ChemComm

Cite this: *Chem. Commun.*, 2011, **47**, 8043–8045

www.rsc.org/chemcomm

COMMUNICATION

pH-controlled reversible drug binding and release using a cytosine-rich hairpin DNA†

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Received 3rd May 2011, Accepted 3rd June 2011
DOI: 10.1039/c1cc12594j

Here we report that a cytosine-rich DNA carrier, that oscillates between a hairpin and an i-motif structure in its response to pH variation, can be used as a drug binding and release device.

Nucleic acids have been recognized as an attractive building material for nanotechnology and materials science owing to their conformational polymorphism, programmable sequence-specific recognition and robust physicochemical nature. Many nucleic acids-based, artificial structures/devices have been constructed and shown potential applications in miniaturized biosensors, microsurgery, drug delivery, nanorobotics and dynamic nanomaterials.[1] Human telomeric i-motif DNA structure is pH-dependent.[2] At acidic pH, it can fold into a closed four-stranded structure with intercalated CC^+ base pairs,[2] and unfold to a single-stranded form at basic pH. A number of pH-related DNA devices based on i-motif DNA in solution[1,2b] or on a surface[2c] have been reported. Although these DNA nanodevices are promising, it is still challenging how to make them to realize sophisticated functions in this field. To this end, much effort has been made.[1e–g] Recently, the Li group[3] reported an i-motif-containing DNA device that can break down certain forms of Watson–Crick interaction under isothermal conditions in its response to pH variation. Because many anticancer drugs can interact with duplex DNA,[4] we then sought to take advantage of this i-motif unique feature to design a DNA drug carrier in pH-controlled reversible binding and releasing manner using a cytosine-rich hairpin DNA.

Controlled drug-release systems are one of the most promising applications for human health care and represent an ever-evolving field for biomedical materials science. ''On-demand'' release systems that respond to a range of stimuli, including redox, pH or temperature, enzymes, competitive binding, and photoirradiation have recently been reported.[5]

Despite these achievements, many of the existing systems have disadvantages such as the use of stimuli that are complicated and/or difficult to apply, poor applicability in aqueous solutions and biocompatibility, and the toxicity agents used. In particular, these previously reported systems mostly have poor drug selectivity. Herein we design and construct a straightforward proton-driven human telomeric cytosine-rich hairpin DNA for drug binding and release, in which DNA is used as both carrier and drug-release device. Because many therapeutic agents that bind to DNA have sequence- and/or structure-specific preference,[6] the drug-release device we design here could select corresponding drugs to deliver. The anticancer agent, doxorubicin (DOX) and the potential anticancer agent, Hoechst 33258, were chosen for our model system. DOX[4a] is a well known anticancer drug which has shown efficacy against a range of neoplasms.[4,7] It is known that DOX can intercalate into a DNA strand (preferentially bind to double-stranded 5′-GC-3′ or 5′-CG-3′ sequences) due to its flat aromatic rings and positive charge. Hoechst 33258 is a positively charged, bisbenzimidazole molecule that binds strongly in the minor groove region of duplex DNA with predominant specificity for AT rich sequences.[8] The bisbenzimidazole family of molecules are potential anticancer drugs.[9]

The working principle of our pH-driven cytosine-rich DNA drug release device is illustrated in Scheme 1. Two oligonucleotides of 28-mer gcDNA (CGC CCC TAA CCC TAA CCC TAA CCC T GCG) and 28-mer atDNA (AAA CCC TAA CCC TAA CCC TAA CCC T TTT) are accordingly designed. Under neutral/basic conditions, the cytosine-rich segment of gcDNA will position itself randomly and the two 3-base termini (GCG-CGC) are complementary to each other, so 28-mer gcDNA forms a stem-and-loop conformation (Scheme 1a). The anticancer drug, positively-charged doxorubicin can bind to the GCG-CGC duplex stem. However, under acidic conditions, due to cytosine protonation (dC^+), an i-motif structure is expected to be formed by the C-rich segment[10] which would break down the hydrogen bonds between the partial duplex stem part (GCG-CGC) (Scheme 1b). So, the positively-charged DOX would be released (Scheme 1c). The positively-charged Hoechst 33258 follows the same way as DOX binding to gcDNA, except for Hoechst 33258 binding to the DNA minor groove.

