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A Unimolecular G-Quadruplex that Functions as a Synthetic Transmembrane Na^+ Transporter

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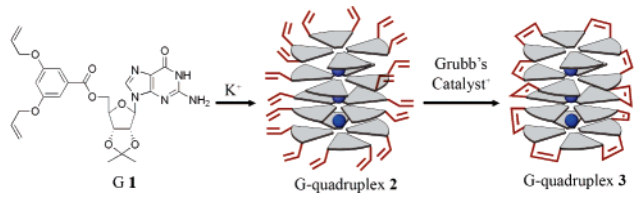
There is a growing appreciation that nucleobases can be used to build supramolecular systems with potential applications.[1] We,[2] and others,[3] have proposed that the G-quartet, a motif found in nucleic acid assemblies,[4] may serve as a scaffold for building synthetic transmembrane ion channels. As the first step toward achieving that goal we show that a strategy combining noncovalent synthesis and post-assembly modification of a G-quadruplex generates a unimolecular ion transporter that moves Na^+ across a phospholipid bilayer.[5] Synthetic pores and transmembrane transporters may ultimately provide new sensors and antimicrobial agents.[6]

We have previously shown that 5′-*tert*-butyldimethylsilyl-2′,3′-isopropylidene guanosine forms a lipophilic G-quadruplex of formula $[G]_{16}\cdot 3K^+\cdot Cs^+\cdot 4pic^-$.[7] One remarkable feature of this structure are four collinear cations that give the appearance of an ion channel. This G-quadruplex has dimensions (26 Å × 30 Å × 30 Å) that approach those needed to span a bilayer membrane. For instance, the gramicidin dimer, a Na^+ channel, has been estimated to be 26 Å thick.[8]

Despite the thermodynamic stability of this noncovalent assembly, individual guanosine subunits are in dynamic equilibrium between "monomer" and hexadecamer when lipophilic G-quadruplexes are in solution.[9] To circumvent problems posed by such kinetic instability we decided to use olefin metathesis to cross-link subunits that had been organized within a G-quadruplex (Scheme 1).[10] We reasoned that such post-assembly modification might

Scheme 1



provide a unimolecular G-quadruplex that would be stable and functional within the hydrophobic phospholipid membrane. As detailed below, this synthetic strategy appears to have been successful.

The precursor, 5′-(3,5-bis(allyloxy)benzoyl)-2′,3′-isopropylidene G **1** was prepared in two steps from guanosine. G **1** was outfitted with two meta-substituted allyl ethers, with the notion that such a pattern would permit olefin metathesis both within an individual G-quartet and between layers. Also, the 3,5-diallyl ethers would be unlikely to undergo intramolecular cyclization to give a strained cyclophane. Solid−liquid extraction of potassium 2,6-dinitrophenolate (K^+DNP^-) by G **1** in CH_2Cl_2 gave quantitative formation of the G-quadruplex **2** as determined by CD and NMR spectroscopy. G-quadruplex **2** had the characteristic positive CD band, centered at 258 nm, for a structure with stacked G-quartets.[7,11] The two sets of ^{1}H NMR signals in a 1:1 ratio, the 4:1 ratio of NMR signals for G **1** and DNP^- anion, and signals for hydrogen-bonded N1 amide

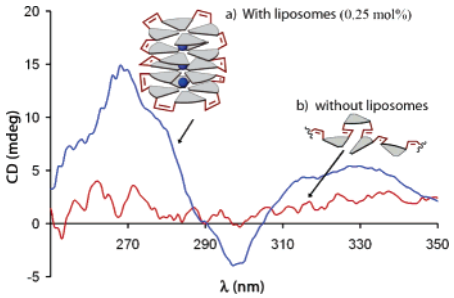


Figure 1. CD spectra of unimolecular G-quadruplex **3** (0.05 mM) in 10 mM sodium phosphate (pH 6.4) with (a) and without (b) EYPC liposomes (100 nm).

