogen bond pairing between the Asp carboxyl side group and the guanine N1 imino and N2 amino protons under conditions where both Trp indole rings sandwich the base by stacking interaction.

Thus, in this paper, we deal with the spectroscopic study on the interaction of a series of *N*-acetylTrp-(Gly)$_m$-Asp-(Gly)$_n$-TrpNHCH$_3$ ($m=0$—2, $n=0$—2) with guanine bases, in order to find out the most suitable spacing between the Trp and Asp residues for revealing the base specificity.

Experimental

Syntheses of Protected Oligopeptides A series of *N*-acetylTrp-(Gly)$_m$-Asp-(Gly)$_m$-TrpNHCH$_3$ ($m=0$—2, $n=0$—2) were all synthesized by usual liquid phase peptide condensation. In order to mimic the peptide fragment in a protein or to suppress the effects of N-terminal amino and C-terminal carboxyl groups on the interaction, both terminal ends were blocked with acetyl and methylamide groups, respectively. The peptides synthesized were purified by gel chromatography on Sephadex LH20 and by HPLC on an ODS column (Capcell Pack C18, Shiseido) in the same manner as a previous paper.[1)] 7-Methylguanosine (m7GuO) was synthesized as formate salt from guanosine (GuO) according to Kamiichi *et al.*[6)]

Fluorescence Experiments Fluorescence spectra were measured on a JASCO FP-770F spectrometer (Nihon Bunko) using a Hg–Xe arc lamp, where a 10-nm slit and 1-cm path length were employed. The temperature of a sample solution was kept at 20 °C by circulating the thermostatically regulated water. The intensities of emission spectra excited at 290 nm were measured at respective λ_{max} near 353 nm.

The sample solution for the fluorescence experiment was prepared according to a previous paper[1)] by using either a 20 mM Tris–HCl (pH = 7.5) or 0.1 M KCl–HCl (pH = 2.0) buffer solution. The association constants K_a of peptide–guanine pairs were evaluated by the Eadie–Hofstee equation[7)];

$$\Delta F = -\frac{1}{K_a}\cdot\frac{\Delta F}{[\mathrm{GMP}]} + \Delta F_c ,$$

where ΔF is the difference (quenching) between the fluorescence intensities of the peptide in the presence (F_a) and absence (F_o) of GMP ($\Delta F = F_o - F_a$), and ΔF_c is the quenching of the peptide completely complexed with GMP (guanosine-5′-monophosphate) molecule. The slope of the Eadie–Hofstee plot was determined by least-squares linear regression analysis. By adding 3.75 to 37.50 μl aliquots of 10 mM GMP to a 3 ml solution of 5 μM peptide, the concentration ratio of [GMP]/

[peptide] was changed from 2.5 to 25.0. All fluorescence measurements were corrected for the sample dilution in the course of the titration experiment.

^{1}H-NMR Experiments ^{1}H-NMR measurements were done on a Varian XL-300 spectrometer (300 MHz for proton) at 20 °C. In order to monitor the chemical shift changes of guanine N2 amino and H8 protons, dimethylsulfoxide (DMSO)-d_6 was used as solvent and the chemical shifts were measured as the difference from an internal standard TMS (tetramethylsilane). Because of the solubility, GuO was used instead of GMP. As a substituent for GuO under acidic conditions, m7GuO was used. The ammonium salt of peptide was used in the NMR study to ensure an anionic state of the Asp carboxyl side group. The association constants were evaluated from the chemical shift changes of guanine N2 amino and H8 (for CuO) or N7 methyl (for m7GuO) protons[8] by using the Eadie–Hofstee equation:

$$\frac{1}{\Delta\delta} = -\frac{1}{K}\cdot\frac{\Delta\delta}{[\text{peptide}]} + \Delta\delta_c ,$$

where $\Delta\delta = \delta_o - \delta$ and $\Delta\delta_c = \delta_c - \delta$, and δ_o, δ and δ_c are the chemical shifts of protons of guanine bases in the absence and presence of peptide, and completely complexed with peptide, respectively. NMR titration was performed by adding 5—50 μl aliqouts of 250 mM peptide to a 5 ml solution of 5 mM GuO or m7GuO, so as to change the ratio of [peptide]/[GuO or m7GuO] from 0.5 to 5.0 with an interval of 0.5.

