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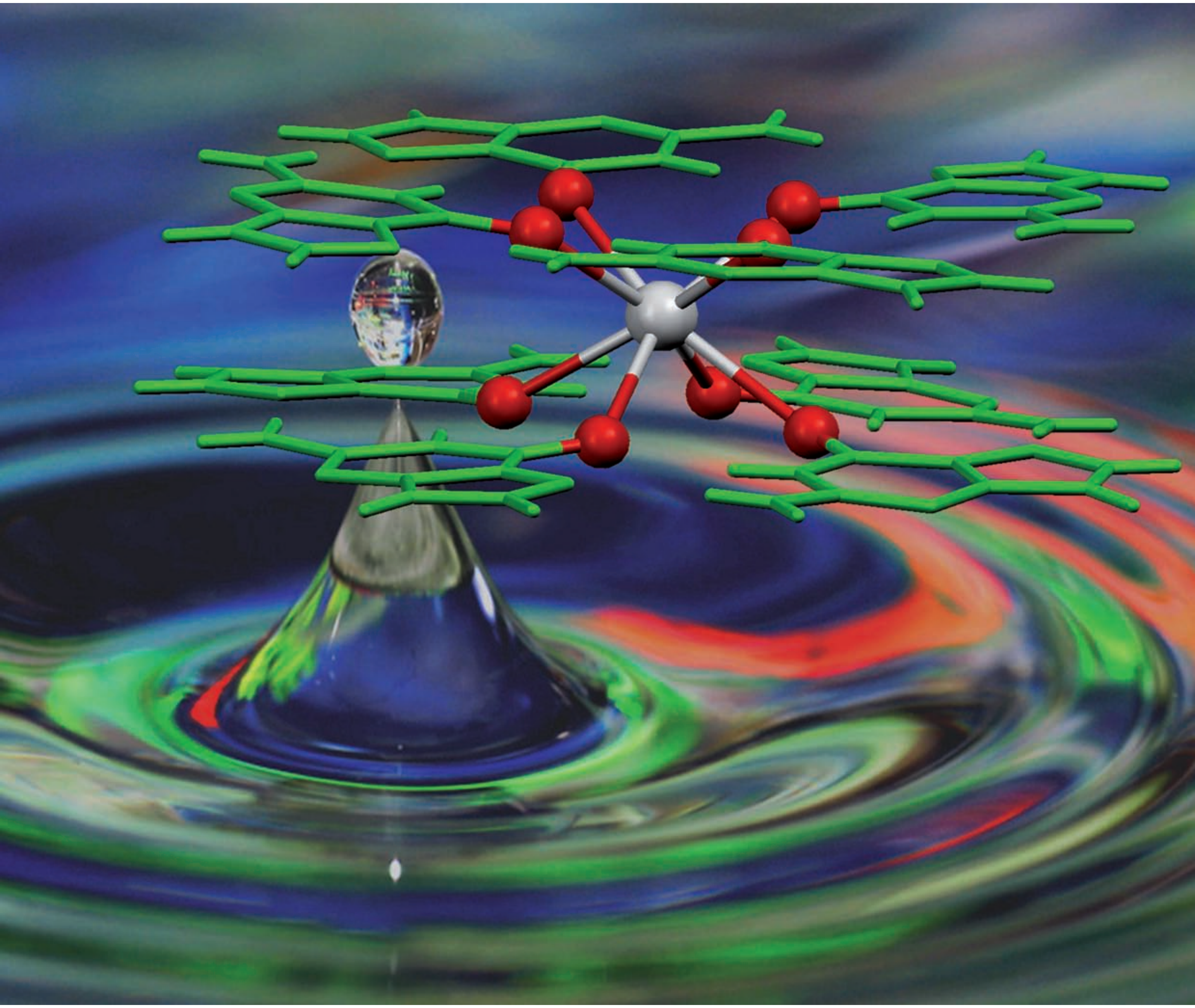

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Direct NMR evidence for Ca^{2+} ion binding to G-quartets†

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We report the first ^{1}H and ^{43}Ca NMR characterization of Ca^{2+} ion binding to G-quartets.

