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Single atom modification leads to enhanced nucleotide self-assembly: the role of cation bridging†

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We report that the ability of disodium 5′-deoxy-5′-thioguanosine-5′-monophosphate, Na₂(5′-GSMP), to self-assemble into a helical G-quadruplex structure in aqueous solution at pH 8 is significantly higher than that of disodium guanosine-5′-monophosphate, Na₂(5′-GMP), which supports our earlier hypothesis regarding the importance of cation bridging.

Guanine (G) nucleotides (DNA and RNA) are known to be able to fold into G-quadruplexes in the presence of certain cations such as K⁺ or Na⁺.1,2 Although all G-quadruplex structures utilize the same basic building block, a hydrogen bonded G-tetramer known as the G-quartet, they exhibit a remarkable structural diversity. For example, the four-repeat human telomeric DNA sequence, d[AGGG(TTAGGG)₃], can fold into drastically different G-quadruplex structures, depending on whether Na⁺ ions or K⁺ ions are present.3–7 To date, the exact role that cations play in G-quadruplex polymorphism has not been fully understood. Like G-rich DNA or RNA sequences, G mononucleotides can also self-assemble into similar G-quadruplex structures at neutral pH.8–14 This is quite puzzling because the phosphate group of a mononucleotide is doubly charged at neutral pH and, as such, the repulsion between them is significantly stronger than the situation seen in DNA or RNA. Yet, as we have shown, guanosine 5′-monophosphate (5′-GMP) can self-aggregate into a G-quadruplex structure of length on the order of several nanometres.15 In a more recent study, we reported a structural elucidation that illustrates how 5′-GMP molecules utilize Na⁺ ions to self-assemble into a right-handed helix in aqueous solution at pH 8.16 In that study, we also hypothesized that a Na⁺ ion must bridge between two adjacent phosphate groups, i.e., P–O−Na⁺−O–P, in order to reduce the strong repulsion between them. In the present work, we further test this hypothesis of cation bridging by examining how the nucleotide self-assembly is affected when the 5′-phosphoryl bridge is slightly lengthened with replacement of one oxygen atom (5′-O) in 5′-GMP by a sulfur atom (5′-S); see Scheme 1.

Fig. 1 shows a comparison between the physical states of Na₂(5′-GMP) and Na₂(5′-GSMP) aqueous solutions at pH 8.† It is striking that, while Na₂(5′-GMP) remains a liquid even at a high concentration of 1.70 M, gelation occurs for Na₂(5′-GSMP) despite the 10-fold dilution. The gel formation of Na₂(5′-GSMP) itself immediately suggests that the self-aggregation of 5′-GSMP is drastically enhanced compared with 5′-GMP. Because the pKₐ values of 5′-GMP and 5′-GSMP are 6.0 and 5.1, respectively,† both 5′-GMP and 5′-GSMP are doubly charged at pH 8. To gain insights into the structural basis of 5′-GSMP gelation, we performed a series of solid-state (¹³C, ²³Na, ³¹P) NMR, FTIR, and powder X-ray diffraction experiments.§

Fig. 2 shows the magic-angle spinning (MAS) solid-state ²³Na NMR spectra of Na₂(5′-GSMP) and Na₂(5′-GMP). Our earlier work has established that a narrow ²³Na NMR signal at −18 ppm is the spectral signature for Na⁺ ions residing inside a G-quadruplex channel.18,19 Indeed, a narrow ²³Na NMR signal at −18 ppm is observed for the Na₂(5′-GSMP) gel, suggesting that the gelation is due to G-quadruplex formation. This is further confirmed by the powder X-ray diffraction pattern, which exhibits the characteristic peak corresponding to a separation of 3.25 Å between adjacent G-quartets.† As also noted in Fig. 2, the ²³Na NMR signal for