Results

Fluorescence Titration Generally, the fluorescence intensity of Trp indole ring decreases by the stacking interaction with nucleic acid base.[9] Thus, the fluorescence quencing of Trp residue in peptide was measured as a function of GMP concentration, and the strength of the interaction was estimated by the analysis of Eadie–Hofstee plots. An example for the WGDW–GMP pair in Tris–HCl buffer (pH 7.5) is shown in Fig. 1.

Since it is known that the protonation of guanine base strengthens the stacking interaction with a Trp indole ring,[10] the interaction was also investigated using KCl–HCl buffer solution (pH = 2.0). The association constants obtained are summarized in Table I, where respective standard errors are given in parentheses.[11]

^{1}H-NMR Titration The interaction between the guanine base and peptide was investigated using the different behaviour of guanine N2 amino and H8 (for GuO) or N7 methyl (for m7GuO) protons.[12] As is shown in Fig. 2a, the chemical shift of guanine N2 amino protons shift downfield in proportion to the peptide concentration. This is generally accepted as an indication of participation of this amino group in hydrogen bond formation with the peptide acceptor group. On the other hand, the chemical shift of GuO H8 or m7GuO N7 methyl protons experience the upfield shift depending on the peptide sequence and its concentration, as is shown in Fig. 2b. This is due mainly to the ring current effect by the guanine base-Trp indole ring stacking interaction. The shift changes of these protons at the coexistence of 10 times molar excess peptide are listed in Table II. The association constants were obtained for the interaction pairs showing the good

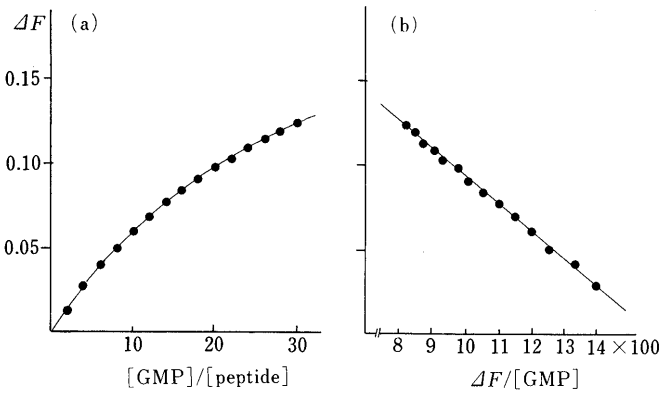

Fig. 1. Trp Fluorescence Quenching of WGDW as a Function of GMP Concentration (a) and Its Eadie–Hofstee Plot (b) in Tris–HCl Buffer (pH 7.5)

TABLE I. Association Constants ($K_a \times 10^3$, M^{-1}) between GMP and Peptides, under Neutral (pH 7.5) and Acidic (pH 2.0) Conditions, Determined by Fluorescence Titration[a]

	WDW	WGDW	WDGW	WGDGW	WGGDW	WDGGW
pH 7.5	4.7 (3)	9.9 (3)	5.0 (4)	5.0 (2)	4.6 (1)	4.9 (2)
pH 2.0	7.9 (3)	12.1 (3)	7.6 (3)	8.5 (4)	8.2 (4)	7.5 (4)

a) The standard errors are given in parentheses. Amino acid residues of each peptide are expressed with one-letter-code, and the description of N-terminal acetyl and C-terminal methylamide groups is ommited.