For demonstration of the DNA conformational change when pH is varied from 7.0 to 5.0, UV melting, circular dichroism (CD) and polyacrylamide gel electrophoresis (PAGE) were carried out. Fig. 1A and B show the UV melting profiles of gcDNA and atDNA under different pH conditions.

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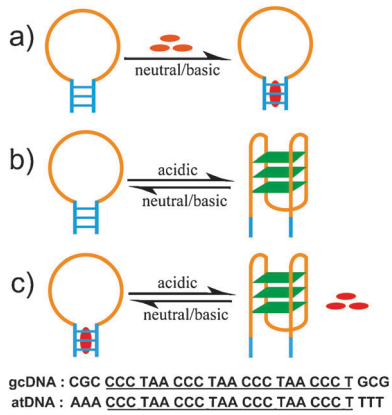
† Electronic supplementary information (ESI) available. See DOI: 10.1039/c1cc12594j



Scheme 1 Schematic representation of our approach for designing cytosine-rich DNA drug binding and release devices capable of breaking down certain forms of Watson–Crick interactions. The sequence with underlining stands for a cytosine-rich segment which could form an i-motif structure under acidic conditions. (a) Drug binding at the stem of hairpin structure under neutral/base conditions. (b) Formation and disintegration of i-motif structure as the pH value of its environment varies. (c) Reversible cycling of drug binding and releasing processes as the pH value of its environment varies.

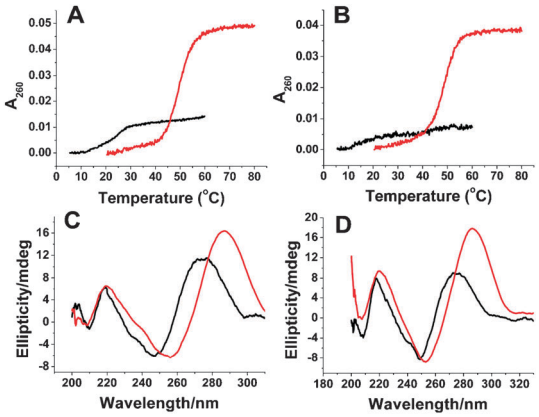


Fig. 1 DNA UV meltings and CD spectral changes at different pH. (A) UV melting profiles of gcDNA at pH 7.0 (black line) or pH 5.0 (red line) in cacodylic buffer. (B) UV melting profiles of atDNA at pH 7.0 (black line) or pH 5.0 (red line) in cacodylic buffer. (C) CD spectra of gcDNA in pH 7.0 (black line) or pH 5.0 (red line) cacodylic buffer at 10 °C. (D) CD spectra of atDNA in pH 7.0 (black line) or pH 5.0 (red line) cacodylic buffer at 10 °C. (1 mM cacodylic acid/sodium cacodylate/100 mM NaCl). DNA concentration was 1 μM (UV meltings) or 2 μM (CD spectral) in strand.

