Isostructural Self-Assembly of 2′-Deoxyguanosine Derivatives in Aqueous and Organic Media

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Water plays a central role in how weak noncovalent interactions dictate the outcome of vital processes such as protein folding as well as the formation of protein–protein and protein–nucleic acid complexes.[1] Recently, there has been an increased interest in the construction of synthetic self-assembled supramolecular nanostructures in water due to their potential use in biomedical applications.[2] Even though the field of supramolecular chemistry has made great strides in the construction of complex self-assembled structures in organic media, similar advances in aqueous environments have lagged behind.[3] Nonetheless, progress in this area has been achieved primarily in the construction of stacked and micellar assemblies of indefinite sizes.[4] The synthesis of discrete and well-defined structures in water has been mostly limited to systems that use coordinative covalent bonds for their formation, in particular, in the construction of capsular supramolecules.[5] In contrast, examples of discrete supramolecular assemblies made exclusively with noncovalent interactions have remained somewhat elusive.[5c]

Recently, Schrader and co-workers[6] reported an elegant system based on aminopyrazole peptides that self-assembled into discrete hexameric nanorosettes in water. Unfortunately, the prevalence of Shrader's hexamer, even at 275 K, was just 40% at 45 mM and 15% at 23 mM in peptide monomers. This example underscores the challenges of developing self-assembling small molecules from scratch. Herein we report the self-assembly of a hydrophilic 8-(*m*-acetylphenyl)-2′-deoxyguanosine (**mAG**) derivative into a discrete and thermally stable hexadecameric supramolecule in aqueous media. Furthermore, we demonstrate that this hexadecamer is isostructural to the one formed by a related lipophilic **mAG** derivative in organic media.

We have previously demonstrated[7] that the self-assembly of lipophilic 8-phenyl-2′-deoxyguanosine derivatives into G-quadruplexes[8] (GQs) could be modulated by replacement of the H8 in the guanine base with a functionalized phenyl group (Figure 1). The position of the functional group in the phenyl ring enables the modulation of the molecularity and thermal stability of the resulting supramolecules. In particular, the **mAG** scaffold forms hexadecamers in organic media very reliably, regardless of the group attached to the sugar. We recently reported taking advantage of this strategy to construct hexadecameric self-assembled dendrimers.[7b]

In this study, we aimed at elucidating if the **mAG** scaffold could also self-assemble in water, and if it did, what would it form. We hypothesized that, because the GQs were held together by a combination of noncovalent interactions,[9] the self-assembly in water was possible as long as we could synthesize soluble derivatives. We achieved this by making the dimethylamino derivatives **mAGcat** and the control compound **dGcat** (which has no 8-phenyl substitution) using a synthetic methodology similar to that previously described by our group (Scheme S1).[7] At neutral pH, the positively charged ammonium groups render both derivatives soluble in water.
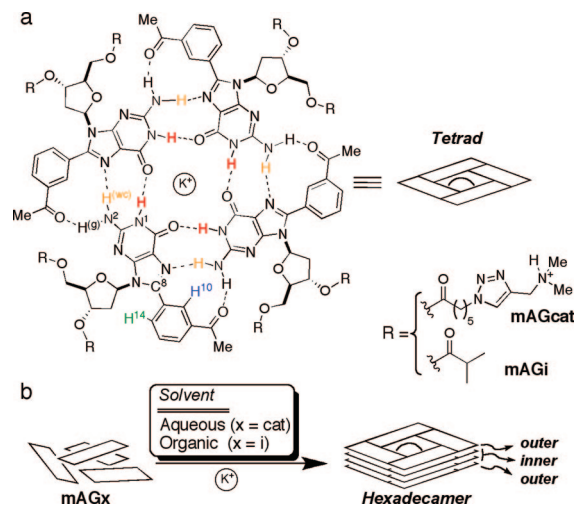

Figure 1. (a) Chemical structure of the **mAG** tetrad. (b) Cartoon representation for the formation of a hexadecamer formed by **mAGx** derivatives in aqueous or organic media. A hexadecamer is composed of two sets of tetrads that occur in distinct chemical environments.
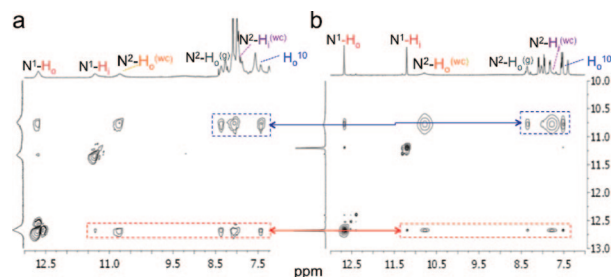

Figure 2. ^{1}H NMR and partial 2D NOESY spectra showing signature cross-peak patterns of (a) **mAGcat** (10 mM) in H_2O-D_2O (9:1) with KCl; (b) **mAGi** (50 mM) in CD_3CN saturated in KI (o = outer, i = inner). (See Figure S24 for more details.)

