Thermodynamic Effect of Complementary Hydrogen Bond Base Pairing on Aromatic Stacking Interaction in the Guanine–X-Trp Complex (X = Adenine, Guanine, Cytosine, Thymine)

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Four kinds of X-Trp (X = adenine, guanine, cytosine, thymine) were synthesized as model compounds to investigate the effect of complementary hydrogen bond base pairing on the stacking interaction of Trp with nucleic acid base. Association constants (K_a) of these compounds with two guanine derivatives (9-ethylguanine and 9-ethyl-7-methylguanine) were determined by Eadie–Hofstee plots of ^{1}H-NMR titration experiments, and the thermodynamic parameters (ΔH, ΔS and ΔG) for the respective complexes were obtained by van't Hoff analyses based on the temperature dependence of the K_a values. The complexes were characterized by enthalpy/entropy compensations, where the interaction of cytosine-Trp with guanine derivatives was largely enthalpy-driven, accompanied by a small entropy component, whereas those of remaining complexes were all associated with a large increase in entropy, accompanied by a small positive enthalpy component. The present insight on the binding of X-Trp with a guanine base provides a thermodynamic basis for the importance of cooperative hydrogen bond pairing and aromatic stacking interactions in the specific recognition of a nucleic acid base pair by protein.

Key words thermodynamic parameter; stacking interaction; base pairing; tryptophan; ^{1}H-NMR; cooperative interaction

Hydrogen bonding and aromatic stacking interactions between nucleic acids or between a nucleic acid and an amino acid play an important role in determining the structural basis for molecular conformation and recognition. We have studied the physicochemical/stereochemical properties of these interactions by spectroscopic[1] and X-ray crystallographic investigations[2] using various model compounds or complexes, and have placed special emphasis on the stacking interaction between the tryptophan indole ring and a nucleic acid base. We have elucidated the quarternization of guanine N7 atom by protonation or alkylation as an important tool for the selective molecular recognition of nucleic acid base by protein, because it enhances significantly the stacking interaction with a tryptophan indole ring[3]; this is applicable to the specific interaction of the mRNA cap structure, characterized by an N7-methylated guanine base, with the eukaryotic initiation factor protein-4E.[4]

As a model to show the importance of the cooperative hydrogen bond pairing and aromatic stacking interactions for specific and selective molecular recognition, we reported the interaction between C-Trp (cytosine-1-yl-ethyl-L-tryptophan) and 9-ethylguanine (e^9G) or 9-ethyl-7-methylguanine (e^9m^7G) (Fig. 1) by ^{1}H-NMR and fluorescence experiments,[5] where the cooperation of the complementary hydrogen bond base pairing of guanine-cytosine and the aromatic stacking of guanine base-tryptophan indole ring was shown to cause much stronger molecular interaction than the simple summation of their respective interactions.

To further elucidate the structural basis for the specific and selective molecular recognition in terms of the effect