protons (δ 11.83 and 11.70) and N2 amino protons (δ 9.72 and 9.56) were characteristic of a D_4-symmetric hexadecamer **2** of formula $[G\ \mathbf{1}]_{16}\cdot 4K^+\cdot 4DNP^-$. Finally, diffusion NMR experiments in CD_2Cl_2 confirmed that this species was indeed a hexadecameric G-quadruplex **2**.[9,12]

Olefin metathesis of the self-assembled G-quadruplex **2** (8 mM), using Grubb's second-generation catalyst, $(H_2IMes)(PCy_3)(Cl_2)$-Ru=CHPh (10 mol % per alkene unit),[13] was complete within 48 h in CH_2Cl_2 at 35 °C. Metathesis was monitored by tracking the disappearance of the ^{1}H NMR signals for the terminal olefins in G-quadruplex **2** and the appearance of new signals for the secondary olefins (cis/trans mix) within the metathesis product **3**. Importantly, no olefin metathesis was observed when "monomeric" G **1** (130 mM) was treated with Grubb's catalyst under similar conditions. Thus, cation-templation of the noncovalent G-quadruplex **2** is necessary for the subsequent covalent capture step.

The structure of the metathesis product **3** was confirmed using mass spectrometry, diffusion NMR, and CD spectroscopy. ESI-MS showed a parent peak at m/z = 2766.3 consistent for a triply charged species of formula $C_{384}H_{400}N_{80}O_{128}\cdot 3K^+$ and molecular weight of 8299 amu. Other ESI-MS experiments also showed that **3** runderwent Na^+/K^+ cation exchange, providing the first hint that this unimolecular G-quadruplex could mediate ion exchange. Pulsed-field gradient NMR experiments in DMSO-d_6 confirmed that metathesis product $[G]_{16}$ **3** was much larger than both precursor **G 1** and adenosine standard A **4**. Thus, the ratio of the experimental diffusion coefficients (D_s(A **4**)/$D_s[G]_{16}$ **3**) = 0.40 was close to the value expected for a hexadecamer (0.37).[9] Finally, the metathesis product $[G]_{16}$ **3** gave characteristic ^{1}H NMR signals (NH amide signals at δ 11.76 and 11.64) and CD spectra (λ_{max} = 260 nm) in CD_2Cl_2 for a lipophilic quadruplex (see Supporting Information).

Significantly, metathesis product $[G]_{16}$ **3** gave the diagnostic CD spectrum for a G-quadruplex when added to an aqueous solution of EYPC liposomes (Figure 1), indicating that $[G]_{16}$ **3** can fold into this secondary structure within the phospholipid bilayer.[14] In contrast, no active CD bands were observed for $[G]_{16}$ **3** when it was added to a solution of 10 mM sodium phosphate that did not

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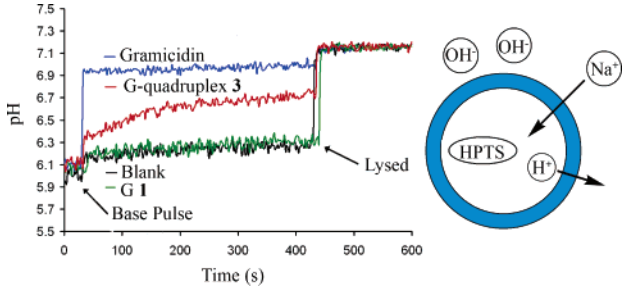

Figure 2. Transport of Na^+ as determined in a pH gradient assay. EYPC liposomes (100 nm) containing HPTS dye (0.1 mM) in 100 mM NaCl, 10 mM sodium phosphate (pH 6.1) were suspended in 100 mM NaCl, 10 mM sodium phosphate (pH 6.1). The compounds, G **1**, G-quadruplex **3**, or gramicidin, were added at $t = 0$ s as DMSO solutions to give a 1:100 ligand-to-lipid ratio. The addition of NaOH solution at $t = 40$ s established a pH gradient of about 1 pH unit. At $t = 430$ s the liposomes were destroyed with Triton-X detergent. Measurement of the fluorescence of the trianionic and tetraanionic forms of HPTS dye allowed determination of the liposomal pH.

contain liposomes, indicating that G **3** is either unstructured or insoluble in aqueous solution.

