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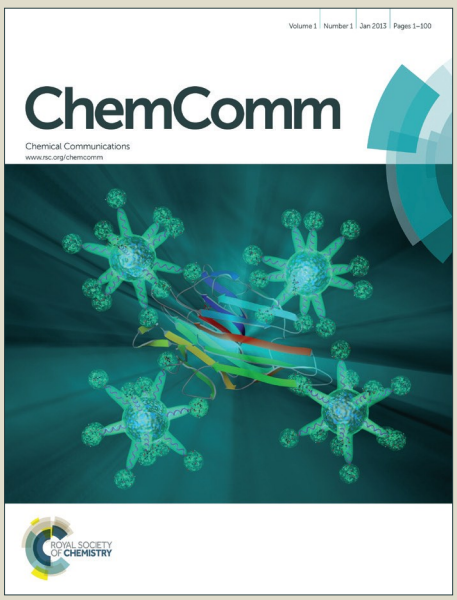

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Incorporation of O^6-methylguanine restricts conformational conversion of human telomere G-quadruplex under molecular crowding

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

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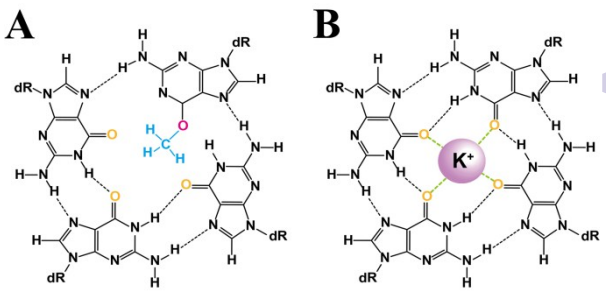
Here we systematically studied incorporation of O^6-methylguanine (6mG) into different position of human telomere G-quadruplex. In contrast to natural G-quadruplex, the 6mG incorporated G-quadruplexes impeded the conformational conversion of G-quadruplex from hybrid to parallel structure under molecular crowding condition in K^+ containing buffer.

Cellular DNA is always subjected to intracellular and extracellular chemical and physical agents that can modify DNA bases or sugar-phosphate backbone.[1] Alkylating agents are one type of these agents which are prevalent in the environment that can lead to DNA damage.[2] Among the damage, methylation of O^6-position in guanine residue (O^6-methylguanine, abbreviated as 6mG, Scheme 1A) has drawn considerable attention since the appearance of 6mG in DNA is always associated with the induction of mutations and cancers.[3] What's more, DNA methylation rates are notably higher in repetitive DNA sequences than in nonrepetitive DNA.[4] Recently, Mekmaysy et al. studied the effect of 6mG on the stability of G-quadruplex.[5] They found that incorporation of one 6mG into a stack of quartet doesn't devastate the structure of G-quadruplex, although incorporation of 6mG indeed perturbs the stability of G-quadruplex. However, as far as we know, there is no report to study the effect of methylation on the structure of G-quadruplex under cell-like crowding condition. Thus, exploring how 6mG incorporated G-rich strands to affect G-quadruplex structure and stability under molecular crowding condition is important and demanding for understanding the biological processes and diseases related to DNA methylation.

Increasing evidence shows that specific guanine (G)-rich DNA can fold into non-canonical four-stranded structures called G-quadruplex which formed from stacking of several G-quartets and stabilized by metal cations (such as K^+ and Na^+). A G-quartet is composed of four coplanar Hoogsteen hydrogen bonds stabilized guanines and coordinated by metal cations at the O^6 position of guanine (Scheme 1B).[7] Bioinformatic studies indicate that G-quadruplex forming sequences are widespread in human genome and particularly prevalent in proximal promoter regions of regulatory genes and gene bodies.[8] Several groundbreaking studies have demonstrated that G-quadruplexes are incontrovertibly existed in human genomic DNA, including telomeres which protect the chromosomes from DNA repair and degradation.[9] What's more, the formation and conformation transition of G-quadruplexes play important role in cell metabolism.[10]



Scheme 1. (A) Schematic diagram showing potential effect of 6mG on hydrogen bonding and ion binding within a G-quartet. (B) Schematic representation of a G-quartet.