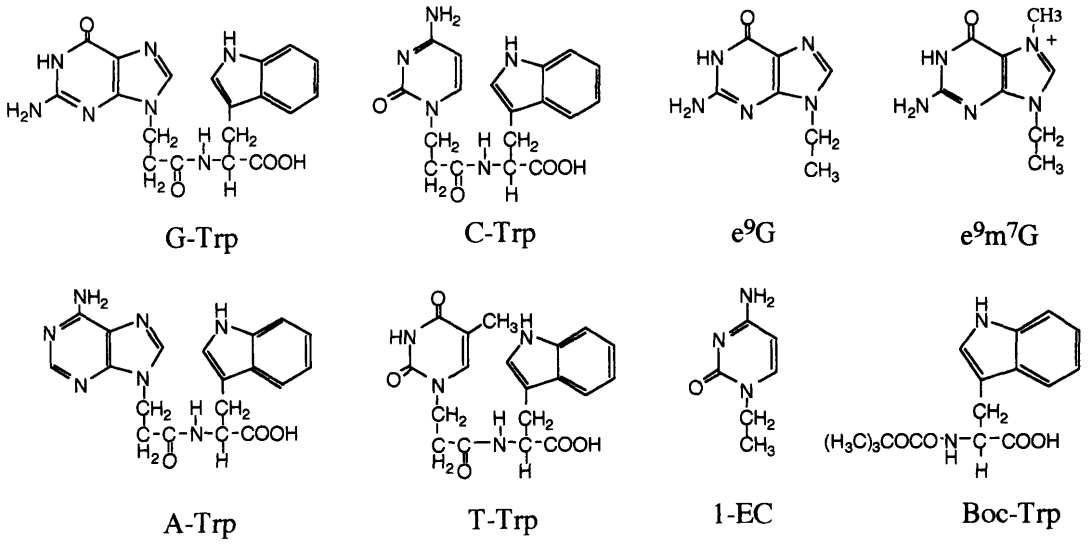

Fig. 1. Chemical Structures of Model Compounds Used in This Work

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of complementary hydrogen bond pairing on the stacking interaction between a guanine base and tryptophan, three kinds of X-Trp, *i.e.*, adenine-9-ylethyl-L-tryptophan (A-Trp), guanine-9-ylethyl-L-tryptophan (G-Trp) and thymine-1-ylethyl-L-tryptophan (T-Trp) (Fig. 1), were synthesized, and their association constants and thermodynamic parameters were determined for the interaction with two guanine derivatives, e^9G and e^9m^7G, hoping that these thermodynamic parameters would clarify the structural/thermodynamic requisite for the strict molecular recognition.

Experimental

Synthesis To synthesize X-Trp, it was necessary to prepare the intermediate compounds, 9-carboxyethyladenine (9-CEA), 9-carboxyethylguanine (9-CEG), 1-carboxyethylthymine (1-CET) and 1-carboxyethylcytosine (1-CEC).

9-Carboxyethyladenine (9-CEA) or 1-Carboxyethylthymine (1-CET) Adenine (6.8 g) or thymine (6.3 g) was dissolved in *N*,*N*-dimethylformamide (50 ml) containing 2-chloropropionic acid ethyl ester (10 ml), and stirred for 24 h at 70 °C in the presence of potassium carbonate (23 g).[6] Potassium carbonate was then removed by filtration and solvents were removed from the filtrate *in vacuo*, leaving a yellow oil. This residue was recrystallized from methanol–water, yielding a white solid. To a solution of this white solid in 30 ml of methanol was added 5 ml of 1 N sodium hydroxide. The solution was neutralized by potassium hydrogen sulfate, and a white powder was precipitated which was washed with water. The yields were 8.4 g (85%) of 9-CEA and 2.1 g (20%) of 1-CET.

9-Carboxyethylguanine (9-CEG) A guanine precursor, 2-amino-6-chloropurine (8.5 g), was carboxyethylated as above and refluxed in 100 ml of 0.1 N hydrochloric acid for 5 h.[6] The clear solution was cooled and neutralized with sodium hydroxide, yielding 6.9 g (62.1%) of an off-white solid.

1-Carboxyethylcytosine (1-CEC) 1-CEC was synthesized according to the previously described method.[7]

X-Trp 9-CEA, 9-CEG, 1-CET or 1-CEC was coupled with L-tryptophan methylester by the usual peptide condensation after protection of the amino group with dimethoxytritylchloride (DMTr-Cl). Deprotection of the DMTr and the methylester group by 80% acetic acid and 1 N sodium hydroxide, respectively, produced the desired compounds, A-Trp (55.0%), G-Trp (33.8%), T-Trp (70.6%), C-Trp (9.8%).

E^9G or E^9m^7G e^9G and e^9m^7G were synthesized as described.[3b,6]

1-Ethylcytosine (1-EC) Cytosine (5.5 g) was dissolved in 100 ml of dimethyl sulfoxide (DMSO) containing ethylchloride (7 ml), and stirred for 24 h in the presence of potassium carbonate (23 g) at room temperature.[6] Potassium carbonate was removed by filtration and solvents were removed from the filtrate *in vacuo*, leaving an amorphous off-white solid. This residue was dissolved in hot 50% methanol, and cooled to 5 °C, yielding 4.1 g (65%) of white plates.

The purities of these synthetic compounds were confirmed by HPLC on an octadecyl silica (ODS) column and the peak assignment of ^{1}H-NMR spectra.