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The phosphate-bound Na\(^+\) ions in Na\(_2\)(5\(^0\)-GSMP) is shifted toward a higher frequency by ca. 5 ppm compared to the corresponding signal in Na\(_2\)(5\(^0\)-GMP) \((\text{vide infra})\). The solid-state \(^{13}\text{C} N\)MR spectra obtained for Na\(_2\)(5\(^0\)-GSMP) and Na\(_2\)(5\(^0\)-GMP) are nearly identical, except for the signals of C5\(^0\), which is expected.\(^{†}\) This observation supports the assertion that the 5\(^0\)-GMP and 5\(^0\)-GSMP G-quadruplex structures are similar. Further evidence comes from FTIR spectra\(^{20}\) obtained for solid Na\(_2\)(5\(^0\)-GSMP) and Na\(_2\)(5\(^0\)-GMP),\(^{†}\) which confirm that both G-quadruplex structures contain mixed C2\(^-\)endo and C3\(^-\)endo sugar puckering conformation. This is a unique feature of the 5\(^0\)-GMP G-quadruplex helix.\(^{16}\) The solid-state \(^{31}\text{P} \)NMR spectrum of Na\(_2\)(5\(^0\)-GSMP) reveals a \(^{31}\text{P} \)chemical shift of 19.3 ppm, which is shifted toward a higher frequency from the 17.6 ppm observed for 5\(^0\)-GSMP monomers in solution.

To aid the proper interpretation of the observed \(^{23}\text{Na}\) and \(^{31}\text{P} \)chemical shifts for Na\(_2\)(5\(^0\)-GSMP), we performed \textit{ab initio} quantum mechanical calculations.\(^{†}\) First, we built a CH\(_3\)–S–PO\(_3\)\(^2\)– fragment whose geometry was fully optimized at the B3LYP/6-31G(d) level. Second, we constructed a cluster model containing two CH\(_3\)–S–PO\(_3\)\(^2\)– groups bridged by a Na\(^+\) ion. The Na\(^+\) ion is coordinated to four additional water molecules to form an octahedral coordination shell. Thus the total cluster model is denoted [CH\(_3\)–S–PO\(_3\)–Na(H\(_2\)O)\(_4\)–O\(_3\)P–S–CH\(_3\)]\(^3\)\(^+\); structural details are given in ESI.\(^{†}\) Third, we performed chemical shift calculations as the separation between CH\(_3\)–S–PO\(_3\)\(^2\)– and Na\(^+\) is varied. The computational results are shown in Fig. 3. We note that \(\delta^{(23}\text{Na})\) displays greater sensitivity toward the Na–O distance change than does \(\delta^{(31}\text{P})\). We also found that \(\delta^{(23}\text{Na})\) does not seem to be sensitive to the nature of the phosphate group, because a cluster model of [CH\(_3\)–O–PO\(_3\)–Na(H\(_2\)O)\(_4\)–O\(_3\)P–O–CH\(_3\)]\(^3\)\(^+\) produced identical results. As indicated in Fig. 3, the computational results suggest that the observed \(^{23}\text{Na}\) chemical shift change of 5 ppm between Na\(_2\)(5\(^0\)-GSMP) and Na\(_2\)(5\(^0\)-GMP) can be interpreted as due to a shortening of the Na–O distance in Na\(_2\)(5\(^0\)-GSMP) by about 0.1 Å. The observed \(^{31}\text{P} \)chemical shifts are also consistent with this interpretation. Although these results should be treated as qualitative, the trend is quite clear in that shorter Na–O distances give rise to larger \(\delta^{(23}\text{Na})\) and \(\delta^{(31}\text{P})\) values (signals shifting toward higher frequency). Shortening of the Na–O distance by 0.1 Å in Na\(_2\)(5\(^0\)-GSMP) is also in agreement with the structural data listed in Scheme 1. As a consequence of the Na–O distance shortening, the Na–P distance in Na\(_2\)(5\(^0\)-GSMP) should also be shortened by approximately 0.1 Å. In principle, this Na–P distance shortening may be detectable by \(^{23}\text{Na}\)\(^{(31}\text{P})\) REDOR experiments. However, our previous experience showed that it is rather difficult to accurately assess a Na–P distance change of this small magnitude by REDOR for nucleotides.\(^{22}\) Nonetheless, all our results strongly suggest that the enhanced cation bridging between phosphate groups in 5\(^0\)-GSMP is responsible for the observed gelation.