Mounting evidence including biophysical and structural studies demonstrated that the conformation of G-quadruplexes is polymorphic.[11] Except DNA sequence, other cellular environmental factors, such as cation species, DNA concentrations, and most importantly, molecular crowding can induce and regulate the conformational conversion of G-quadruplex.[12] A substantial amount of large biomolecules (such as proteins, nucleic acids and complex sugars) pervade the intracellular environment, and make the interior crowded. Until now, several studies have demonstrated that molecular

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Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/x0xx00000x

crowding can induces hybrid G-quadruplex to parallel G-quadruplex;[11c, 11d, 13] moreover, even a single base difference has a drastic effect on the structure of G-quadruplex under molecular crowding condition;[14] what's more, Trent and Chaires have indicated that hydration determines the stability and conformation of G-quadruplex.[15] Taken together, these studies clearly suggest that the structure of G-quadruplex under molecular crowding condition can be different from the one under dilute condition. Thus, it's valuable to explore the structure of 6mG incorporated G-quadruplex under molecular crowding condition.

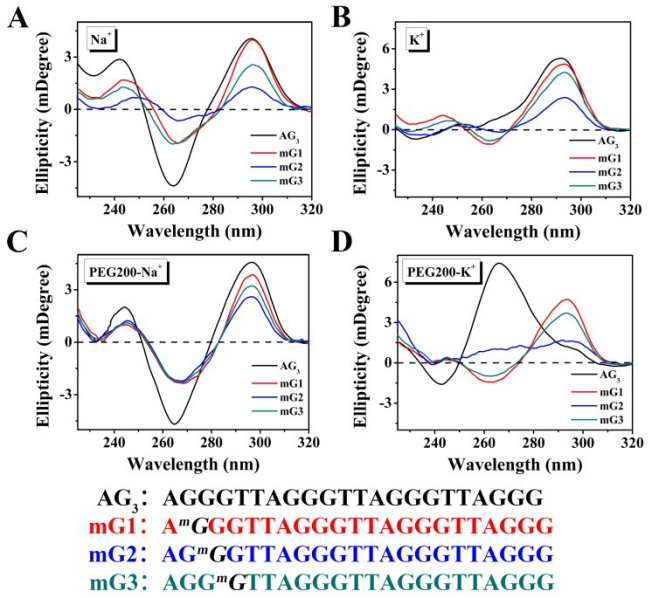


Fig. 1 CD spectra of AG3, mG1, mG2 and mG3 measured in different buffer conditions at 25 ºC. (A) 10 mM Tris buffer (pH 7.2) containing 100 mM NaCl. (B) 10 mM Tris buffer (pH 7.2) containing 100 mM KCl. (C) 10 mM Tris buffer (pH 7.2) containing 100 mM NaCl and 40% PEG200. (D) 10 mM Tris buffer (pH 7.2) containing 100 mM KCl and 40% PEG200. The sequences of the four DNA used in our experiment were listed in the bottom of the panel. The mG represents the position of 6mG. The DNA concentration was 3 μM/Strand.

First of all, circular dichroism (CD) spectroscopy was used to characterize the structure of modified and unmodified DNA sequences (Table S1) under dilute and crowding conditions. As shown in Fig. 1A, under dilute condition in the presence of Na^+, the CD spectrum of AG_3 showed a positive peak at 295 nm and a negative peak at 260 nm, which was the representative characters of antiparallel G-quadruplex.[12a, 16] Meanwhile, the CD spectra of mG1, mG2 and mG3 were similar to AG_3, with a decrease in the magnitude of the CD and a slight shift in the negative peak (Fig. 1A). It should be noted that the magnitude of the decrease of CD varied according to the position of 6mG, and the one located in the central G-quartet (mG2) perturbed the CD spectrum most. In contrast with the dilute condition, the CD spectra of the four sequences in the presence of Na^+ under crowding condition showed similar but increased characteristic signals (Fig. 1C). What's more, the differences among the CD spectra of mG1/mG3 and mG2 were obviously diminished, which suggested molecular crowding stabilized the G-quadruplex of mG2 more effectively than mG1 and mG3.