^{1}H-NMR Titration and Association Constant (K_a) The ^{1}H-NMR spectra were determined using a Varian XL-300 (300 MHz for ^{1}H, Fourier transform mode) instrument fitted with a variable-temperature probe (accuracy to 1 °C). DMSO-d_6 was used as solvent because of the solubility limit of each sample in another solvent such as D_2O. The chemical shifts were measured with tetramethylsilane (TMS) as an internal standard. Titration experiments were performed 3 times at each temperature (298, 308, 323, 331 and 338 K) by adding 2.5—50 μl aliquots of 500 mM X-Trp to a 500 μl solution of 5 mM guanine derivative. Association constant (K_a) was estimated from the chemical shift changes of the C_8 proton of the guanine derivative using the Eadie–Hofstee plot[8]:

$$\Delta\delta = \frac{1}{K_a}\cdot\frac{\Delta\delta}{[M]} + \Delta\delta_c \quad (1)$$

where $\Delta\delta = \delta_0 - \delta$, $\Delta\delta_c = \delta_c - \delta$, and δ_0 and δ are the chemical shifts of C_8-proton of the guanine derivative in the absence and presence of X-Trp at the concentration of [M], respectively, and δ_c is that of guanine derivative completely complexed with X-Trp.

Thermodynamic Parameters The thermodynamic parameters of enthalpy change (ΔH^0), entropy change (ΔS^0) and Gibbs free energy change (ΔG^0) of interactions between X-Trp and guanine derivative were obtained from the following equations:

$$\Delta G^0 = -RT\ln K_a \quad (2)$$

$$\Delta G^0 = \Delta H^0 - T\Delta S^0 \quad (3)$$

Then,

$$\ln K_a = -\Delta H^0/RT + \Delta S^0/R \quad (4)$$

where R is the gas constant (1.987 cal mol^{-1} deg^{-1}), T is the temperature in Kelvin, and K_a is the association constant determined from Eq. 1. The value of ΔH^0 was evaluated from the slope of the van't Hoff relation of Eq. 4, and then ΔG^0 and ΔS^0 were calculated from Eqs. 2 and 3, respectively.

Results and Discussion

Figure 2 shows the ^{1}H-NMR spectral change of e^9G (a) and e^9m^7G (b) as a function of C-Trp concentration; the spectral changes were also observed for G-Trp, A-Trp and T-Trp, though their patterns were different depending on the respective interaction pairs. No notable nuclear

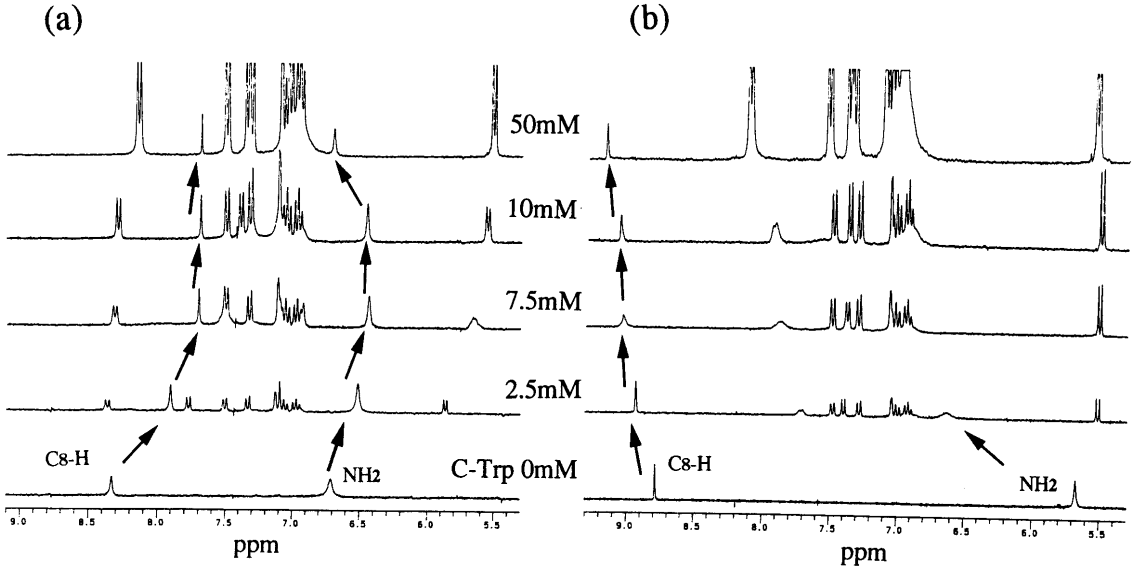

Fig. 2. ^{1}H-NMR Spectra of e^9G (a) and e^9m^7G (b) as a Function of C-Trp Concentration in DMSO-d_6

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Overhauser effects (NOEs) were observed between the interaction pair, and this may be due to the relatively weak complex formation in the DMSO solution. However, the spectral change of the respective protons suggested the complex formations of all interaction pairs through the aromatic stacking interaction characterized by the upfield shift of the proton and the hydrogen bonding formation by the downfield shift of the proton.