G-Quartets have attracted considerable attention in various areas of research ranging from molecular biology to nanotechnology.[1,2] G-Quartet formation generally requires the presence of metal ions and is known to be promoted by monovalent (Na^+, K^+, Rb^+, NH_4^+, Tl^+) and divalent (Sr^{2+}, Ba^{2+}, Pb^{2+}) cations. Recently, we showed that trivalent lanthanide (La^{3+}, Eu^{3+}, Tb^{3+}, Dy^{3+}, Tm^{3+}) metal ions can also template G-quartet formation[3] and Neidle and co-workers[4] reported the first crystallographic evidence for Ca^{2+} ions occupying the central cavity of a DNA G-quadruplex formed by d(TG_4T). Here we report direct ^{1}H and ^{43}Ca NMR characterization of Ca^{2+} ion binding to the G-quartet structure formed by self-assembly of 2′,3′,5′-*O*-triacetylguanosine (TAG) in solution.

Fig. 1 shows the ^{1}H NMR spectra of monomeric TAG in DMSO and TAG–Ca^{2+} complex in $CDCl_3$.‡ The ^{1}H NMR signals for the imino (N^1H) and one of the amino protons (N^2H_A) are significantly shifted toward high-frequency positions (with larger chemical shift values) in the TAG–Ca^{2+} complex. Two sets of signals (in a 1 : 1 ratio) are observed for each proton, indicating that both *anti* and *syn* conformers are present in solution, as previously observed by Davis and co-workers.[5] All these spectral features are characteristic of G-quartet formation. Comparison of the integrated areas for the TAG signals and the picrate signal (δ = 8.94 ppm) suggests a molecular ratio of 4 : 1 between TAG and picrate, thus a ratio of 8 : 1 between TAG and Ca^{2+}. This indicates that the basic unit of the TAG–Ca^{2+} complex is an octamer containing two G-quartets and a central Ca^{2+} ion, *i.e.*, [TAG_8Ca^{2+}]. This stoichiometry was found to be independent of the picrate concentrations in the aqueous phase during the extraction process. To further confirm G-quartet formation in the TAG–Ca^{2+} complex, we obtained 2D NOESY spectra of the complex. As seen in Fig. 2, cross peaks are observed between N^2H_A and H8 protons and between N^1H and H8 protons. These NOE cross peaks arise from intra-base close contacts, which are the spectral signatures of G-quartet formation. We also performed electrospray ionization mass spectrometry (ESI-MS) and tandem mass spectrometry (MS/MS) experiments for the TAG–Ca^{2+} complex.† The MS and MS/MS results prove unambiguously that $[TAG_8Ca]^{2+}$ (*m*/*z* 1656.424) is the predominant species in the gas phase. Because the ionic radius of Ca^{2+} (1.12 Å for a coordination number of 8) is similar to those of Na^+ (1.18 Å), K^+ (1.33 Å) and Sr^{2+} (1.26 Å),[6] it is not surprising that a Ca^{2+} ion can fit into the central cavity between two G-quartets.

Now that we have established the G-quartet formation of TAG promoted by Ca^{2+} ion binding, we attempt to obtain the ^{43}Ca NMR signature for Ca^{2+} ions residing inside the cavity between G-quartets. ^{43}Ca (spin 7/2, natural abundance 0.145%, $Q = -40.8 \times 10^{-31}$ m^2, ν_0 = 40.4 MHz at 14.1 T) is among a group of low-γ quadrupolar nuclei that are notoriously difficult to study by NMR spectroscopy.[7] In the past three decades, ^{43}Ca NMR spectroscopy has been used to study Ca^{2+} binding in inorganic materials,[8–12] simple organic salts,[13] and in proteins.[14–20] Fig. 3 shows the natural abundance ^{43}Ca NMR spectra of $CaCl_2$(aq), $CaPic_2$(aq) and TAG–Ca^{2+} in $CDCl_3$ at 14.1 T.§ A ^{43}Ca NMR signal at −43 ppm is clearly observed for the [TAG_8Ca^{2+}] octamer. To establish a relationship between the observed ^{43}Ca chemical shift and ion coordination geometry, we performed extensive quantum chemical shielding calculations.¶ We constructed four molecular models, each containing two stacking G-quartets and a central Ca^{2+} ion. In Model I, we used the crystal structure of a Sr^{2+} complex of 5′-silyl-2′,3′-*O*-isopropylidene guanosine[23] and simply replaced the Sr^{2+}