When it came to K^+ containing buffer, however, some interesting phenomena were observed. Under dilute condition in the presence of K^+, the CD spectrum of AG_3 displayed a negative peak near 240 nm and a positive peak near 290 nm (Fig. 1B), which was typical for a G-quadruplex with a hybrid sturcture.[12a] However, the CD spectra of mG1 and mG3 were distinct from AG_3, and the major difference lied in the decreased CD near 260 nm. Besides, the CD spectrum obtained from mG2 showed a notable shift in the negative peak compared with mG1 and mG3, which implied the 6mG located in the inner quartet disturbed G-quadruplex most under our experimental conditions as detailed in Fig. 1. Under molecular crowding condition in the presence of K^+, AG_3 had the characteristic CD signals of parallel G-quadruplex, indicating the conformational conversion of AG_3 happened under molecular crowding condition (Fig. 1D).[11d] On the other hand, the CD spectra of mG1 and mG3 under molecular crowding condition were similar to the one obtained from dilute condition, indicating mG1 and mG3 under crowding condition were different from AG3, and did not change to parallel G-quadruplex. In other words, incorporation of 6mG restricts the conformational conversion of G-quadruplex under molecular crowding condition. For mG2, however, its CD spectrum showed two positive peaks around 265 nm and 295 nm, indicative of a new DNA structure different from parallel/hybrid G-quadruplex formed by mG2 under molecular crowding condition. Since Pedroso et al. have reported that substituting the central guanine in intramolecular G-quadruplex to adenine resulted in the formation of intermolecular G-quadruplex which was characterized by a CD signal with two positive peaks centered at 265 nm and 295 nm,[17] we speculate that mG2 may form intermolecular G-quadruplex under molecular crowding condition. To investigate the conformation of mG2 under molecular crowding condition in detail, we measured the CD spectra of mG2 in K^+ containing buffer with different content of PEG200 (Fig. S1). Clearly, with the content of PEG200 increased, the intensity of the CD band around 265 nm increased gradually from negative to positive, indicating that it is the molecular crowding condition produced by PEG200 that induces the conformational conversion of mG2. What's more, the CD spectra of mG1, mG2 and mG3 measured at physiological temperature (37 ºC) under molecular condition didn't show palpable changes compared with the one obtained at room temperature (Fig. S2), indicating all of the DNA structures formed by mG1, mG2 and mG3 were stable at physiological temperature. Overall, the impact of 6mG substitution on G-quadruplex depended on their position in K^+ as in Na^+; molecular crowding could effectively weakens the difference between the CD of mG1 and mG3; mG2 could form new DNA structure under molecular crowding condition; more importantly, molecular crowding did not alter the

conformation of mG1 and mG3 under our experimental condition.

Furthermore, the CD spectra of AG_3, mG1, mG2 and mG3 were measured under other molecular crowding conditions in K^+ to investigate whether the size and shape of crowding reagent have effect on the conformational conversion. As shown in Fig. S3, the CD spectra of mG1 and mG3 obtained from other molecular crowding conditions (40% PEG1000, PEG2000, PEG6000) resembled the one obtained from 40% PEG200, which further demonstrated incorporation of 6mG impeded the conformation conversion of G-quadruplex under molecular crowding conditions irrespective of the shape and size of crowding reagent. The CD spectra of mG2 obtained from 40% PEG1000, PEG2000 and PEG6000 also showed a positive peak around 265 nm. Besides, we also measured the CD spectra of the four DNA in the presence of acetonitrile which was recognized as an excellent dehydration reagent[15] (Fig. S3). The CD spectrum of mG2 displayed a positive peak around 290 nm and a negative peak around 260 nm in the presence of acetonitrile, which was similar with the CD spectra of mG1 and mG3 obtained under the same condition, indicating the structure of mG2 was similar with mG1 and mG3 under this condition. Obviously, this result was different from the CD spectra obtained from crowding conditions (40% PEG200, PEG1000, PEG2000 and PEG6000). As a dehydration reagent, acetonitrile just affected the osmotic stress of the solution whilst PEG not only influenced the osmotic stress but also gave rise to macromolecular crowding. Hence, we can conclude that it is the macromolecular crowding that induces the structure conversion of mG2.

