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Quartet formation of a guanine derivative with an isopropyl group: crystal structures of "naked" G-quartets and thermodynamics of G-quartet formation†

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The formation of guanine quartets with 9-isopropylguanine (^{i}PG) is discussed in organic solvents. Crystal structures of the ^{i}PG quartets were determined by X-ray crystallography with template cations (Na^+ and Ca^{2+}) and the structure without a template cation was also obtained by virtue of the stabilization by intermolecular hydrogen bonding with water molecules of crystallization. The difference in the quartet formation of ^{i}PG in the presence and absence of a template cation was clearly demonstrated by ^{1}H NMR measurements in $CDCl_3$–CH_3OH mixed solvents. The quartet formation is mainly governed by the enthalpy gain due to the electrostatic interaction between the O6 oxygen in ^{i}PG and the template cations in the presence of the cations rather than the intermolecular hydrogen bonding, while desolvation of ^{i}PG is the dominant factor for the formation in the absence of cations. In the presence of Na^+ and Ca^{2+}, ΔH and ΔS values in the formation of ^{i}PG-4–Na^+ and ^{i}PG-4–Ca^{2+} complexes were determined to be $\Delta H = -8.4$ kcal mol^{-1} and $\Delta S = +50$ cal mol^{-1} K^{-1} for Na^+ and $\Delta H = -12.9$ kcal mol^{-1} and $\Delta S = +34$ cal mol^{-1} K^{-1} for Ca^{2+} on the basis of van't Hoff plots attained from the results of temperature-dependent UV-Vis spectroscopic measurements.

Introduction

Since Gellert and coworkers suggested the guanine tetrad structure was linked by intermolecular hydrogen bonding in 1962,[1] the characteristic guanine-quartet (hereafter referred to as G-4) structure (Fig. 1) has attracted a great deal of attention of researchers in many different areas.[2–8] Especially, integrated G-4 structures which exist at the edge of the DNA chain, called guanine quadruplexes (hereafter denoted as G-quadruplex), have been reported in large numbers.[9–15] So far, many kinds of G-quadruplexes included in higher-order structures have been crystallised with various template cations.[16,17] In parallel, studies on the interaction of molecules to show a certain selectivity for the part of the G-quadruplex have been conducted.[18–27] The main purpose of those studies has been the acquirement of an anticancer effect. The effect is based on inhibition of telomerase binding to the G-quadruplex moiety of DNA by protecting the moiety with the molecules. G-quadruplexes have also been utilized to develop functional biomaterials: the cavity of G-quadruplex can include guest ions, which can be applied to ion-conducting materials and so on.[28,29]

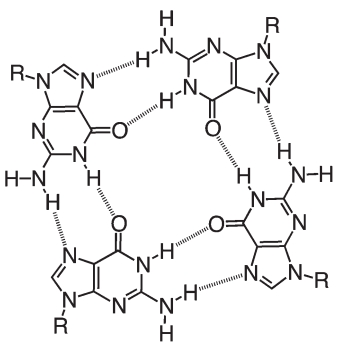

Fig. 1 Schematic description of G-quartet (G-4).

The study on solution behaviour of guanine and its derivatives in organic solvents should be important for elucidating the dynamics of the G-4 structure formation by intermolecular hydrogen bonding in detail. However, the formation dynamics of a discrete G-4 structure is yet to be well investigated. The

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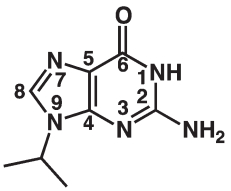


Fig. 2 The structure of 9-isopropylguanine (^{i}PG).

lack of the thermodynamic data on the formation of a discrete and single-layer G-4 structure has hampered the discussion on the details of the G-4 formation, including the driving force of the formation. In fact, G-quadruplex structures involve too many complicated factors including noncovalent intermolecular interactions[30] provided by many functional groups such as sugar and phosphate moieties, which cover the part of G-4 cores.[31,32] Therefore, interactions of external molecules with a G-quadruplex have been observed as interactions with the peripheral groups, not direct interactions with the G-4 units. In addition, the conformational regulation of the peripheral moieties has been considered to be difficult for the formation of supramolecular assemblies of guanine derivatives linked only by intermolecular hydrogen bonding in solutions.[33–35] Thus, G-4 formation with the use of a guanine derivative without complicated peripheral groups is indispensable to clarify the intrinsic thermodynamics of the complementary hydrogen bonding and interaction of G with a template cation in a discrete and single-layer G-4 structure.