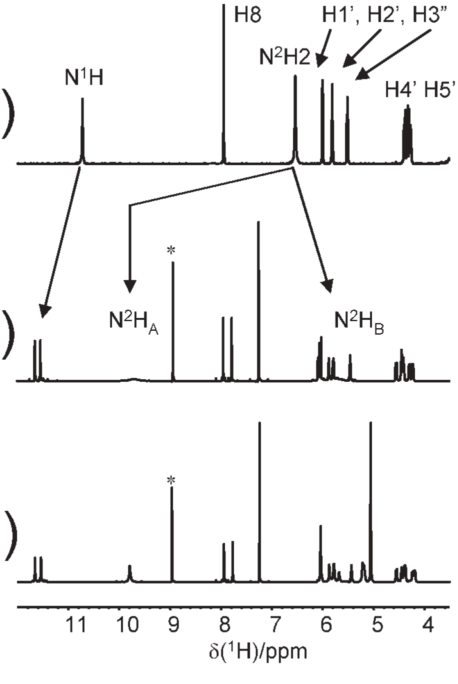

Fig. 1 Regions of ^{1}H NMR spectra of (A) TAG in DMSO at 298 K, (B) TAG–Ca^{2+} complex in $CDCl_3$ at 298 K, and (C) TAG–Ca^{2+} complex in $CDCl_3$ at 268 K. * marks the signal from picrate; see text for discussion.

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† Electronic supplementary information (ESI) available: Electrospray ionization MS and MS/MS spectra; link to PDB 2GW0. Complete 2D ^{1}H NOESY spectrum. See DOI: 10.1039/b714803h

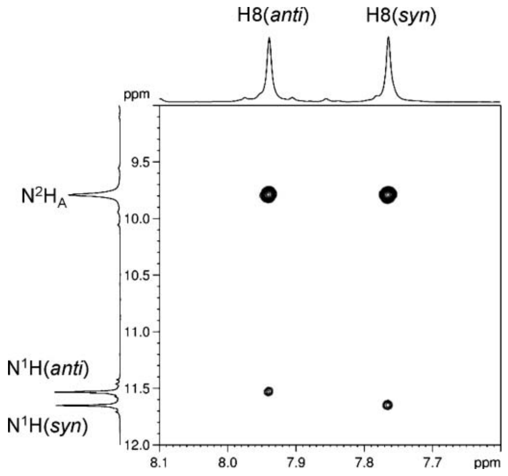

Fig. 2 The G-quartet formation signature region of the 2D ^1H NOESY spectrum of TAG–Ca^{2+} complex in $CDCl_3$ at 268 K.

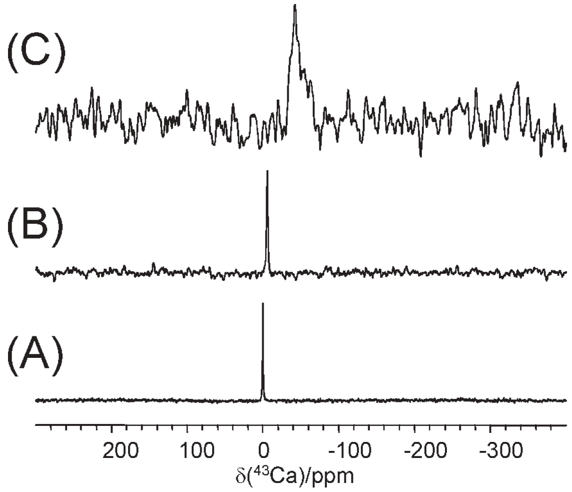

Fig. 3 Natural abundance ^{43}Ca NMR spectra of (A) $CaCl_2(aq)$ (400 transients, 0.25 s recycle delay), (B) $CaPic_2(aq)$ (3757 transients, 0.25 s recycle delay) and (C) TAG–Ca^{2+} complex in $CDCl_3$ (approx. 60 mM, 544 890 transients, 0.25 s recycle delay). All ^{43}Ca chemical shifts are referenced to $CaCl_2(aq)$ at $\delta = 0$ ppm.