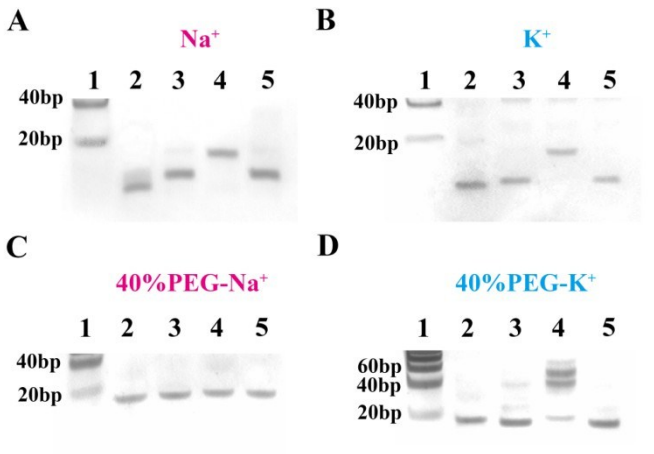
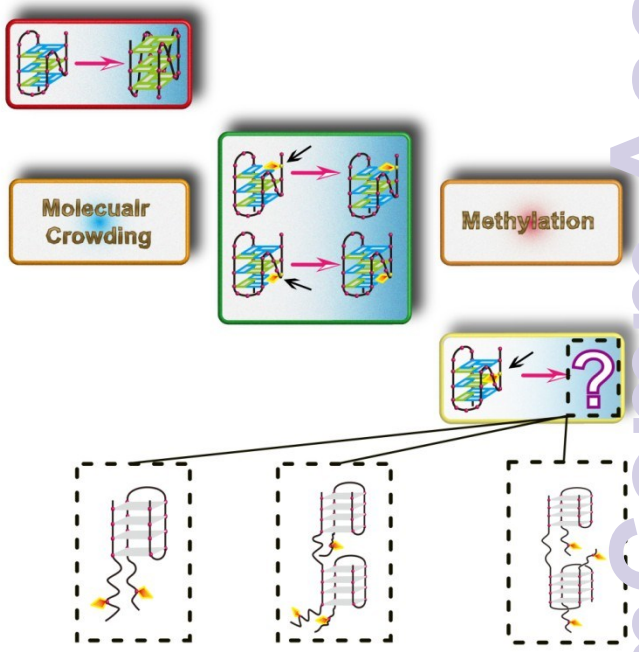


Fig. 2 Native 12% PAGE images of the four DNA in Na^+ (A) buffer and K^+ (B) buffer. Native 12% PAGE containing 40% PEG200 images of the four DNA in Na^+ (C) buffer and K^+ (D) buffer. Lane 1: DNA ladder, Lane 2: AG_3, Lane 3: mG1; Lane 4: mG2; Lane 5: mG3.

Next, we performed the PAGE experiments to further explore the structure of the four DNA under dilute and molecular crowding conditions. As shown in Fig. 2A and 2C, G-quadruplex formed by AG_3 migrated faster than G-quadruplex formed by the other sequences in Na^+ containing gel, indicating the structure of AG_3 G-quadruplex was more compact than the other G-quadruplexes.[18] In the meantime, the G-quadruplex formed by mG2 migrates slower than the other G-quadruplexes, indicative of the fact that G-quadruplex formed by mG2 was looser than the other G-quadruplex. In Na^+ containing gel added with 40% PEG200, the difference between the migration distances of the four G-quadruplex were evidently diminished compared with the one obtained under dilute condition, which further demonstrated that molecular crowding could stabilize the G-quadruplex of mG2 more effectively than mG1 and mG3 (Fig. 2C). On the other hand, in K^+ containing gel added with 40% PEG200, two new phenomena were observed (Fig. 2D). First, the migration distance of the G-quadruplex formed by AG_3 decreased compared with mG1 and mG3, which demonstrated the conformational conversion of AG_3 under molecular crowding condition in the presence of K^+. Second, the electrophoretic band of mG2 was not single, which suggested that mG2 could form multiple intermolecular G-quadruplex under molecular crowding condition in the presence of K^+. Besides, according to the DNA ladder used in the experiment, we speculate the intermolecular G-quadruplex may be formed by two and three DNA sequences (Scheme 2). To investigate the conformational conversion, K^+ containing gel added with 10% PEG200, 20% PEG200 and 30% PEG200 were carried out. As shown in Fig. S4 a new band formed by AG_3 appeared in the presence of PEG200 and the content of the new band was increased with the increase of the PEG200. Meanwhile, the electrophoretic band of mG2 changed from single to multiple. Hence, the results obtained from PAGE experiments further supported the data obtained from CD experiments.



