Dynamic sol–gel interconversion by reversible cation binding and release in G-quartet-based supramolecular polymers

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Received (in Cambridge, UK) 6th September 2005, Accepted 14th October 2005
First published as an Advance Article on the web 1st November 2005
DOI: 10.1039/b512527h

The bis-guanine monomer G-G forms highly cross-linked, K^+ stabilized, polymeric hydrogels that can be reversibly interconverted between gel and sol state *via* sequential binding and release of K^+ by a cryptand undergoing protonation/deprotonation.

The implementation of supramolecular chemistry in materials science has led to the development of supramolecular materials, such as right from the start,[3] supramolecular polymer and liquid crystals, where the components are held together by sets of complementary noncovalent interactions.[1,2] In particular, from the start,[3] polymeric and liquid crystalline supramolecular species have been obtained through connections involving complementary hydrogen bonding patterns between derivatives or analogues of nucleobases (*e.g.* uracil, adenine, diamino-pyridine, *etc.*).[4]

On the other hand, a particularly intriguing type of hydrogen bonded association is the formation of G-quartets (G_4's) resulting in a supramolecular macrocycle of four guanine bases each interacting *via* four Hoogsteen type hydrogen bonds.[5,6] The stabilization of such G_4 units in aqueous solution requires binding of a cation such as Na^+ or K^+.

G-quartets play a very important role in biology, in particular in nucleic acid telomers.[5–7] They also offer the possibility of generating supramolecular materials such as gels and columnar discotic liquid crystals[5,6,8] as well as, in principle, highly cross-linked supramolecular polymers.[9] Remarkably stable dynamic hydrogels have been obtained, that display gelation-driven component selection.[10] Furthermore, the participation of metal cations in the G_4 array provides a means of regulating its formation.[4g]

We describe here 1) the formation of gels of supramolecular polymers based on G_4 cores from linear components bearing terminal guanine groups; 2) the effect of chemical and physical parameters on these gels; 3) the regulation of gel formation through reversible sol–gel interconversion *via* cation complexation and release.

The ditopic α,ω -bisguanine monomer **G-G** was synthesized in 36% overall yield by TOTU induced condensation of the protected guanyl acetic acid derivative **1**[11] with the 1,14-diamino-3,6,9,12-tetraoxatetradecane **2**[12], followed by deprotection, as described.[13,14a] The monotopic compound **3** was obtained in 40% overall yield by condensation of **1** with an excess of **2** (2 eq.) followed by deprotection.[4b]

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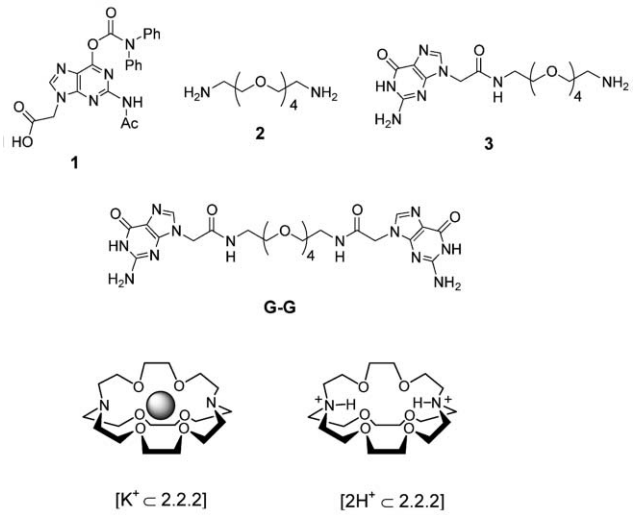

The properties of **G-G** in aqueous medium as a function of various parameters were examined.[15] Already a 5 mM aqueous solution of **G-G** forms a stable hydrogel in presence of 10 equivalents (50 mM) of KCl. In contrast, the monotopic compound **3** is very soluble in water and at 15 mM concentration, in presence of a large excess of KCl (> 150 mM), the solution remains clear and transparent with no sign of gel formation.