In this work, 9-isopropylguanine (^{i}PG)[36] having the isopropyl group at the 9-N position was used to form the G-4 structure (Fig. 2). The introduction of the small isopropyl group allowed us not only to improve the solubility into organic solvents but also to accommodate the crystallization of "naked" G-4 supramolecules, without any steric protection of the G-4 core by such deoxyribose-phosphate moieties in guanosine derivatives reported so far. The strategy presented herein makes it possible for the first time to discuss the intrinsic characteristics of the G-4 structure and the detailed thermodynamics of the G-4 formation to elucidate the role of the template cations. Based on the observations, we would clarify the switching of the driving force of the G-4 formation from desolvation of the guanine molecule in the absence of template metal ions to electrostatic attractive interactions between ^{i}PG and metal ions in the presence of the template cations.

Results and discussion

1. Crystal structures of G-quartets

In all cases, crystallization of G-4 assemblies was conducted in methanol solutions with vapour diffusion of diethyl ether. Crystal structures of the G-4 assemblies made of ^{i}PG are shown in Fig. 3. Even in the absence of a template cation, it was clearly demonstrated that ^{i}PG could form the quartet

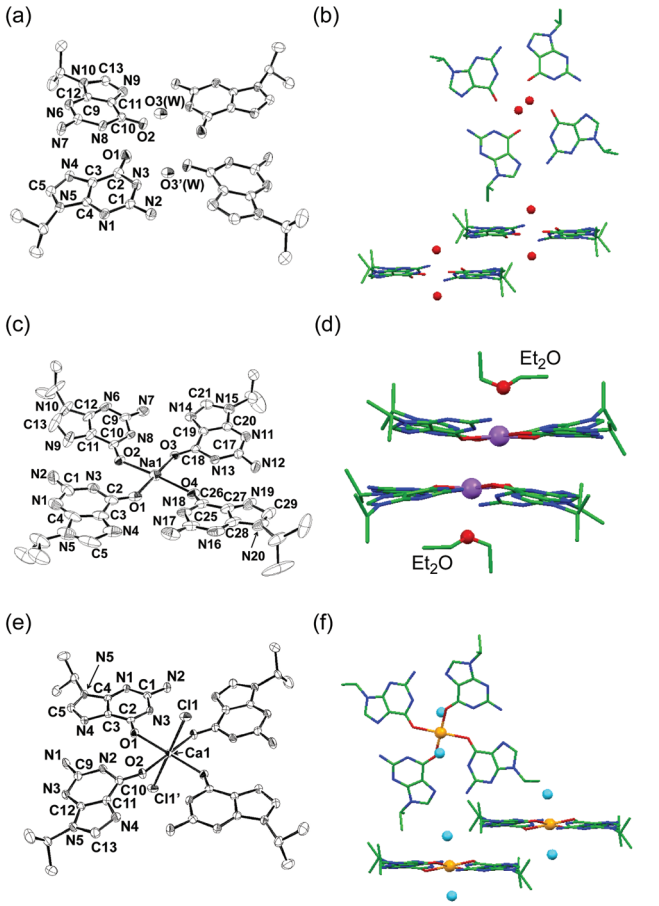


Fig. 3 Crystal structures of quartets of ^{i}PG with and without template cations: (a) ORTEP drawing of the ^{i}PG-4 structure without template cations (70% probability thermal ellipsoids); (c) that with Na^{+} derived from $NaBPh_4$ (30% probability thermal ellipsoids). BPh_4^{-} was omitted for clarity; (e) that with Ca^{2+} (70% probability thermal ellipsoids). Partial packing structures for (a), (c), and (e) are depicted in (b), (d) and (f), respectively.