Having shown that metathesis product $[G]_{16}$ **3** forms a G-quadruplex in phospholipid liposomes, we next evaluated its ability to function as a transmembrane ion transporter. Initial studies used a standard base-pulse assay to indirectly measure Na^+ transport across liposomal membranes.[15] Liposomes (100 nm) containing the pH-sensitive dye, HPTS, were suspended in a solution of 75 mM Na_2SO_4, 10 mM sodium phosphate (pH = 6.0). As shown in Figure 2, addition of exogeneous base led to a rapid increase in the internal pH of these liposomes when they were in the presence of metathesis product $[G]_{16}$ **3** (1 mol %). In sharp contrast, no pH change occurred when either G **1** or the noncovalent assembly $[G\ \mathbf{1}]_{16}\cdot 4K^+\cdot 4DNP^-$ was added to the HPTS-loaded liposomes. Gramicidin was a positive control in these experiments. The pH increase mediated by $[G]_{16}$ **3** is consistent with Na^+ influx across the phospholipid bilayer.

Direct evidence for transmembrane cation transport down a Na^+ concentration gradient, as facilitated by unimolecular G-quadruplex **3**, was obtained from ^{23}Na NMR experiments.[16] EYPC liposomes (200 nm) containing 130 mM LiCl in 10 mM lithium phosphate (pH = 6.4) were suspended in a solution containing 100 mM NaCl, 10 mM sodium phosphate (pH = 6.4). Addition of the NMR shift reagent, $Dy(PPPi)_2^{-7}$, caused the "outer" ^{23}Na peak to move upfield to δ −7.00, distinguishing it from any "internal" ^{23}Na at δ 0.24. After incubation of the LiCl-filled liposomes with the metathesis product $[G]_{16}$ **3** (0.1 mol %) for 10 min, ^{23}Na NMR analysis showed that equilibrium had been achieved between the internal and external Na^+ populations (Figure 3). Importantly, under the same conditions no Na^+ transport was mediated by the precursor G **1** or the noncovalent assembly **2** $[G\ \mathbf{1}]_{16}\cdot 4K^+\cdot 4DNP^-$ (1 mol %). Again, gramicidin served as a positive control in these Na^+ transport experiments.

In conclusion, we have used a strategy that combines noncovalent synthesis and covalent capture to prepare a functional supramolecular assembly, unimolecular G-quadruplex **3**, in just two steps from a guanosine derivative. The unimolecular G-quadruplex **3** apparently folds into a conformation that allows it to transport Na^+ ions across phospholipid bilayer membranes. We are now focused on determining the cation selectivity (Na^+ vs K^+) and mechanism of transport (carrier vs channel) for this transmembrane ion transporter.

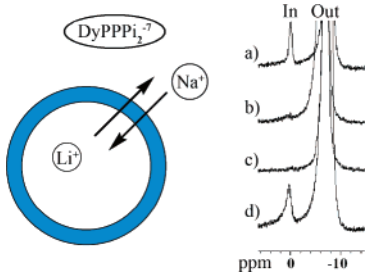

Figure 3. A series of ^{23}Na NMR spectra 10 min after addition of (a) metathesis product **3**, (b) G **1**, (c) DMSO blank, and (d) gramicidin to a solution of EYPC liposomes (200 nm) that initially contained 100 mM LiCl, 10 mM lithium phosphate suspended in an extravesicular buffer containing 100 mM NaCl, 10 mM sodium phosphate. Transport of Na^+ across the bilayer is indicated by a ^{23}Na NMR peak at δ 0.24 ppm.