^{1}H NMR experiments with **mAGcat** in H_2O-D_2O (9:1, with water suppression) reveal many of the signatures of a quadruplex assembly in a solution containing KCl (1 M).[10] The presence of two peaks between 11 and 13 ppm is characteristic of the N^1H protons, which are in slow exchange with the solvent (Figure 2). A comparison with the spectrum of **mAGi** in CD_3CN reveals striking similarities between the two species in key areas of their spectra. In contrast, control experiments with the parent compound **dGcat** show no evidence of self-assembly even at higher concentrations (Figure S18).[11] These findings underscore the importance of the 8-phenyl group in the self-assembly, which enhances the noncovalent interactions such as hydrophobic contacts, stacking, and H bonds.

The CD spectra of both **mAGcat** and **mAGi** exhibit very similar signatures with a negative band near 330 nm and a positive band
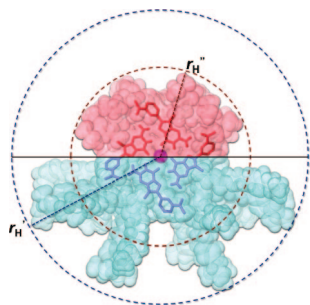
Figure 3. Comparison of the relative sizes for the hexadecamers of **mAGcat** (blue, r'_H) and **mAGi** (red, r''_H) highlighting the hydrodynamic radii. Only the top tetrads are highlighted for clarity. The purple sphere represents a potassium cation.[14]

near 290 nm (Figure S14). Despite their different environments, the close correlation of their CD spectra is a strong indication they share a similar structure at the core.[12]

The hexadecamers of both **mAGcat** and **mAGi** display parallel signature cross-peak patterns in their 2D NOESY spectra in H_2O-D_2O (9:1) and CD_3CN, respectively (Figure 2). These experiments offer further evidence to support that **mAGcat** and **mAGi** form supramolecules of the same molecularity (hexadecamers). Furthermore, parallel to the CD results, the NOESY spectra indicate that the stereochemical arrangement of the **mAG** moieties within the core of the quadruplex is nearly identical (i.e., isostructural).

In order to get a sense of the size of the $(\text{mAGcat})_{16}\cdot 3\text{KCl}$, we performed ^{1}H DOSY NMR experiments to determine its molecular translational coefficient (*D*). We found that $D = (0.776 \pm 0.020) \times 10^{-10}\ \text{m}^2\ \text{s}^{-1}$ at 298 K, which corresponds to a hydrodynamic radius (r'_H) of 25.5 Å.[13] This value is in close agreement with 24.8 Å, which was obtained from a molecular model of the **mAGcat** hexadecamer (Figures S25 and S26).[14] In contrast, $(\text{mAGi})_{16}\cdot 3\text{KI}$ has a $D = (6.430 \pm 0.114) \times 10^{-10}\ \text{m}^2\ \text{s}^{-1}$ at 298 K in CD_3CN, which corresponds to a smaller r''_H of 10.0 Å. The differences in sizes are due to the shorter isopropyl side chains in **mAGi** and the fact that the side chains in **mAGcat** are more extended due to the repulsion between the cationic ammonium groups (Figure 3).

Analysis of the melting curve of **mAGcat**, obtained by variable temperature (VT) NMR, reveals a T_m of 60.7 °C, which is once again remarkably close to the value obtained for **mAGi** in CD_3CN of 56.4 °C (Figure S27).[7] A detailed thermodynamic profile using differential scanning calorimetry (DSC) shows a monophasic transition at 55.1 °C with a favorable ΔG (293 K) value, -2.1 kcal/mol ($\Delta H = -33.7$ kcal/mol, $T\Delta S\ -31.6$ kcal/mol) for the formation of the quadruplex and results from the compensation of a favorable enthalpy with an unfavorable entropy contribution (assembly of 16 subunits and 3 cations).[15] Compared to **dGcat**, **mAGcat** has a larger surface area and greater hydrophobicity, both of which have been shown to stabilize supramolecular structures in water due to enhanced van der Waals and π-stacking.[16] The **mAGcat** tetrads can also form up to four additional H bonds due to the expanded Hoogsteen edge (Figure 1a).[7,17]

In conclusion, the **mAG** moiety represents a remarkable example of a small-molecule recognition motif that is capable of assembling isostructurally in organic or aqueous media with high fidelity and stability. We expect that this and related derivatives will offer important insights into the rules that govern the self-assembly of small molecules in aqueous media. We are currently using this and related compounds to construct self-assembled nanostructures in aqueous media for biomedical applications.[2]

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