(^{i}PG-4) structure in the crystal with the aid of two water molecules (^{i}PG-4–H_2O; Fig. 3(a) and Fig. S1 in ESI†). As observed in the G-quadruplex, the G-4 assembly was formed by the complementary intermolecular hydrogen bonding between the O6 and N1*H* atoms and that between the 2-amino group and the 7-nitrogen atom. Intermolecular hydrogen bonding was observed among the water molecule of crystallization and the oxygen atoms at the 6-position in ^{i}PG showing the interatomic distance (O1···O3(W) and O2···O3(W), 2.85 and 3.03 Å, respectively). This hydrogen bonding could assist the G-4 formation to compensate for the lack of stabilization by template cations. In the packing structure, the ^{i}PG-4 planes overlap partially due to CH/π and π–π interactions (Fig. 3(b)). The integration of the ^{i}PG-4 planes by stacking as seen in the G-quadruplex could not be observed, because the two hydrogen atoms of the water molecule of crystallization can direct only to one ^{i}PG-4 plane and the water molecule cannot link two ^{i}PG-4 planes. Although a G-4 structure without template cations has been reported,[37,38] no report has appeared on "naked" G-4

Table 1 Summary of the interatomic distances related to template cations, those of N1H···O6 as intermolecular hydrogen bonding in ^{i}PG-4 structures, and interplane distances between two G-4 assemblies

^{i}PG-4–H_2O		^{i}PG-4–Na^+		^{i}PG-4–Ca^{2+}	
Water···O (Å)			Cation···O (Å)		
O3···O1	2.852(1)	Na1···O1	2.257(4)	Ca1···O1	2.3212(9)
		Na1···O2	2.300(3)		
O3···O2	3.030(1)	Na1···O3	2.320(4)	Ca1···O2	3.3232(9)
		Na1···O4	2.357(3)		
		N*H*···O (Å)			
N8···O1	2.849(2)	N18···O1	2.876(5)	N8···O1	2.941(2)
		N3···O2	2.878(6)		
N3···O2	2.782(1)	N8···O3	2.872(4)	N3···O2	2.943(1)
		N13···O4	2.906(5)		
		G-4 plane···G-4 plane (Å)			
3.597		3.626		3.652	
		Selected bonds			
		Na1···O4′	2.729(4)	Ca1···Cl1	2.6980(4)
		Na1···O5	2.609(7)		

structures without steric protection by large substituents attached to the guanine scaffold.

In the presence of sodium cation derived from $Na[B(C_6H_5)_4]$ ($NaBPh_4$), the crystal structure of ^{i}PG clearly demonstrated the formation of the G-4 dimer. The Na^+ ion resided nearly in the mean plane of the ^{i}PG-4 assembly. Two of the G-4 assemblies formed a dimer by the electrostatic interaction between Na^+ and O6 of guanine in the other ^{i}PG-4 plane, showing the interatomic distance of Na···O4′ to be 2.729(4) Å (Fig. 3(c, d) and Fig. S2 in ESI†). One diethyl ether molecule was found to bind to the Na^+ ion with the interatomic distance of 2.609(7) Å to cap the dimeric structure, preventing further stacking with the ^{i}PG-4 assemblies. In the dimeric unit in the crystal, π–π interactions are operating with the closest interatomic distance of 3.422(8) Å.

We also examined the formation of the ^{i}PG-4 structures in the presence of a calcium ion derived from $CaCl_2$. In this case, a ^{i}PG-4 structure was formed and the Ca^{2+} ion also resided in the mean plane of the ^{i}PG-4 structure. Two chloride counter anions interacted with the calcium ion with the Ca^{2+}···Cl^- distance of 2.6980(4) Å both above and below the mean plane of the ^{i}PG-4 bound to Ca^{2+} (^{i}PG-4–Ca^{2+}) (Fig. 3(e) and Fig. S3 shown in ESI†). The packing structure is similar to that of the water-assisted ^{i}PG-4 assembly: As well as in the case of the water-assisted ^{i}PG-4, two of the ^{i}PG-4–Ca^{2+} assemblies stack *via* CH/π and π–π interactions to form a dimeric motif in the crystal (Fig. 3(f)).

Each distance between the cation and O6 of guanine in the corresponding ^{i}PG-4 plane and that between N1H and O6 for the hydrogen bonding to form the ^{i}PG-4 structure were slightly different among three kinds of ^{i}PG-4 structures. However, the distances fall in the normal range of those reported in G-quadruplexes, *i.e.*, 2.3 Å and 2.9 Å, respectively,[39] as summarized in Table 1. Thus, the G-4 formation can be assisted by electrostatic interactions of O6 in guanine with a cationic entity or water molecules having polarized O–H bonds, regardless of the steric protection by bulky peripheral substituents.