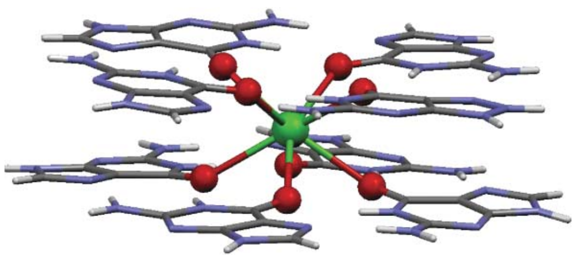

Fig. 4 Illustration of the [TAG_8Ca^{2+}] octamer model used in quantum chemical calculations.

Table 1 Calculated and experimental ^{43}Ca chemical shifts (in ppm) for four models, each containing two G-quartets and a central Ca^{2+} ion[a]

Method/basis set	Model I	Model II	Model III	Model IV
HF/6-311++G**	−38.1	−62.8	−75.0	−63.9
HF/cc-pVTZ	−28.8	−50.8	−63.8	−53.4
HF/Sadlej pVTZ	−34.5	−55.6	−66.8	−57.4
B3LYP/6-311++G**	−37.2	−63.0	−74.1	−62.9
B3LYP/cc-pVTZ	−26.4	−50.2	−63.5	−52.2
B3LYP/Sadlej pVTZ	−37.9	−61.3	−73.5	−63.4
Expt	−43 ± 2			

[a] See text for model descriptions and footnote for computational details.

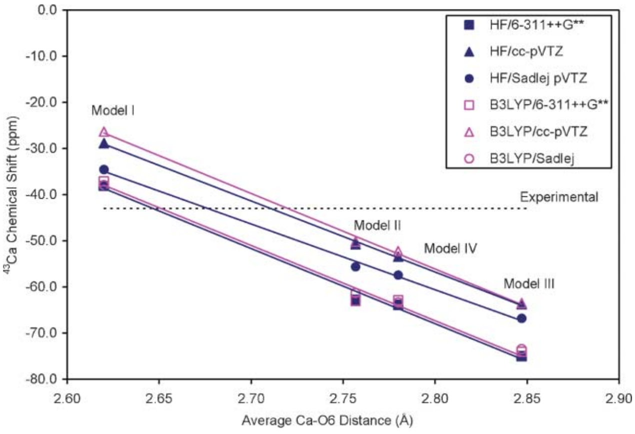

Fig. 5 Relationship between computed ^{43}Ca chemical shifts and average Ca–O6 distances.

ion by a Ca^{2+} ion. In Model II, we used one of the Ca^{2+} sites (Ca55) in the new crystal structure of d(TG_4T) (PDB entry: 2GW0†).[4] In Model III, we used the crystal structure of a Na^+ complex of 5′-silyl-2′,3′-*O*-isopropylidene guanosine[24] and replaced the Na^+ ion by a Ca^{2+} ion. Model IV consists of two idealized G-quartets (geometry optimized at B3LYP/6-311G**)[25] separated by 3.32 Å and a central Ca^{2+} ion. In each model, the Ca^{2+} ion is coordinated to eight guanine carbonyl oxygen (O6) atoms in a bipyramidal antiprism fashion, as illustrated in Fig. 4. Among these models, the geometry of individual G-quartets is essentially the same. The main difference lies in the size of the central cavity between G-quartets, which is reflected by the different Ca–O6 distances. Model I should most resemble the situation in [TAG_8Ca^{2+}]. The computed ^{43}Ca chemical shifts from these models are shown in Table 1. As shown in Fig. 5, we found a correlation between calculated ^{43}Ca chemical shifts and average Ca–O6 distances among the four octamer models. This correlation is in excellent agreement with recent findings in both inorganic[11] and organic systems.[13] In addition, it is comforting to see that the ^{43}Ca calculations with different methods and basis sets predict chemical shifts within 10 ppm, which is much smaller than the entire ^{43}Ca chemical shift range of *ca.* 200 ppm. Using this observed correlation, we estimate the average Ca–O6 distance in [TAG_8Ca^{2+}] to be approximately 2.70 ± 0.05 Å, which is quite reasonable compared to that found at the Ca site in d(TG_4T), 2.76 Å.[4] We also calculated the electric field gradient at the Ca^{2+} site for all the models. The computations consistently predict that the magnitude of the ^{43}Ca nuclear quadrupole coupling constant is less than 1 MHz. This explains why a relatively small line width (*ca.* 600 Hz) was observed in the ^{43}Ca NMR spectrum of [TAG_8Ca^{2+}].

