Interaction Modes between *N*7-Quarternized Guanine and Cytosine-Containing Dipeptides

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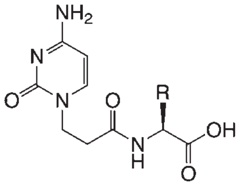
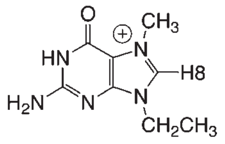
(Received July 29, 2002; CL-020626)

7-Methyl-9-ethyl-guanine (^{7m}G) was titrated by eleven cytosine-containing dipeptides (C-Xxx) monitoring the chemical shift of H8 proton. Amino acids incorporated to C-Xxx were related to the molecular ratio and association constant of the complex. In the Eadie-Hoffstee plots, the dipeptides of which side chains could form hydrogen bonds showed two phases. The highest association constant was observed for C-Asn, and its level was 20 times greater than that of C-Trp.

The cytosine-incorporated dipeptides (**1**) have been designed to investigate the cooperative interactions between nucleic acid and dipeptides, and it was presumed that the interactions between cytosine and guanine bases were strengthened by incorporated amino acids.[1,2] We found the various conformations of **1**[3–5] and its affinities for 7-methy-9-ethyl-guanune (**2**).[6] These suggest a possibility to develop the probes interacting with *N*7-quarternized guanines. These probes support the interpretation for the recognition system of proteins, initiation factor 4E[7] and snurportin,[8] which are primarily recognizing *N*7-quarternized guanines.



C-Xxx (**1**)



^{7m}G (**2**)

In this study, eleven amino acids (Gly, Ala, Ile, Glu, Ser, Thr, Asn, Gln, Phe, Tyr and Trp) were incorporated into **1**, and **2** was titrated by these analogues monitoring the chemical shift of H8.[9] The binding stoichiometry and Eadie-Hofstee plots are shown in Figures 1 and 2, respectively. The association constants were obtained from the Eadie-Hofstee plots and summarized in Table 1.

The $F_{\max}$ values of 0.5 were observed for Gly, Ala, Ile, and Trp-incorporated dipeptides (**1a–c** and **k**). These made a complex with **2** at 1 : 1 molecular ratio. The R_c values over 1.0 were observed for the other dipeptides incorporating Glu, Ser, Thr, Asn, Gln, Phe and Tyr (**1d–j**), indicating that these dipeptides accept more than one molecule of **2** (Table 1). Although the differences between K_1 and K_2 are relatively small for **1h**, **1i** and **1j**, these **1d–j** show two phases in the Eadie-Hofstee plots (Figure 2).

The maximum association constant was observed for **1g** at higher peptide concentration ($K_1 = 6.65 \times 10^6\,\mathrm{M}^{-1}$), its value was approximately 20 times of **1k** ($K = 0.34 \times 10^6\,\mathrm{M}^{-1}$). It is surprising, because the guanine base was directly interacted with Trp and Glu residues in the complex of initiation factor and *N*7-methyl guanosine triphosphate, but not with any Asn residue.[7]