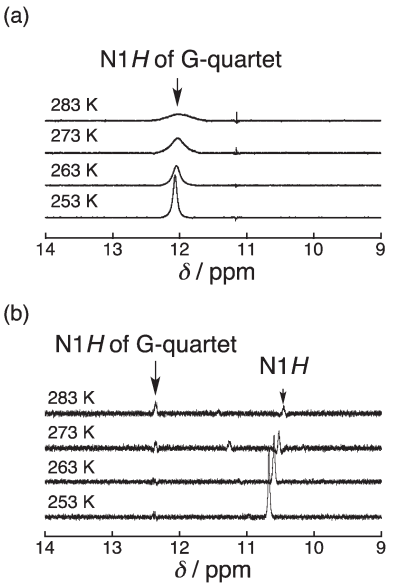


Fig. 4 Temperature dependent ^{1}H NMR studies of ^{i}PG with $NaBPh_4$. Conditions: (a) ^{i}PG (19 mM) and $NaBPh_4$ (5.4 mM) in $CDCl_3$ and CH_3OH (600 μl (30 : 1 v/v)); (b) ^{i}PG (3.6 mM) in $CDCl_3$ and CH_3OH (600 μl (5 : 1 v/v)).

2. Discussion of G-quartet formation in organic solvents

Observations of the ^{i}PG-4 structure formation in organic solvents were carried out using ESI-MS, UV-Vis and ^{1}H NMR measurements. Since ^{i}PG is capable of forming a variety of supramolecular structures by virtue of intermolecular hydrogen bonding, including a ribbon structure as shown in Fig. S4 in ESI.† Thus, the regulation of the supramolecular structure should be carried out under strictly optimized conditions in organic solvents to form the G-4 structure selectively.

In general, the following issues have been considered: (1) the proton of N1*H* fixed into a G-4 assembly by intermolecular hydrogen bonding does not undergo exchange with deuterium of solvents, and (2) noncovalent intermolecular interactions including hydrogen bonding and electrostatic interaction in G-4 become stronger at low temperature, and then the G-4 structure is stabilized by the decrease of enthalpy beyond the decrease of entropy.[40] In the presence of Na^+, the proton signal of N1*H* of ^{i}PG forming the ^{i}PG-4 assembly can be detected around 12 ppm in the mixed solvent of CH_3OH and $CDCl_3$. The signal became clearer on cooling down to 253 K (Fig. 4(a)). On the other hand, in the absence of Na^+, the ^{i}PG-4 structure could not be formed even on cooling down to 253 K. Instead, the chemical shift of the proton of N1*H* of ^{i}PG without forming the ^{i}PG-4 structure (δ = 10.5 ppm) could be observed clearly at low temperature. In addition, on cooling, the signal of N1*H* showed a downfield shift due to intermolecular hydrogen bonding probably between the guanine derivative and methanol (Fig. 4(b)). This result indicates that, in the absence of electrostatic interaction with template cations, the solvation of the ^{i}PG molecule by methanol exerts a stronger effect in the methanol solution of ^{i}PG rather than intermolecular hydrogen bonding to form the ^{i}PG-4 structure. This result

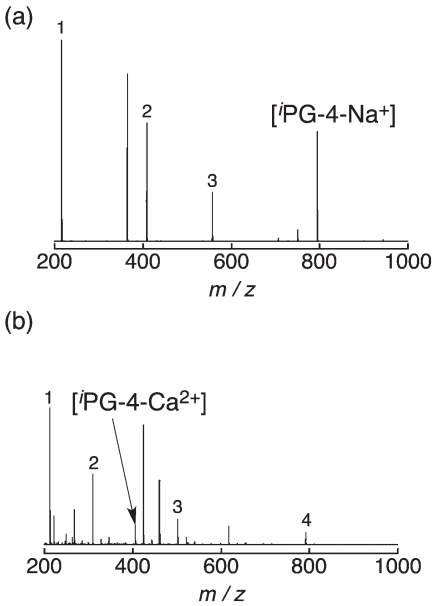


Fig. 5 ESI-MS spectra of self-assembled species of ^{i}PG with (a) $NaBPh_4$ and (b) $CaCl_2$; in methanol at room temperature under N_2. Conditions: (a) ^{i}PG (15 mM), $NaBPh_4$ (6.8 mM); (b) ^{i}PG (18 mM), $CaCl_2$ (8.4 mM). Peaks: (a) 1: ^{i}PG–Na^+ (m/z = 216.0), 2: ^{i}PG-2–Na^+ (m/z = 409.1), 3: ^{i}PG–$NaBPh_4$–Na^+ (m/z = 558.1); (b) 1: ^{i}PG-2–Ca^{2+} (m/z = 213.0), 2: ^{i}PG-3–Ca^{2+} (m/z = 309.6), 3: ^{i}PG-5–Ca^{2+} (m/z = 502.7), 4: ^{i}PG-8–Ca^{2+} (m/z = 792.3).

suggests that the gain of enthalpy by $Na^+\cdots O$ interaction can compensate for the instability caused by desolvation.