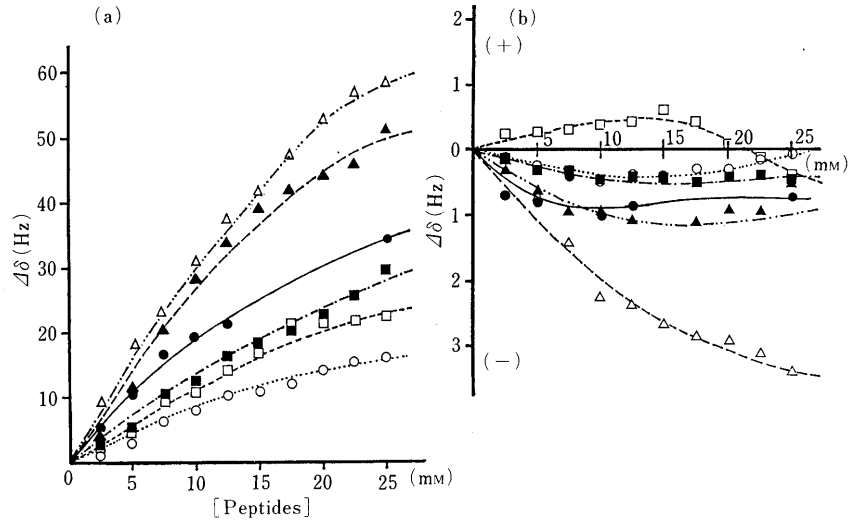

Fig. 2. Chemical Shift Change of GuO N2 (a) and H8 (b) Protons as a Function of Peptide Concentration
—●—, WDW; --△--, WGDW; —·—▲—·—, WGGDW; --□--, WDGW; —·—■—·—, WDGGW; ---○---, WGDGW.

TABLE II. Difference (in Hz)[a] between Guanine N2 Amino and H8 (for GuO) or N7 Methyl (for m7GuO) Proton Chemical Shifts in Absence and Presence[b] of Peptide, Together with Association Constants (K_a, M^{-1}) in Parentheses[c]

		WDW	WGDW	WDGW	WGDGW	WGGDW	WDGGW
GuO	NH_2	30.8	57.9	22.5	16.4	50.9	29.9
	H8	−1.0	−3.4	−0.4	−0.1	−0.4	−0.3
m7GuO	NH_2	282.1	343.3	223.1	170.4	231.3	174.0
		(212.2)	(266.7)	(159.3)	(113.3)	(168.0)	(107.1)
	CH_3	−3.2	−6.6	−3.0	−5.8	−4.6	−4.3
		(120.6)	(182.8)	(83.3)	(102.5)	(82.5)	(76.3)

a) The values are the averaged one of duplicates. The mean error for the value is less than ±0.2 Hz. *b*) The concentration of peptide was 10 times molar excess, compared to that of GuO or m7GuO. *c*) The mean errors of the association constants were all within 0.5.

linearities of $|r|>0.9$, and are listed in parentheses in Table II. Since the chemical shift changes of GuO NH_2 and H8 protons were not so significant, the association constants could not be determined accurately.

Discussion

Previous spectroscopic studies[1,5] evidenced that it is very profitable for the specific recognition of guanine base to utilize the hydrogen bond pairing ability of acidic amino acid such as Asp or Glu and the stacking ability of Trp. The present study lies on the line of designing the best-fit peptide to guanine base by utilizing the cooperation of both the interaction abilities.

A series of peptides used appear to form 1 : 1 complexes with guanine, respectively, because the Job plot[13] using the chemical shift change of GuO N2 amino or m7GuO N7 methyl protons interacting with WGDW peptide (data are not shown) showed the maximum at the molar fraction of [peptide]/[GuO or m7GuO + peptide] = 1/2, similar to that previously observed for the m7GuO–Trp–Glu pair.[5]

Since the stacking complex of tryptophan with a nucleic acid base has no fluorescence intensity, the association constants determined from such a quenching degree reflect the strength of Trp indole–guanine stacking interaction. Although no significant difference was observed among the K_a values in Table I, however, a reproducible preference was observed for the GMP–WGDW pair in Tris–HCl buffer slution; its K_a is about two times larger than the others. It is interesting to note that the interaction force with the guanine base appears to be sequence-dependent, and the position of Asp residue is important for revealing guanine specificity under the condition where both of the terminal ends of peptide are occupied with Trp residues. A similar tendency was also observed in an acidic KCl–HCl solution (pH = 2.0), where the N7 position of guanine base is in a protonated state ($pK_a = 3.3$) and the carboxyl group of the Asp side chain is in a neutral state (pK_a 3.86). Compared with those in the neutral solution, the association constants are meaningfully increased for all pairs because of the strengthening of the stacking interaction caused by the guanine base protonation.[10] Although the hydrogen bonding mode of the Asp carboxyl side group with a guanine base is probably different in the neutral and acidic solutions, it is obvious that Asp residue plays an important role in revealing the guanine specificity.

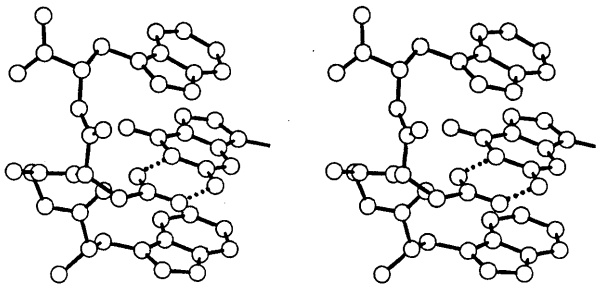

Fig. 3. A Stereoscopic View of Possible Binding Mode of WGDW Tetrapeptide for Guanine Base

The dotted lines represent hydrogen bonds.