The melting temperature T_{gel} of the gel formed by **G-G** was obtained by visual determination of the temperature at which the gel flows on vial inversion. T_{gel} increases markedly on increasing the concentration of **G-G** in 200 mM KCl at pH 7.3–7.8 (Fig. 1a), as well as on increasing the concentration of KCl for a 15 mM solution of **G-G** (Fig. 1b). The proton NMR spectrum of the gel allows the determination of the gelled fraction by integration of the proton signals with respect to an internal or an external *tert*-butanol reference signal. Only the signals of the "free" **G-G** are

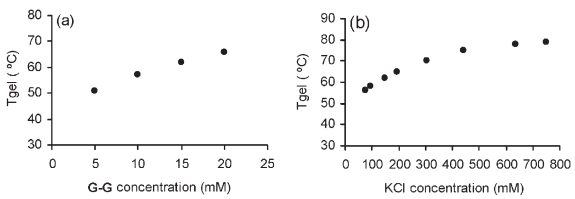

Fig. 1 Temperature of gelation T_{gel} (°C) determined visually, (a) as a function of **G-G** concentration in 200 mM KCl at pH 7.3–7.8 (51 °C and 66 °C at 5 and 20 mM respectively) and (b) as a function of KCl concentration for a 15 mM **G-G** solution (56 °C and 78 °C at 75 and 636 mM respectively).

detected, whereas those of **G-G** engaged in the gel are broadened beyond detection.[10] The percentage of free **G-G** decreases markedly as a function of **G-G** concentration (in 200 mM KCl, Fig. 2a), as well as on increasing the KCl concentration at constant 15 mM of **G-G** (Fig. 2b).

The gelation properties of **G-G** may be attributed to the formation of extended supramolecular polymeric assemblies based on the formation of hydrogen bonded G-quartet macrocycles stabilized by binding of K^+ cations[5,6] and presenting probably multiple cross-linking interconnections. The networks formed may be considered to encompass the various superstructures resulting from a combination of a chain of G_4 units interconnected in a double-linear fashion and of a fully cross-linked array (Fig. 3). In addition, assemblies of internally-bridged G-quartets [(**G-G**)$_2$, K^+] may be considered, as the spacer in **G-G** is long enough for bridging two G groups of a G_4 core (Fig. 3a). Furthermore, stacking of G_4 cores may also take place, as is known in the formation of extended stacks of G quartets formed by derivatives containing a single G head group.[5,6]

The addition of up to 10 eq. of the monotopic compound **3**, as end-capping agent, to a solution of **G-G** (10 mM) in presence of a large excess of KCl (310 mM) gives a white opaque gel. At higher concentrations of **3** (about 20 eq. or above), no gel formation is observed. In comparison, **G-G** (10 mM) in 480 mM KCl gives a gel even in presence of 32 eq. of cytidine, the nucleoside complementary to guanosine, but the gel formed more slowly (in a few minutes).

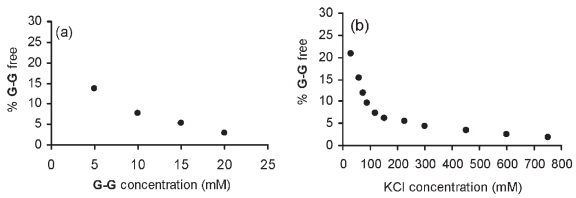

Fig. 2 Investigation of the gelation of **G-G** by ^{1}H NMR spectroscopy. a) Fraction of free **G-G** as a function of concentration of **G-G** at 200 mM KCl and pH 7.3–7.8. b) Fraction of free **G-G** as a function of concentration of KCl at 15 mM **G-G**.