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Supporting Information Available: Experimental protocols and selected spectroscopic data. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Reviews: (a) Sessler, J. L.; Jayawickramarajah, J. *Chem. Commun*. **2005**, 1939. (b) Sivakova, S.; Rowan, S. J. *Chem. Soc. Rev*. **2005**, *34*, 9.
(2) Davis, J. T. *Angew. Chem., Int. Ed.* **2004**, *43*, 668.
(3) (a) Chen, L.; Sakai, N.; Moshiri, S. T.; Matile, S. *Tetrahedron Lett.* **1998**, *39*, 3627. (b) Datta, B.; Bier, M. E.; Roy, S.; Armitage, B. A. *J. Am. Chem. Soc.* **2005**, *127*, 4199. (c) Krishnan-Ghosh, Y.; Stephens, E.; Balasubramanian, S. *J. Am. Chem. Soc.* **2004**, *126*, 13895.
(4) For discussion of the ion channel nature of DNA G-quadruplexes: (a) Hud, N. V.; Schultze, P.; Sklenar, V.; Feigon, J. *J. Mol. Biol.* **1999**, *285*, 233. (b) Wong, A.; Wu, G. *J. Am. Chem. Soc.* **2003**, *125*, 13895. (c) Wong, A.; Ida, R.; Wu, G. *Biochem. Biophys. Res. Commun.* **2005**, *337*, 363.
(5) For other covalent modifications of G-quartets: (a) Sreenivasachary, N.; Lehn, J.-M *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 5938. (b) Krishnan-Ghosh, Y.; Whitney, A. M.; Balasubramanian, S. *Chem. Commun.* **2005**, 3068.
(6) For a review: Matile, S.; Som, A.; Sorde, N. *Tetrahedron* **2004**, *60*, 6405.
(7) Forman, S. L.; Fettinger, J. C.; Pieraccini, S.; Gottarelli, G.; Davis, J. T. *J. Am. Chem. Soc.* **2000**, *122*, 4060.
(8) Urry, D. W. *Biochim. Biophys. Acta* **1972**, *265*, 115.
(9) Kaucher, M. S.; Lam, Y. F.; Pieraccini, S.; Gottarelli, G.; Davis, J. T. *Chem. Eur. J.* **2005**, *11*, 164.
(10) For examples of olefin metathesis fixing noncovalent assemblies: (a) Clark, T. D.; Ghadiri, M. R. *J. Am. Chem. Soc.* **1995**, *117*, 12364. (b) Cardullo, F.; Calama, M. C.; Snellink-Ruel, B. H. M.; Weidmann, J. L.; Bielejewska, A.; Fokkens, R.; Nibbering, N. M. M.; Timmerman, P.; Reinhoudt, D. N. *Chem. Commun.* **2000**, 367. (c) Wang, L. Y.; Vysotsky, M. O.; Bogdan, A.; Bolte, M.; Böhmer, V. *Science* **2004**, *304*, 1312.
(11) Gottarelli, G.; Masiero, S.; Spada, G. P. *Enantiomer* **1998**, *3*, 429.
(12) For a review on diffusion NMR in supramolecular chemistry: Cohen, Y.; Avram, L.; Frish, L. *Angew. Chem., Int. Ed*. **2005**, *44*, 520.
(13) Scholl, M.; Ding, S.; Lee, C. W.; Grubbs, R. H. *Org. Lett.* **1999**, *1*, 953.
(14) For a recent example where CD was used to detect a supramolecular assembly in a membrane: ten Cate, M. G. J.; Crego-Calama, M.; Reinhoudt, D. N. *J. Am. Chem. Soc*. **2004**, *126*, 10840.
(15) (a) Kano, K.; Fendler, J. H. *Biochim. Biophys. Acta* **1978**, *509*, 289. (b) Sakai, N.; Brennan, K. C.; Weiss, L. A.; Matile S. *J. Am. Chem. Soc.* **1997**, *119*, 8726.
(16) (a) Gupta, R. K.; Gupta P. *Biophys. J.* **1982**, *37*, 76. (b) Pike, M. M.; Simon, S. R.; Balschi, J. A.; Springer, C. S. *Proc. Natl. Acad. Sci. U.S.A.* **1982**, *79*, 810.

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