In summary, we have presented the first ^{1}H and ^{43}Ca NMR characterization of Ca^{2+}-templated G-quartet formation. The ^{43}Ca NMR signature observed for Ca^{2+} ions residing inside the central cavity between G-quartets provides a benchmark for future studies of Ca^{2+} ion binding in DNA G-quadruplex systems. Because Ca^{2+} ions are ubiquitous in cells, the biological implication of Ca^{2+} binding in DNA G-quadruplexes should be further investigated. Previous studies by Sugimoto and co-workers[26] have indeed shown that Ca^{2+} ions may play an important role in the structural polymorphism of certain DNA G-quadruplexes. Our finding suggests that ^{43}Ca NMR spectroscopy will be a useful tool in these studies. For large DNA G-quadruplexes, it will almost certainly be necessary to use ^{43}Ca isotope enrichment to increase NMR sensitivity.

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Notes and references

‡ 2′,3′,5′-*O*-Triacetylguanosine (TAG, 98% purity) was purchased from Sigma-Aldrich and used without further purification. TAG–Ca^{2+} complex was prepared using a liquid–liquid extraction method using CHCl$_3$ and an aqueous solution of calcium picrate. *Warning*: Caution should be exercised when handling picrates. After 24 h of extraction, the organic phase was collected and subsequently dried under vacuum. All solution-state ^{1}H NMR spectra were recorded on a Bruker Avance 600 MHz spectrometer (14.1 T). The 2D NOESY spectra were performed using the pulse program NOESYGPPH (Bruker XWINNMR Version 3.5) with mixing times between 100–400 ms and the phase-sensitive TPPI mode. The data were collected using a 90° pulse of 10.0 μs and a relaxation delay of 3.0 s. A total of 8 scans were collected for each time increment. The final data matrix was 2048 (F_2) × 1024 (F_1).

§ All solution ^{43}Ca NMR experiments were performed on a Chemagnetics-Varian Infinity 600 (14.1 T) spectrometer operating at 40.386 MHz for ^{43}Ca nuclei. A Varian T3 rotor (9.5 mm diameter) was used to increase the NMR sensitivity. The strength of the radio-frequency field was approximately 14 kHz. A spectral window of 50 kHz was used. All ^{43}Ca chemical shifts are referenced to the signal from 1 M CaCl$_2$(aq), $\delta(^{43}\mathrm{Ca}) = 0$ ppm.

¶ Quantum chemical calculations were performed using the Gaussian 03 suite of programs[21] on a SunFire 6800 symmetric multiprocessor system. Each of the four nodes is equipped with a 24 × 1.05 GHz (8 MB E-Cache) UltraSPARC-III processor and 96 GB of RAM. For the central Ca atom, we chose three different all-electron basis sets: 6-311++G**, cc-pVTZ and Sadlej pVTZ.[22] A 6-31G* basis set was used for all other non-metal atoms. Each octamer model consists of 129 atoms. Chemical shielding calculations were performed at both Hartree–Fock (HF) and density-functional theory (DFT) levels using the GIAO method as implemented in Gaussian 03. The computed absolute chemical shielding constant (σ) was converted to the chemical shift scale (δ) using $\delta = \sigma_{\mathrm{ref}} - \sigma$, where σ_{ref} is the absolute chemical shielding constant for the reference sample, a fully hydrated cluster, $[\mathrm{Ca(H_2O)_8}]^{2+}$ (MP2/6-311++G** fully optimized geometry: Ca–O$_\mathrm{W}$: 2.480 Å).

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