The hydrogen bond in which the guanine N2 amino protons participate could be monitored by the lowfield change of these chemical shifts. As is given in Table II, a notable shift change was observed for the WGDW–GuO pair. As a large change was also observed for WGGDW, the sequence of GDW may be convenient for forming a stable hydrogen bond of the Asp carboxyl side group and the guanine base. On the other hand, the H8 proton experienced the upfield shift change upon the stacking formation with the Trp indole ring. Although its change was not so significant, a notable shift was observed only for the WGDW–GuO pair. Thus, the NMR experiments suggest that the WGDW fragment is also preferential for the stacking formation with the guanine base, and this is in agreement with the fluorescence result.

M7GuO was used as a model compound of N7-protonated guanine base. Although the existence of a guanine N7 methyl group is thermodynamically inconvenient for the stacking structure itself with the indole compound,[6] the tendency of stacking formation was significantly elevated compared with the case of GuO (see K_a values in Table II). The preference of WGDW for interaction with m7GuO was suggested from the shift changes and K_a values of N2 amino and N7 methyl protons.

The present study indicated the highest specificity of WGDW peptide for the guanine base, together with the importance of (a) intimate cooperation of Asp hydrogen bonding and Trp stacking interactions and (b) their spatial arrangements for the guanine base recognition. In order to consider what kind of binding mode is actually possible for WGDW–guanine base interaction, a model building was attempted using the energy optimization technique by molecular mechanics calculations (program MMFF[14]) operating on an IRIS 2400 turbo graphic computer. A model satisfying the spectroscopic data in the neutral condition is shown in Fig. 3, where the guanine base is sandwiched with two terminal Trp residues and the N1 imino and N2 amino protons of the base are hydrogen-bonded to the carboxyl group (anionic form) of Asp side chain. This type of binding mode would also be possible in an acidic solution, although the protonated carboxyl group of Asp could form a 'cyclic'-type hydrogen bond pairing with guanine N1 and O6, N2 and N3, or O6 and N7 atomic pairs.[15] It is interesting to note that the length of the Asp side chain is important for guanine recognition, because the WGEW peptide is no longer specific for the guanine base.[16]

Stereospecific interaction between the cognate species is the essence of strict molecular recognition. The spatially correlated cooperation of the hydrogen bond pairing and stacking interactions was shown by the present study to provide an effective circumstance of such a molecular recognition. The molecular design of peptide in which the spatial cooperation of electrostatic and hydrophobic interactions is further taken into consideration would give much higher selectivity for the recognition of a specific nucleic acid base compared with the present one.

References and Notes

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11) As judged from the association constants measured for some interaction pairs, the binding preference of GuO against these peptides appears to be essentially the same as that of GMP in both the neutral and acidic solutions.
12) Under the experimental conditions used in this work, no notable nuclear Overhauser effect (NOEs) were observed between the protons of both molecules.
13) According to Job plot (P. Job, *Comput. Rend.*, **180**, 928 (1925)), the stoichiometries of the WGDW–GuO and –m7GuO were determined by plotting the GuO NH_2 and m7GuO CH_3 proton chemical shifts as a function of molar fraction {[GuO or m7GuO]/[WGDW+GuO or m7GuO]}, where the total concentration of [GuO or m7GuO]+[WGDW] was kept constant (=20 mM).
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15) As was already pointed out (S. E. Carberry, R. E. Rhoads and D. J. Goss, *Biochemistry*, **28**, 8078 (1989)), the O6 atom of m7GuO would take the OH form, where the N1 atom is deprotonated. Thus, a 'cyclic-type' hydrogen bond pairing *via* N1 and O6 atomic pairs could be possible. Also, similar O6 enolate form would be probable for GuO in the acidic solution.
16) Although detailed spectroscopic studies on the interaction of a series of *N*-acetylTrp–$(Gly)_m$–Glu–$(Gly)_n$–$TrpNHCH_3$ ($m=0$—2, $n=0$—2) with guanine base are now in progress, the preference of WEGW, rather than WGEW, has been suggested. A part of this work was presented at the 112th Annual Meeting of the Pharmaceutical Society of Japan, Hakata, March 1992.