Determination of the K_a value was done using the chemical shift change of guanine C_8-proton.[9] As exemplified in Fig. 2, the C_8-proton of e^9G shifted upfield with the increase of X-Trp, due to the current effect[11] of the indole ring of X-Trp stacked on e^9G. In the case of e^9m^7G, however, the downfield shift of the C_8-proton was observed, which is due to an electrostatic interaction between the electron-poor C_8-proton and the π-electron-rich indole ring of X-Trp; the N7-methylation of the guanine base is known to increase the electron positivity of C_8-proton.[3b] Thus, the K_a value obtained from the chemical shift change of this proton reflects the strength of the molecular association based on the different binding force in the X-Trp–e^9G and e^9m^7G pairs.

Figure 3 gives the representative Eadie–Hofstee plots of e^9m^7G C_8-proton as a function of X-Trp concentration at 298 K; the K_a values were determined from respective slopes, calculated by the least-squares linear regression analysis (correlation coefficient >0.94). Table 1 gives the K_a values of X-Trp–e^9G and e^9m^7G pairs at each temperature. Although a quantitative discussion concerning the structural preference of e^9G or e^9m^7G for the interaction with X-Trp is impossible based on these K_a values alone, it is obvious that the interaction between C-Trp and the guanine bases increases proportionally as the temperature decreases; this reflects clearly that the association process of the guanine base–C-Trp pair is exothermic and is different from the other pairs, all of which are endothermic.

To clarify what factor induces such a difference, the thermodynamic parameters were measured for respective interacting pairs. Figure 4 shows van't Hoff plots (Eq. 4)[12]; the plots showed good linearities in the temperature range investigated here (correlation coefficient >0.90), indicating that ΔH^0 is independent of the temperature. It is noteworthy that only C-Trp shows a positive linear equation, while the others show a negative inclination. The thermodynamic parameters are given in Table 2. Since

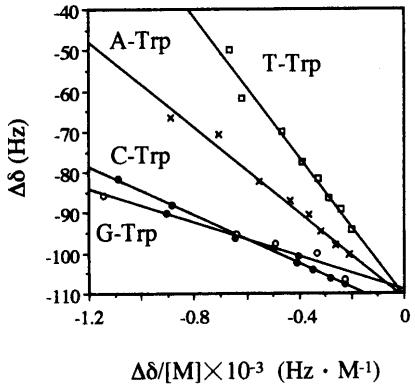

Fig. 3. Eadie-Hofstee Plots of Chemical Shift Changes of e^9m^7G C_8-Proton as a Function of X-Trp Concentration

Table 1. Association Constants (K_a) between X-Trp and e^9G or e^9m^7G in DMSO-d_6[a]

	e^9G ($\times 10^{-3}$ M)					e^9m^7G ($\times 10^{-2}$ M)				
	298 K	308 K	323 K	331 K	338 K	298 K	308 K	323 K	331 K	338 K
C-Trp	11 (1)	10.0 (5)	5.9 (3)	5.8[b]	—[c]	3.2 (2)	2.2 (3)	2.02 (7)	1.89 (7)	1.65 (8)
G-Trp	—[c]	—[c]	—[c]	—[c]	—[c]	4.6 (2)	5.1[b]	5.4[b]	5.5 (6)	6.0[b]
A-Trp	—[c]	4.9[b]	—[c]	8.4 (5)	8.9 (6)	1.89 (4)	2.2 (1)	2.31 (5)	2.6 (1)	2.64 (9)
T-Trp	1.6[b]	2.2 (2)	2.96 (9)	3.5[b]	4.3 (1)	1.2 (1)	1.3 (1)	1.38 (3)	1.3[b]	1.47 (3)
Boc-Trp	0.035[a]	0.042[b]	0.055[b]	0.064[b]	0.068[b]	0.022[b]	0.023[b]	0.025[b]	0.026[b]	0.027[b]

a) The estimated standard deviations for K_a values are given in parentheses. *b*) The average of two reliable values. *c*) Accurate K_a value was not obtained.