The hypothesized cation bridging in the 5\(^0\)-GSMP and 5\(^0\)-GMP helices is illustrated in Fig. 4. Our earlier model suggests

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**Fig. 1** Illustration of the drastically different physical states of (a) Na\(_2\)(5\(^0\)-GMP) (1.70 M) and (b) Na\(_2\)(5\(^0\)-GSMP) (0.17 M) at pH 8 in aqueous solution.

**Fig. 2** Solid-state \(^{23}\text{Na} \)NMR spectra obtained under the MAS condition at 14.1 T. The sample spinning frequency was 10 kHz. High power \(^1\text{H} \)decoupling was applied during acquisition. In each case, 64 transients were collected with a recycle delay of 5 s.

**Fig. 3** Dependence of computed \(^{23}\text{Na} \) (closed circles) and \(^{31}\text{P} \) (open circles) chemical shifts for the [CH\(_3\)–S–PO\(_3\)–Na(H\(_2\)O)\(_4\)–O\(_3\)P–S–CH\(_3\)]\(^3\)\(^+\) cluster model on the Na–O distance.

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that cation bridging occurs along the helical axis between a phosphate group linked to a C2'-endo sugar and the next phosphate which is connected to a C3'-endo sugar.\textsuperscript{16} It is important to point out that any enhancement of cation bridging between two phosphate groups would be amplified four times in a G-quadruplex helix. Cation bridging appears to depend on a delicate balance between cation size and phosphate separation, because it is well known that replacement of Na\textsuperscript{+} by K\textsuperscript{+}, Rb\textsuperscript{+} or NH\textsubscript{4}\textsuperscript{+} results in different, yet unknown, 5'-GMP G-quadruplex structures.\textsuperscript{10,23,24} For monovalent cation binding to the phosphate groups of the 5'-GMP helix, we have determined the following sequence of affinity: Li\textsuperscript{+} > NH\textsubscript{4}\textsuperscript{+} > Na\textsuperscript{+} > Cs\textsuperscript{+} > Rb\textsuperscript{+} > K\textsuperscript{+}.\textsuperscript{25,26} We also observed that addition of Mg\textsuperscript{2+} to 5'-GMP promotes crystallization of the monomeric form rather than the G-quartet-based aggregation. We should point out that the cation bridging between doubly charged phosphate groups in 5'-GMP and 5'-GMP helices is reminiscent of the P-O-H⋯O=P hydrogen bonding linkage proposed by Davies and coworkers to describe the continuous helical structure formed by acidic 5'-GMP.\textsuperscript{27}

In summary, while 5'-GSMP and 5'-GMP differ by just one atom, their abilities to self-assemble in aqueous solution are drastically different. Replacement of O by S at the 5' position introduces a slightly extended phosphate group, making it possible to facilitate much stronger cation bridging. Our results strongly support the concept that cation bridging plays a crucial role in the formation of G-quadruplex structures from G mononucleotides. This work was supported by NSERC of Canada. I.C.M.K. thanks NSERC for a Canada Graduate Scholarship (CGS). R.J.D. thanks EPSRC for a DTA studentship. All quantum chemical calculations were performed at the High Performance Computing Virtual Laboratory (HPCVL) at Queen’s University.

Notes and references

\textsuperscript{1} Hydrated disodium guanosine 5'-monophosphate (99% purity) was purchased from Sigma-Aldrich and used without further purification.\textsuperscript{2} Solid-state NMR experiments were performed on Bruker Avance-500 and Avance-600 NMR spectrometers. See ESI for more details.\textsuperscript{3} Quantum chemical calculations were performed using Gaussian 03 suite of programs\textsuperscript{10} on a SunFire 6800 symmetric multiprocessor system. See ESI for more details.\textsuperscript{4}