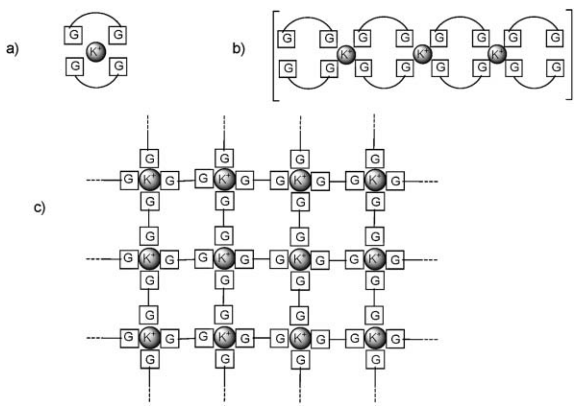

Fig. 3 Possible supramolecular entities formed by **G-G** through association into G-quartets stabilized by K^+ binding: (a) internally-bridged [(**G-G**)$_2$ K^+] assembly; (b) linear chain of doubly-bridged G_4 units; (c) fully cross-linked regular array of G_4 units.

Most interestingly, the stabilization of the G_4 structure by K^+ offers the possibility of inducing reversible gel–sol interconversion by sequential removal and addition of the potassium ions. This may in principle be achieved if a suitable competing K^+ binding agent is available. Indeed, the cryptand [2.2.2] complexes very strongly K^+ even in aqueous solution at neutral or basic pH with formation of the cryptate [$K^+ \subset$ 2.2.2].[16] The bound K^+ ion may be released by protonation of the bridgehead nitrogens to give [$2H^+ \subset$ 2.2.2].[16] Thus, addition of 10 eq. [2.2.2] to the hydrogel formed from an aqueous solution of **G-G** (10 mM) and 10 eq. KCl (100 mM) leads to the disappearance of the gel giving a sol. On addition of 10 eq. HCl, K^+ is released from its cryptate and the gel is regenerated. Adding thereafter 10 eq. NaOH deprotonates [$2H^+ \subset$ 2.2.2] and the liberated cryptand recaptures K^+ with simultaneous gel to sol transformation. The process can be repeated by sequential acidification/neutralisation.

The gel–sol interconversion may be observed visually (Fig. 4, *top*) as well as spectroscopically by ^{1}H-NMR determination of the amount of free **G-G** in the medium (Fig. 5). The sequence of events is represented in Fig. 4 (*bottom*), showing the reversible changes of the probe between gel and sol states as visually observed.

One may note that the process represents a sequential change in the state and corresponding mechanical properties of the sample, fuelled by acid–base neutralization.

The present results lead to the following conclusions: i) ditopic monomers such as **G-G**, bearing two guanine type recognition groups as connectors form supramolecular polymers through hydrogen bonding; ii) the polyassociation involves the formation of cores consisting of G-quartets, hydrogen bonded supramolecular macrocycles stabilized by binding of metal ions such as K^+, resulting in a presumably highly cross-linked polymeric network;

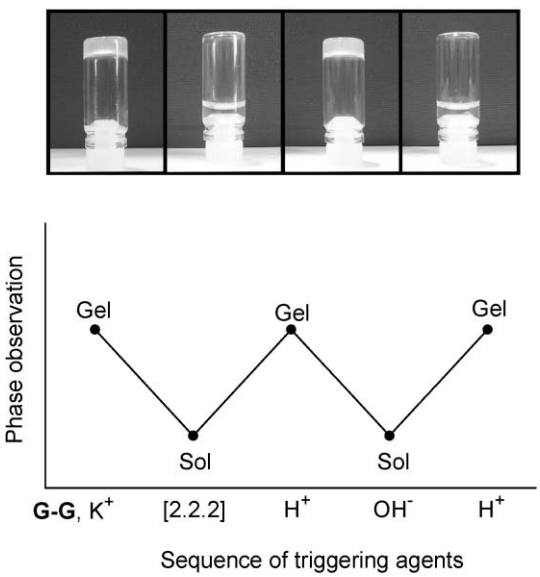

Fig. 4 (*Top*) Visual observation of the reversible gel–sol interconversion of the hydrogel formed by a sample of **G-G** (10 mM) in 100 mM (10 eq.) KCl. From left to right: initial sample; addition of 10 eq. cryptand [2.2.2]; addition of 10 eq. HCl; addition of 10 eq. NaOH; all samples at room temperature (22 °C). (*Bottom*) Schematic representation of the modulation of the gel–sol status induced by the sequence of triggering agents. The samples were warmed and cooled after each addition to ensure homogeneity; total dilution is less than 3%.