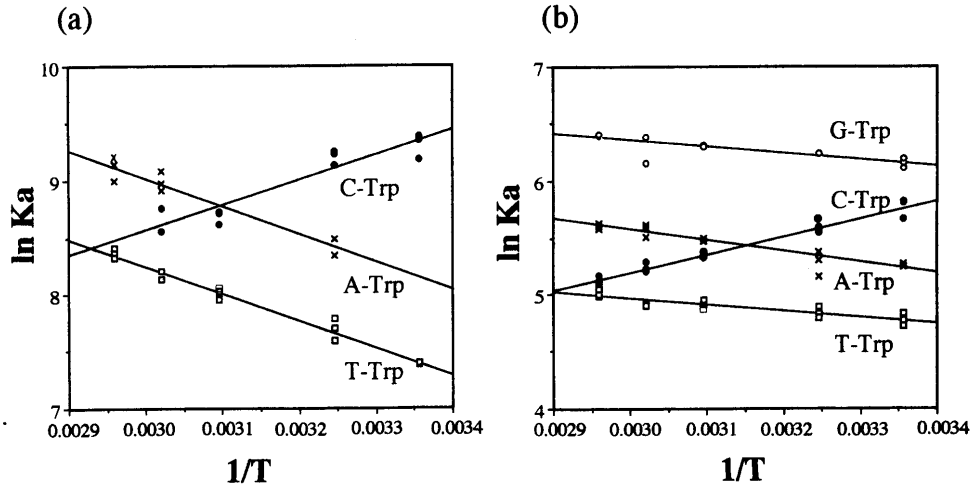

Fig. 4. Van't Hoff Plots of Association Constants between X-Trp and e^9G (a) or e^9m^7G (b)

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Table 2. Thermodynamic Parameters for the Associations between X-Trp and e^9G or e^9m^7G, Determined from ^1H-NMR Titration[a]

	e^9G			e^9m^7G		
	ΔH^0 (kJ/mol)	ΔS^0 (J/mol K)	$\Delta G^0_{(298K)}$ (kJ/mol)	ΔH^0 (kJ/mol)	ΔS^0 (J/mol K)	$\Delta G^0_{(298K)}$ (kJ/mol)
C-Trp	−18.0	16.7	−23.0	−12.1	6.3	−14.2
G-Trp	—	—	—	4.6	66.9	−15.1
A-Trp	20.5	136.8	−20.1	6.7	66.5	−13.0
T-Trp	19.2	126.4	−18.4	3.3	51.5	−11.7
Boc-Trp	11.3	69.0	−9.2	5.4	43.1	−7.5

a) The mean errors are 25% and 20% or less for the data of e^9G and e^9m^7G, respectively.

the ΔG^0 values (at 298 K) for all interaction pairs are negative, one could say that these pairs exist as complexes of stabilization energies of −11.7——23.0 kJ/mol. The ΔG^0 value indicates that e^9G forms the most stable complex with C-Trp, and e^9m^7G with G-Trp, in agreement with the K_a values obtained at 298 K (Table 1). On the other hand, it is noteworthy that the binding of C-Trp to e^9G or e^9m^7G is enthalpy-driven (negative enthalpy), in contrast with that of A-Trp, G-Trp or T-Trp, complex formations of which are all entropy-driven (large positive entropy). This implies that the main factor contributing to complex formation with the guanine base is different between C-Trp and the others.

Understanding of the structural requisite for the specific molecular recognition necessitates elucidation of what kind of interaction mode causes the enthalpy stabilization observed only in the C-Trp–guanine interaction pair. Thus, the associations and thermodynamic parameters of the component molecules of C-Trp, 1-EC and Boc-Trp with guanine derivatives were examined. The results are given in Tables 1 and 2, from which the data of 1-EC were excluded because its interactions were too weak to be considered accurate under the present experimental conditions. Concerning the Boc-Trp–guanine interaction pairs, the complex formations are entropy-driven, although their interactions are much weaker than those of X-Trp. Since it has been generally believed[2,13] that the interaction between the guanine base and tryptophan indole ring is mainly due to the parallel π–π stacking interaction, the structural ordering by such interaction alone without any conformational change could be associated with a negative entropy change. However, the fact that positive entropy change was caused by the complex formation implies a large positive contribution of several sources such as hydrophobic interaction and solvent effects. It would be most reasonable to assume the contribution from the breakage of the ordered structure of the guanine derivative to have been formed in the isolated state by the interaction with Boc-Trp. Also, a positive entropy may be in part due to the usage of DMSO solvent; it is generally accepted that the DMSO tends to break down the aromatic stacking interaction. In any event, the entropy-driven interaction observed in the complex pairs of G-, A-, and T-Trp is related with the aromatic stacking interaction, although the consequent complex structure becomes less ordered.