A striking difference between pH 7.0 and 5.0 was observed. At pH 7.0, UV melting studies show that DNA melting temperatures (T_m) of gcDNA and atDNA are 22.5 °C and 15.6 °C, respectively, however, at pH 5.0, both gcDNA and atDNA show an unambiguous transition with T_m at 48.9 °C. This indicates that the two DNA become more stable at pH 5.0 than at pH 7.0. CD spectra demonstrate that both of the two DNA undergo conformational change when pH is varied from 7.0 to 5.0 at 10 °C. Fig. 1C and D show that the observed DNA structure is pH-dependent. At pH 7.0, gcDNA has a positive band near 275 nm and a negative band near 246 nm, atDNA has a positive band near 275 nm and a negative band near 248 nm, showing that B-form duplex is formed. According to the sequence of DNA, we deduce that both gcDNA and atDNA adopt a hairpin structure with a B-DNA stem, or form an intermolecular B-DNA duplex which was excluded by our next gel experiments. At pH 5.0, two i-motif DNA characteristic CD bands were observed for both gcDNA and atDNA.[11,12] The positive band of gcDNA was blue-shifted to 286 nm and the negative band shifted to 256 nm. For atDNA, the positive band was shifted to 286 nm and the negative band shifted to 253 nm. This clearly indicates that both C-rich gcDNA and atDNA form an i-motif structure and have the potential ability to break down hydrogen bonds of the B-DNA stem or intermolecular B-DNA duplex.

Polyacrylamide gel electrophoresis (PAGE) experiments were carried out to further study the conformational change under different pH conditions at 4 °C (Fig. 2). dT_{22} and dT_{12} DNA were used as markers. Comparison of Fig. 2A with Fig. 2B clearly shows that the DNA has different electrophoretic mobility at pH 5.0 and pH 7.0. At pH 7.0, both gcDNA (28 mer) and atDNA (28 mer) migrated slightly faster than dT_{22} (Fig. 2A), indicating that the two DNA adopt a compact hairpin structure rather than the intermolecular duplex (Scheme 1a). In contrast, when the pH value was lowered to 5.0, the two DNA migrated much faster than dT_{22} and almost as fast as dT_{12}. This observation was consistent with the suggestion that the two DNA formed a more compact i-motif structure at pH 5.0 (Scheme 1b). The results unambiguously show that the DNA device can adopt different conformations corresponding to the pH conditions. We then investigated the operability of the designed DNA devices. Using gcDNA–doxorubicin and atDNA–Hoechst 33258 as model systems, we evaluated whether the DNA device can reversibly capture and release drug molecules under control. Fluorescence titration[13] was used to determine drug binding to DNA, in which fixed concentrations of either DOX or Hoechst 33258 were titrated with increasing DNA concentrations (Fig. S1). According to the titration, the binding ratio of DOX to gcDNA is 3 : 1 and that of Hoechst 33258 to atDNA is 2 : 1 (Fig. S1 B and D). The calculated thermodynamic parameters are summarized in Table S2. Nonlinear least-squares analysis[13] of the fluorescence titration data (details in supporting information) yielded the binding constants of 3.73×10^5 and 2.32×10^4 M^{-1} for DOX to gcDNA and Hoechst 33258 to atDNA, respectively. We also studied drug binding to DNA at different pH using UV melting, CD spectra (Fig. S2) and PAGE (Fig. 2). UV melting studies show that at

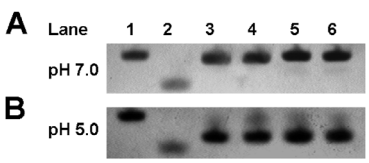


Fig. 2 Native gel electrophoretic analysis (15% PAGE) of gcDNA in the absence/presence of doxorubicin and atDNA in the absence/presence of Hoechst 33258. The gels were run in tris-boric acid buffer at 4 °C. (A) at pH 7.0. Lane 1: dT_{22}; Lane 2: dT_{12}; Lane 3: gcDNA; Lane 4: gcDNA + doxorubicin; Lane 5: atDNA; Lane 6: atDNA + Hoechst 33258. (B) at pH 5.0. Lane 1: dT_{22}; Lane 2: dT_{12}; Lane 3: gcDNA; Lane 4: gcDNA + doxorubicin; Lane 5: atDNA; Lane 6: atDNA + Hoechst 33258.