ESI-MS measurements of ^{i}PG in the presence of template cations allowed us to detect the formation of the ^{i}PG-4 structure clearly even in the methanol solution. Upon addition of $NaBPh_4$ to provide Na^+ as a template cation, a peak cluster was observed at m/z = 795.2 (calcd, 795.4), assignable to that derived from ^{i}PG-4–Na^+ (Fig. 5(a) and Fig. S5 in ESI†). In the presence of Ca^{2+}, a peak cluster assigned to $[^{i}\text{PG-4–}Ca^{2+}]^{2+}$ was observed at m/z = 406.1 (calcd, 406.2; see Fig. S6 in ESI†), also confirming its formation.

Further scrutiny was given to the formation of ^{i}PG-4 in the presence of the template cations in CH_3OH–$CHCl_3$ mixed solution by UV-Vis absorption spectroscopy. The absorption spectra of ^{i}PG showed red shifts in the course of addition of $NaBPh_4$ or $CaCl_2$ (Fig. 6(a) and see Fig. S7(a) in ESI†). The shift was assigned to form G-4–cation complexes: a Job's plot for ^{i}PG and $NaBPh_4$ gave the maximum at 0.2 mole fraction, indicating the 4 : 1 complexation of ^{i}PG with sodium ion (Fig. 6(c)). In the presence of an excess amount of Na^+, however, the presence of a 1 : 1 complex of ^{i}PG with Na^+ was observed by ESI-MS spectrometry (see Fig. S8 in ESI†). In contrast, the Job's plot for ^{i}PG and $CaCl_2$ afforded two maximums at 0.2 and 0.5, indicating that ^{i}PG-4–Ca^{2+} is formed at lower concentration of Ca^{2+}, however, a 1 : 1 complex (^{i}PG–Ca^{2+}) became dominant at higher concentrations of Ca^{2+} (see Fig. S7(b) in ESI†). It should be noted that the Ca^{2+} ion binds to guanine more tightly than the Na^+ ion due to the higher cationic charge, even though the Cl^- anion may interact with the Ca^{2+} ion in solution to reduce the Lewis acidity.

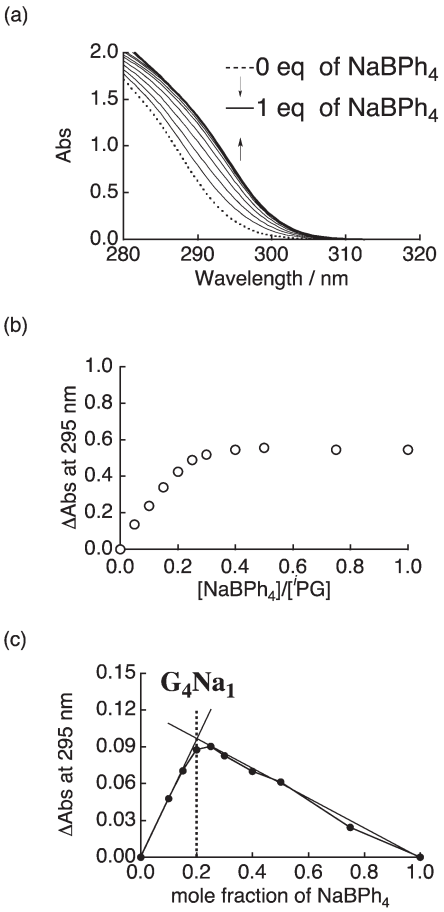
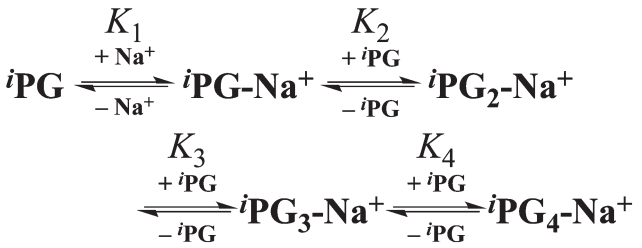