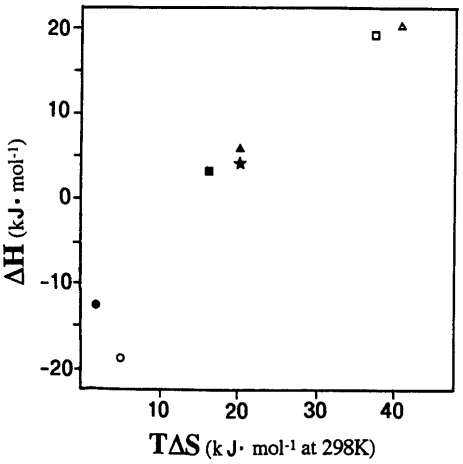

Fig. 5. Enthalpy/Entropy Compensation in the e^9G– and e^9m^7G–X-Trp Interaction Pairs

○, e^9G–C-Trp; ●, e^9m^7G–C-Trp; △, e^9G–A-Trp; ▲, e^9m^7G–A-Trp; □, e^9G–T-Trp; ■, e^9m^7G–T-Trp; ★, e^9m^7G–G-Trp.

The enthalpy/entropy compensation in the X-Trp–e^9G and e^9m^7G interaction pairs is shown in Fig. 5. This figure highlights the difference between factors contributing to the stabilizations of X-Trp–guanine derivative complexes. Respective pairs were classified into three groups according to the difference of interaction mode, *i.e.*, group I (e^9G and e^9m^7G–C-Trp), II (e^9m^7G–A-Trp, G-Trp and T-Trp) and III (e^9G–A-Trp and T-Trp); the difference between groups II and III could be due to the strength of stacking interaction between the Trp indole ring and guanine base.[3] Since the enthalpy/entropy compensation has been used as a clear index for the base-pair mismatches on the complex stability,[14] it is reasonable to assume that while the binding of guanine derivative to G-Trp, A-Trp or T-Trp is strongly affected by the entropy of stacking interaction between the guanine base and indole ring, the enthalpy-driven complex formation of C-Trp originates from the complementary hydrogen bond pairing of cytosine–guanine bases in addition to the stacking interaction of guanine base–indole ring. Furthermore, the significant entropy loss in C-Trp–guanine interaction pair, compared with those of the other pairs, implies that the interaction between C-Trp and the guanine derivative is highly specific, and the complex formation of C-Trp is stereostructurally much more ordered than those of the others. Such a situation could be explained by the synergism of aromatic stacking interaction and complementary hydrogen bond pairing which restricts the motion of the C-Trp molecule by its binding with the guanine base, consequently leading to a large loss of positive entropy of binding and production of large negative enthalpy as a result of enthalpy/entropy compensation. In this sense, the relatively strong association of G-Trp with e^9m^7G, which is dominated by the entropy-driven stacking interaction, could be nonspecific. In conclusion, it could be possible that the change from the entropy-driven complex formation of Boc-Trp–guanine to the enthalpy-driven one of C-Trp–guanine is mainly due to the mutual cooperation of hydrogen bond pairing and stacking interactions.

The true effect of complementary hydrogen bond base

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pairing on the stacking interaction of Trp with nucleic acid base requires that the models be studied in water. Since the problem of solubility forced using the solvent DMSO to measure the spectral changes by ^{1}H-NMR, the results may not reflect the original feature directly. However, we believe that the insights obtained illustrate some interesting aspects of electrostatic and solvophobic effects in mediating recognition.

Some protein–receptor interactions where information transfer is involved are reportedly enthalpy-driven (for example, agonist), and others where information transfer is not involved are entropy-driven (for example, antagonist).[15] Such a thermodynamic phenomenon may be important for the expression and/or transmission of a cellular genome. In this context, the present results provide useful information on the structural/thermodynamic requisite and interaction force which ensure the specific molecular recognition of nucleic acid base or its sequence by a protein.

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