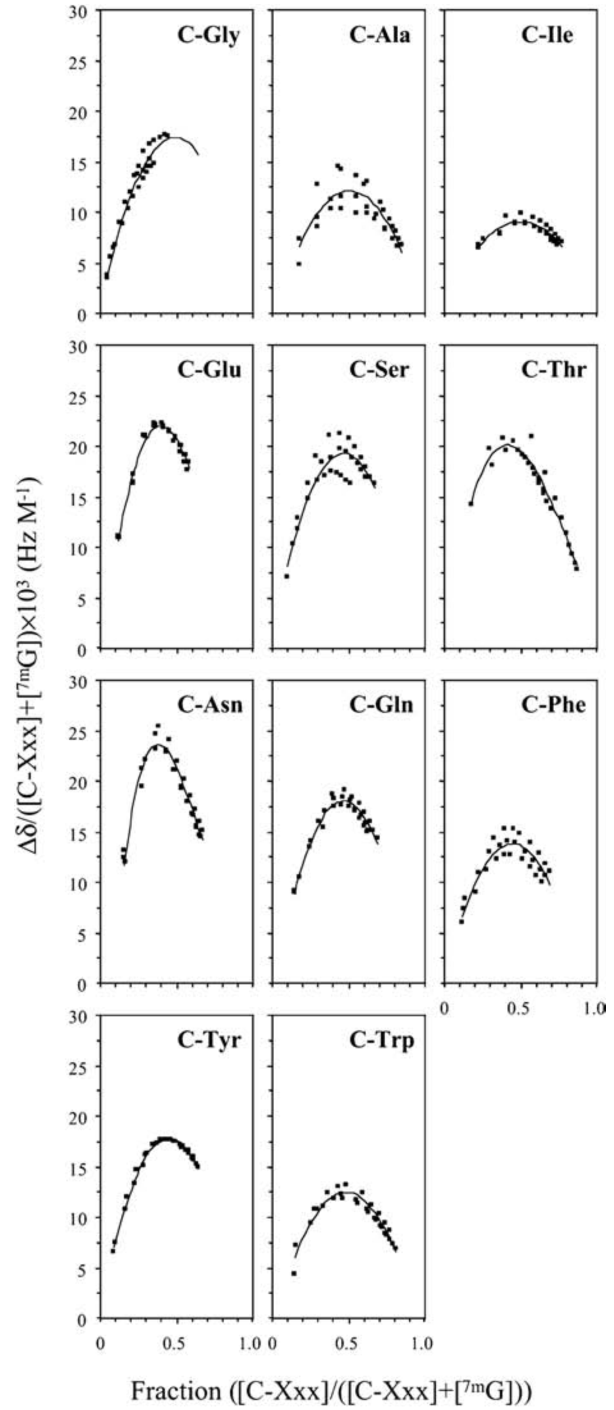


Figure 1. Stoichiometry for the complex of **1** and **2**. Chemical shift changes of **2**-H8 are plotted as a function of molar fraction.[9]

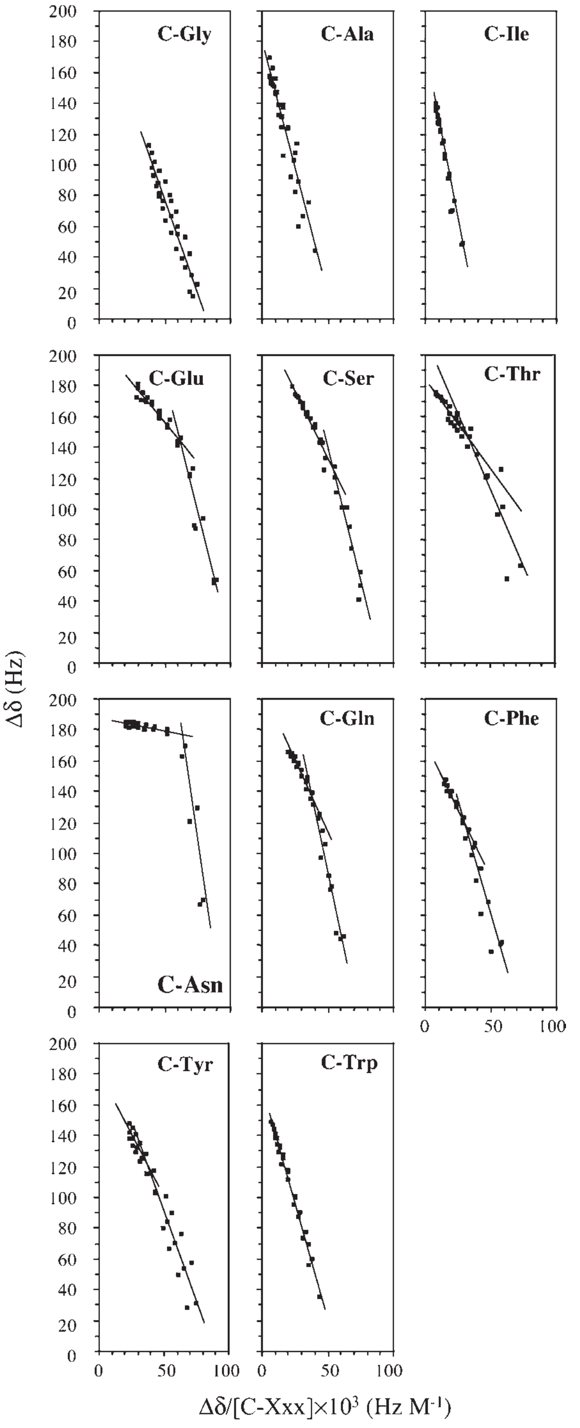


Figure 2. Eadie-Hoffstee plots for each **1** analogues. Chemical shift changes ($\Delta\delta$) of **2**-H8 are plotted as a function of $\Delta\delta$ per dipeptide concentrations.[9]

Trp has been considered to strongly interact with $N7$-quarternized guanines by π–π electron interactions,[10] and **1k** is, therefore, expected to show the higher or highest affinity for **2**. We do not have evidences, but the number of **2** molecules binding to **1g** (R_c = 1.63) suggests that the interaction mode between **1g** and **2** may be different from that observed in the complex structure.