Fig. 6 UV-Vis spectral change by adding (a) $NaBPh_4$, (b) titration curve at 295 nm and (c) Job's plot of the ^{i}PG–Na^+ complex: Conditions: (a) ^{i}PG (207 μM) in CH_3OH and $CHCl_3$ (1 : 74 v/v), the additional $NaBPh_4$ (15.5 mM) in CH_3OH and $CHCl_3$ (1 : 9 v/v); (b) the total mole concentration was adjusted to 41.3 μM in CH_3OH and $CHCl_3$ (1 : 374 v/v), ^{i}PG (0.62 mM) in CH_3OH and $CHCl_3$ (1 : 24 v/v), $NaBPh_4$ (0.62 mM) in CH_3OH and $CHCl_3$ (1 : 24 v/v); all the measurements were carried out at room temperature.

In the range under 0.5 mole fraction in the Job's plots, allosteric effects could not be observed clearly on the sequential process relating to the formation of ^{i}PG-4–cation complexes in both the cases of Na^+ and Ca^{2+} (see Fig. 6(b) and see the inset of Fig S7(a) in ESI†). This indicates that the intermolecular hydrogen bonding to form the G-4 structure is much weaker than the electrostatic interaction between the O6 oxygen of guanine and the template cation. Therefore, in the presence of a template cation, the main driving force of the G-4 formation is the interaction of the guanine O6 oxygen with the template cation.

3. Thermodynamics of the formation of G-quartet analysed by van't Hoff plots

As mentioned above, the electrostatic interaction among the template cation and the O6 oxygen of guanine is much stronger than the intermolecular hydrogen bonding to form the G-4 structure. In addition, the contribution of equilibrium of G-4 formation by the metal-free guanine molecules should be



Scheme 1

negligible. Furthermore, as observed in variable-temperature NMR measurements (Fig. 4), ^{i}PG molecules can form a ^{i}PG-4 structure in CH_3OH–$CDCl_3$ at temperatures above 253 K, however, below 253 K, the formation is negligible. Therefore, in order to simplify the equilibrium of G-4 formation in the presence of a template cation, spectroscopic titration to determine the equilibrium constants of the ^{i}PG-4 formation was conducted at temperatures below 253 K. Under such conditions, the equilibrium can be elucidated as shown in Scheme 1.

Spectroscopic titration to determine the equilibrium constant of the ^{i}PG-4–Na^+ formation was carried out at 253 K or lower and under 0.5 of the equivalence of $NaBPh_4$ relative to ^{i}PG, *i.e.*, under 0.333 of the mole fraction of $NaBPh_4$ for ^{i}PG. At temperatures below 253 K, the plot of UV-Vis absorbance changes at 295 nm by adding $NaBPh_4$ exhibited no sigmoidal change, suggesting the absence of the allosteric effect (Fig. 7). The analysis of the spectral change at each temperature was made to determine an apparent equilibrium constant based on the following equation.

$$K = K_1K_2K_3K_4 = \frac{[^{i}\text{PG-4}-\text{Na}^+]}{[^{i}\text{PG}]^4[\text{Na}^+]} \quad (1)$$

In the case of $CaCl_2$, the treatments were the same as described above for $NaBPh_4$, using $[Ca^{2+}]$ in eqn (1) in place of $[Na^+]$.

Equilibrium constants (K, M^{-4}) of the formation of ^{i}PG-4–Na^+ and ^{i}PG-4–Ca^{2+} at various temperatures are summarized in Table 2. The enthalpy and entropy changes (ΔH and ΔS, respectively) in the formation of ^{i}PG-4–Na^+ were determined to be −8.4 kcal mol^{-1} and +50 cal mol^{-1} K^{-1}, respectively, on the basis of a van't Hoff plot (ln K *vs.* $1/T$) as depicted in Fig. 7(b). Similarly, the ΔH and ΔS values of the ^{i}PG-4–Ca^{2+} formation were also determined to be −12.9 kcal mol^{-1} and +34 cal mol^{-1} K^{-1}, respectively (see Fig. S9 in ESI†). The results indicate that the more positive Ca^{2+} ion binds more tightly to the ^{i}PG-4 assembly to stabilize the quartet structure than the Na^+ ion does.