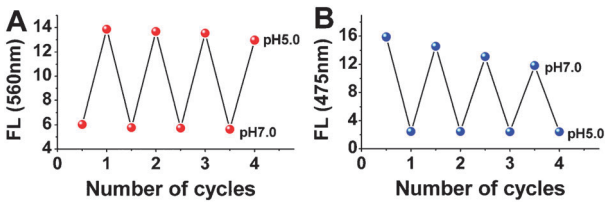


Fig. 3 Switches between drug binding and release monitored by fluorescence spectroscopy while the solution pH value oscillated between 7.0 and 5.0 at 4 °C. (A) Changes of fluorescence emission intensity of gcDNA–doxorubicin system at 560 nm on excitation at 480 nm. (B) Changes of fluorescence emission intensity of atDNA–Hoechst 33258 system at 475 nm on excitation at 346 nm.

pH 7.0, DOX can increase gcDNA melting temperature (T_m) by 16 °C, Hoechst 33258 can increase atDNA melting temperature (T_m) by 14 °C. Clearly, both DOX and Hoechst 33258 can stabilize DNA at pH 7.0. However, at pH 5.0, either positively-charged DOX or Hoechst 33258 does not increase i-motif DNA stability (Fig. S2 C and D). Compared with DNA alone at pH 7.0 (Fig. S2 E and F), drug binding hardly changes the DNA CD spectrum; this is consistent with PAGE results that drug binding does not dramatically affect DNA mobility (Fig. 2). All these results indicate that drug binding at pH 7.0 does not alter DNA conformation.

The reversibility of drug binding and release was studied by measuring drug fluorescence intensity upon pH change (Fig. 3). Changes of emission intensity at (A) 560 nm, the maximum emission wavelength of DOX (excitation at 480 nm), and (B) 475 nm, the maximum emission wavelength of Hoechst 33258 (excitation at 346 nm) were recorded when the designed DNA devices were operated for cycles. Fig. S1 A and C show that in the absence of DNA, drugs are at free state with an intrinsic fluorescence; upon addition of DNA, drug–DNA complex was formed, resulting in a dramatic fluorescence decrease for DOX binding[4a] to gcDNA (Fig. S1 A) or significant increase for Hoechst 33258 binding[8a] to atDNA (Fig. S1 C). Intriguingly, upon pH change, as illustrated in Fig. 3A, the fluorescence intensity of gcDNA–DOX at 560 nm is increased with changing pH from 7.0 to 5.0, and decreased to the initial intensity again when the pH is changed back from 5.0 to 7.0. These results indicated that DOX can bind to gcDNA at pH 7.0 and then be released at pH 5.0. Drug binding and release can be continuously cycled when the environmental pH value oscillates between 7.0 and 5.0. For the atDNA–Hoechst 33258 system, as shown in Fig. 3B, the fluorescence intensity at 475 nm is decreased with changing pH from 7.0 to 5.0, and increased to the initial intensity again when the pH is changed back from 5.0 to 7.0. These results also indicate that Hoechst 33258 can bind to atDNA at pH 7.0 and then be released at pH 5.0, and can be continuously cycled when the environmental pH value oscillates between 7.0 and 5.0. The decreased fluorescence intensity after a number of cycles should be attributed to the photo bleaching of fluorescence dye and the dilution of the solution. UV melting results further supported reversible drug binding and release upon pH change (Fig. S3).

In summary, we have constructed a novel proton-driven DNA drug binding and release device. It can reversibly bind and release anticancer agents by switching between a stem–loop hairpin DNA and an i-motif structure. This kind of DNA-drug releasing device, driven by pH without the need for an external energy source, is robust, highly reversible and does not lead to additional duplex products to undergo unwanted side-reactions in the system. More importantly, our work has demonstrated the successful combination of the operating principles of a DNA-based nanomechanical device with the unique conformation-dependent drug binding and delivery property. This may shed light on the design and construction of easy-to-handle, cost-efficient, reliable and highly efficient functional DNA nanostructures used for controllable drug binding and releasing.

The authors thank Drs J. Geng and Y. Song for their helpful suggestions on this research. This work was supported by 973 Project (20011CB936004), NSFC (20831003, 90813001, 20833006, 90913007, 20903086), and Fund from CAS.

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