Table 1. Summary of the interaction between **1** and **2**

	Xxx	$F_{\max}$	R_c	K, K_1[a]	K_2[a]
1a	Gly	0.50	1.00	0.41	
1b	Ala	0.50	1.00	0.30	
1c	Ile	0.50	1.00	0.22	
1d	Glu	0.40	1.50	0.95	0.30
1e	Ser	0.46	1.17	0.56	0.29
1f	Thr	0.42	1.38	0.86	0.51
1g	Asn	0.38	1.63	6.65	0.16
1h	Gln	0.46	1.17	0.54	0.24
1i	Phe	0.45	1.22	0.55	0.33
1j	Tyr	0.45	1.22	0.62	0.43
1k	Trp	0.49	1.04	0.34	

[a] Unit of association constants is $10^6\,M^{-1}$. K_1 and K_2 were obtained at higher and lower peptide concentration, respectively.

References and Notes

1 P. Narayanan, H. M. Berman, and R. Rousseau, *J. Am. Chem. Soc.*, **98**, 8472 (1976).

2 G. Lancelot, R. Mayer, and C. Helene, *J. Am. Chem. Soc.*, **101**, 1569 (1979).

3 M. Doi, M. Tarui, M. Ogata, A. Asano, and T. Ishida, *Acta Crystallogr.*, **C54**, 1941 (1998).

4 M. Doi, H. Miyako, A. Asano, and T. Ishida, *Anal. Sci.*, **15**, 109 (1999).

5 M. Doi, M. Tarui, and T. Ishida, *Anal. Sci.*, **16**, 557 (2000).

6 M. Tarui, N. Nomoto, Y. Hasegawa, K. Minoura, M. Doi, and T. Ishida, *Chem. Pharm. Bull.*, **44**, 1998 (1996).

7 A. Niedzwiecka, J. Marcotrigiano, J. Stepinski, M. Jankowska-Anyszka, A. Wyslouch-Cieszynska, M. Dadlez, A. C. Gingras, P. Mak, E. Darzynkiewicz, N. Sonenberg, S. K. Burley, and R. Stolarski, *J. Mol. Biol.*, **319**, 615 (2002).

8 J. Huber, U. Cronshagen, M. Kadokura, C. Marshallsay, T. Wada, M. Sekine, and R. Luhrmann, *EMBO J.*, **17**, 4114 (1998).

9 **2** was dissolved in 0.6 ml DMSO-d_6 at approximately 5 mM. Each analogue of **1** was also dissolved in DMSO-d_6 at approximately 200 mM, and 2–5 μl portion of this solution was added to the solution of **2**. Titration was performed up to approximately 10 mM on **1** and repeated three times. The chemical shift of **2**-H8 was measured relative for internal trimethylsilane at 0.0 ppm on Varian Inova-500 at 25 °C. The stoichiometry of interaction between **1** and **2** was calculated from the H8-chemical shift change ($\Delta\delta$/([C-Xxx]+[^{7m}G])) as a function of molar fraction [C-Xxx]/([C-Xxx]+[^{7m}G]). The molar fractions giving the maximum peak ($F_{\max}$) were obtained from the plots (Figure 1), and the molecule ratios of the complex (R_c) were calculated as $R_c = (1 - F_{\max})/F_{\max}$. The R_c value indicates the number of **2** molecules binding to **1**. Association constants (K) were estimated from the H8 chemical shift change by the Eadie-Hofstee plot using an equation: $\Delta\delta = -(1/K)(\Delta\delta/[\text{C-Xxx}]) + \Delta\delta_c$, where $\Delta\delta_c$ represents chemical shift change at completely complexed **1** and **2**. When the linear correlation was not entirely observed in the titration range, the Eadie-Hofstee equation was separately applied and association constants were estimated as K_1 and K_2 for high and low concentrations of dipeptide, respectively.

10 H. Ueda, M. Doi, M. Inoue, T. Ishida, T. Tanaka, and S. Uesugi, *Biochem. Biophys. Res. Commun.*, **154**, 199 (1988).