The enthalpy change can be elucidated as the summation of the stabilization by the coulombic interaction between ^{i}PG and the template cation (Na^+ or Ca^{2+}), intermolecular hydrogen bonding between each ^{i}PG and the desolvation of methanol from ^{i}PG. As noted at the end of the former section, since the intermolecular hydrogen bonding cannot become the main driving force to form ^{i}PG-4, the enthalpy change should be derived from the attractive electrostatic interaction between ^{i}PG and the template-cation. In addition, although the formation of ^{i}PG-4–Na^+ and ^{i}PG-4–Ca^{2+} should be unfavourable in terms of entropy changes, the entropy loss can be compensated for by the desolvation of methanol from ^{i}PG as well as Na^+. The degree of contributions to the entropy change from the desolvation of ^{i}PG and the template cations, however, remains unclear due to the lack of the thermodynamic parameters for the ^{i}PG-4 formation in the absence of a template cation.

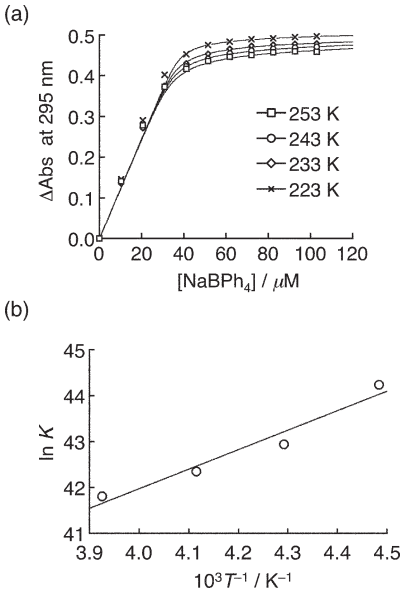


Fig. 7 (a) Absorbance change of ^{i}PG at 295 nm upon addition of $NaBPh_4$ at various temperatures in the course of the formation of ^{i}PG-4–Na^+; (b) a van't Hoff plot for the equilibrium constants. Conditions: ^{i}PG (215 μM) in CH_3OH and $CHCl_3$ (1 : 74 v/v), the additional $NaBPh_4$ (15.5 mM) in CH_3OH and $CHCl_3$ (1 : 9 v/v).

Table 2 Equilibrium constants at each temperature and the enthalpy and entropy of the formation of ^{i}PG-4–Na^+ and ^{i}PG-4–Ca^{2+}

^{i}PG-4–Na^+			
T (K)	K (M^{-4})[a]	ΔH^b (kcal mol^{-1})	ΔS^b (cal mol^{-1} K^{-1})
253	1.4×10^{18}	−8.4	+50
243	2.5×10^{18}		
233	4.7×10^{18}		
223	1.6×10^{19}		
^{i}PG-4–Ca^{2+}			
T (K)	K (M^{-4})[a]	ΔH^b (kcal mol^{-1})	ΔS^b (cal mol^{-1} K^{-1})
308	4.9×10^{16}	−12.9	+34
303	6.3×10^{16}		
298	1.0×10^{17}		

[a] Each equilibrium constant was obtained by temperature-dependent UV-Vis measurements.[41] [b] Each value was estimated by the slope and the intercept of the van't Hoff plot; ln $K = -\Delta H/RT + \Delta S/R$.

Conclusions

In this study, we determined crystal structures of "naked" G-4 assemblies without bulky substituents using ^{i}PG as a building block. In the crystal structure of ^{i}PG-4–H_2O, the quartet structure is stabilized by intermolecular hydrogen bonding among the O6 oxygen of ^{i}PG in the quartet and the water molecules of crystallization. In the structures of ^{i}PG-4–Na^+ and ^{i}PG-4–Ca^{2+}, the template cations such as Na^+ and Ca^{2+} reside at the centre of the quartets. It should be noted that the ^{i}PG-4 structure can be obtained without the presence of the template cations and bulky peripheral substituents to protect the hydrogen-bonded supramolecular G-tetramer.