Scheme 2. The conformation of the G-quadruplexes formed by AG_3, mG1, mG3 and the potential conformation of the G-quadruplex formed by mG2 under molecular crowding condition in K^+ containing buffer. The Anti and syn guanine bases are colored in green and blue, respectively.

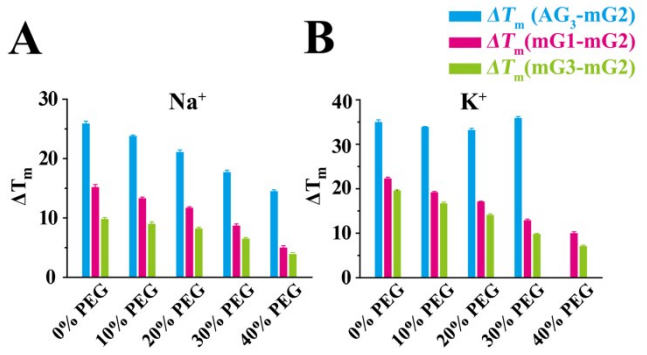


Fig. 3 The difference value (ΔT_m) between the T_m of the G-quadruplex formed by mG2 and other sequences (AG_3/mG1/mG3) under Na^+ (A) or K^+ (B) containing buffers with different PEG200 content. The T_m of the four sequences was obtained from UV melting experiments and DNA concentration was 3 μM/Strand.

Then, UV melting experiments were carried out to evaluate the thermal stability (T_m) of the four sequences under dilute and molecular crowding conditions. Obviously, G-quadruplex formed by AG_3 showed the highest T_m among the four sequences irrespective of the solution conditions (Fig. S5 and S6). In Na^+ containing buffer, the T_m of the four G-quadruplexes increased with the increment content of PEG. Obviously, the ΔT_m between G-quadruplex formed by mG2 and other sequences were evidently decreased with the content of PEG200 increase, which further demonstrated the fact that molecular crowding could stabilize the G-quadruplex of mG2 more effectively than mG1 and mG3. In K^+ containing buffer, however, the ΔT_m of (AG_3-mG2) was not in linear with the content of PEG. According to previous report,[11d] we attributed this result to the fact that the G-quadruplex formed by AG_3 underwent conformational conversion with the content of PEG increase and the T_m of parallel G-quadruplex was higher than hybrid G-quadruplex. Hence, melting experiments further solidified the conclusions obtained from CD and PAGE experiments.

In conclusion, through CD spectra, PAGE experiments and UV melting experiments, we demonstrated that incorporation of 6mG into human telomere G-quadruplex impedes the conformational conversion of G-quadruplex under molecular crowding condition in K^+ containing buffer. mG2 incorporated G-quadruplex may form intermolecular G-quadruplex under molecular crowding condition. Besides, our results further indicate that the stability differences between the 6mG incorporated G-quadruplex and natural G-quadruplex were evidently diminished under molecular crowding condition.

This work was supported by the 973 Project (2011CB936004, 2012CB720602), NSFC (21210002, 21431007, 21533008), and Strategic Research Foundation at Private Universities (2014-2019), Japan, and the Hirao Taro Foundation of KONAN GAKUEN for Academic Research.

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