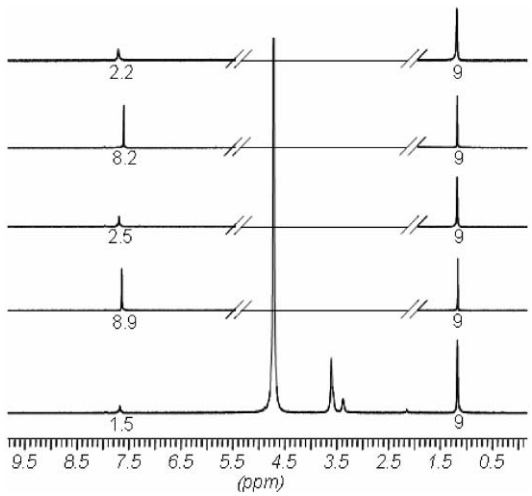

Fig. 5 Observation of the reversible gel–sol interconversion of a solution of **G-G** (10 mM) in 100 mM KCl by integration of the H(9) proton NMR peak of the guanine group at 7.61 ppm, with respect to the signal of *tert*-butanol at 1.15 ppm (at 400 MHz). From bottom trace: initial gel sample; addition of 100 mM cryptand [2.2.2]; addition of 100 mM DCl; addition of 100 mM NaOD; addition of 100 mM DCl. The figures under the signals indicate the relative peak areas. The signals of the solvent and of the cryptates between 2.5 and 5.5 ppm have been removed for clarity.

iii) these features translate into the generation of stable hydrogels; iv) reversible gel–sol interconversion may be achieved by sequential sequestering and release of the core-stabilizing metal ions, by means of a competing ligand, whose cation binding properties may be modulated by external triggers such as protonation/deprotonation (photochemically or electrochemically active triggers may also be considered);[17,18] v) the features displayed by the present system may in principle be extended to other multivalent core groups, such as isoguanosine forming pentets[6] and other metal ions;[5,6] vi) the present system represents a class of supramolecular dynamers,[1c,19] dynamic polymers of supramolecular nature, whose polyassociation may be controlled by external parameters; vii) as the formation of G-quartets is also of great biological significance, *e.g.* in chromosomic telomers,[5–7] interesting biological effects may result from invasion of G-quartets by synthetic agents as well as from binding of the metal ions involved, that would result in destabilisation of the quartet structure.

We thank the European Commission for partial financial support of this work within the AMNA contract.

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14 (*a*) Characterization of compound **G-G**: ^{1}H NMR (DMSO-d_6): 3.25–3.52 (m, 20H), 4.64 (s, 4H, CH_2), 6.48 (s, 4H, NH_2), 7.61 (s, 2H, CH), 8.26 (s (br), 2H, NH), 10.63 (s (br), 2H, NH); ^{13}C NMR (DMSO-d_6): 39.27, 45.21, 69.33, 70.02, 70.16, 70.20, 116.54, 138.78, 151.94, 154.10, 157.41, 166.99; High resolution ES-MS: (**G-G**H$^+$) *m*/*z*: 619.2695; (*b*) Characterization of compound **3**: ^{1}H NMR (DMSO-d_6): 2.92 (tr, 2H), 3.42 (br, 2H), 3.52–3.58 (m, 18H), 4.36 (s, 2H), 6.51 (s (br), 2H), 7.55 (s, 1H); ^{13}C NMR (DMSO-d_6): 39.16, 46.63, 67.98, 69.96, 70.03, 70.12, 116.37, 138.97, 151.78, 153.84, 157.42, 169.88.

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