We also discussed the thermodynamics of the ^{i}PG-4 formation in CH_3OH and CH_3OH–$CH(D)Cl_3$ solutions on the basis of spectroscopic measurements. Concerning the ^{i}PG-4 formation, it is clarified that the electrostatic interactions between the template cations and the O6 oxygen of ^{i}PG contribute more dominantly to the stabilization than the multiple intermolecular hydrogen bonding among the guanine molecules in the ^{i}PG-4 structure. In the absence of the template cations, the ^{i}PG-4 formation is governed by entropy change due to the desolvation of methanol from ^{i}PG. In sharp contrast, in the presence of template cations, the charge of the cations, *i.e.*, the Lewis acidity of the cations, is important to stabilize the ^{i}PG-4 assemblies by virtue of the electrostatic attractive interaction between the template cations and the electrostatically negative O6 oxygen of ^{i}PG. This is clearly reflected on the more negative ΔH values of the ^{i}PG-4–Ca^{2+} formation than that of the ^{i}PG-4–Na^+ formation. Thus, the dicationic Ca^{2+} ion is more favourable to stabilize the ^{i}PG-4 structure thermodynamically than the monocationic Na^+ ion (Fig. S10 in ESI†).

The results obtained in this work provide a solid thermodynamic basis of the driving force of the G-quartet formation without any bulky substituents on the guanine scaffold. This work will also contribute to the manipulation of G-quartet structures toward their further application in the construction of supramolecular materials.

Experimental section

Materials and methods

$CHCl_3$ (Wako Pure Chemical Industries) was purified by distillation with CaH_2. CH_3OH (Wako Pure Chemical Industries) was dried using Mg and I_2. $CDCl_3$ was purchased from Cambridge Isotope Laboratories, Inc. Other chemicals and solvents were purchased from Wako Pure Chemical Industries and Tokyo Chemical Industries. These reagents were used as received. 9-Isopropylguanine was synthesized according to the literature method (see ref. 36 in the text).

Apparatus

^{1}H NMR spectra were measured on a JNM-EX270 spectrometer (JEOL). UV-Vis absorption spectra were recorded on a SHIMAZU UV2450. ESI-MS measurements were recorded on an Applied Biosystems QStar Pulsar i (ESI-TOF; positive mode) spectrometer.

^{1}H NMR measurements

A mixed solvent of $CDCl_3$–CH_3OH was used for measurements and chemical shifts were determined relative to an internal standard (TMS). Variable-temperature NMR measurements were made on a sample containing 9-isopropylguanine and each template cation after incubating for over 3 minutes at a certain temperature.

UV-Vis titration to determine thermodynamic parameters

UV-Vis spectroscopic titrations of ^{i}PG upon addition of template cations were conducted in mixed solvents of $CHCl_3$/CH_3OH. Sodium tetraphenylborate and calcium dichloride were used as the sources of the template cations. In order to determine the K value (expressed by eqn (1) in the text), absorbance at an appropriate wavelength (295 nm for ^{i}PG-4–Na^+ and 288 nm for ^{i}PG-4–Ca^{2+}) was monitored. Thermodynamic parameters were determined using the van't Hoff plot (see the footnote of Table 2).

X-ray crystallography

Single crystals of ^{i}PG-4 assemblies made of ^{i}PG with water molecules of crystallization and template cations were attained by recrystallisation from methanol with vapour diffusion of diethyl ether. The crystallization of the G-ribbon type was carried out in the mixed solvent of methanol and chloroform with vapour diffusion of diethyl ether. All measurements were performed on a Bruker SMART APEX II ULTRA CCD diffractometer at 120 K or 90 K with graphite-monochromated Mo Kα radiation (λ = 0.71073 Å). The structures were solved by direct methods and expanded using Fourier techniques. Non-hydrogen atoms were refined anisotropically including solvents except disordered molecules (ether in the crystal structure of the G-4 with sodium tetraphenylborate). Refinements were carried out by full-matrix least squares techniques on F^2 with scattering factors[42] and including anomalous dispersion effects.[43] All calculations were performed using the Yadokari-XG crystallographic software package,[44] and structure refinements were made by using *SHELXL 97*.[45] All the X-ray crystallographic data are summarized in Table S1 in ESI.†

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$$[\mathrm{Cation}]_{\mathrm{add}} = \frac{\Delta A}{\varepsilon K}\left(\left(\frac{\varepsilon}{\varepsilon[^{\mathrm{i}}\mathrm{PG}]_0 - 4\Delta A}\right)^4 + K\right)$$
$$\left(\varepsilon = \varepsilon_{^{\mathrm{i}}\mathrm{PG}_4} - 4\varepsilon_{^{\mathrm{i}}\mathrm{PG}}\right)$$

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