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The dynamic properties of an intramolecular transition from DNA duplex to cytosine–thymine motif triplex

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We here report that the formation and breakdown of an intramolecular cytosine–thymine (CT) motif DNA triple-helix can be performed repeatedly, quickly and independently of its local concentration without performance reduction over successive cycles; as a consequence, we propose that this set of characteristics makes the DNA duplex–triplex transition an ideal candidate to power simple nanometer-scale devices capable of maintaining effective performance regardless of their local concentration.

Introduction

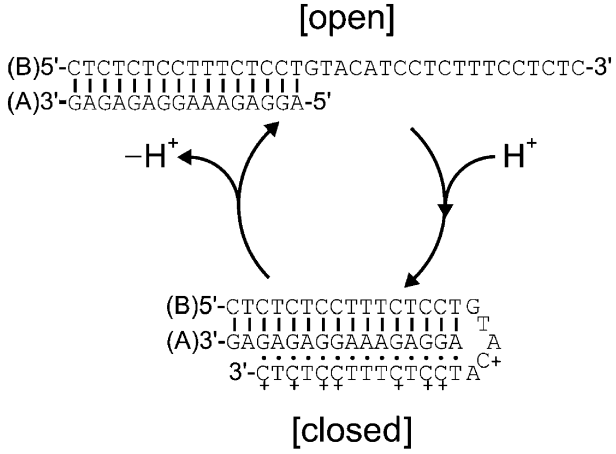
DNA is one of the most promising tools that can be employed to assemble static or dynamic architectures on the nanometer scale.[1] In particular, one field of nanoscience research that has been much investigated recently is the construction of DNA nanomotors capable of moving nanoscale objects under some sort of external input.[2] The most common strategies for obtaining controlled movement of the DNA nanostructures use competing hybridization equilibria which give rise to different topologies,[3] and take advantage of the inherent capability of DNA to dynamically assume different conformations in response to changes in the environment. DNA conformational transitions utilized so far in pursuit of this goal include, among others, the B–Z[4] transition, the duplex–tetraplex[5] transition, and the controlled DNA cruciform migration upon addition of an intercalator.[6]

To be suitable for this kind of implementation, a DNA conformational transition has to satisfy the requirements of being reasonably fast and robust,[3c] that is, to respond to repeated exposure of an appropriate switching stimulus consistently, in the same way, without showing performance change or degradation. While most of the DNA conformational transitions employed in this field so far meet these requirements, several others remain uncharacterized with respect to their speed and robustness. One such DNA conformation shift that has not been utilized so far to generate controlled motion in the nanoscale is that between double helix and CT-motif triple helix. The CT-motif DNA triple helix is a well-documented structure[7] formed by a target duplex and a homopyrimidinic 'triplex forming oligonucleotide' (TFO). The formation of this type of triplex is critically dependent on the protonation of the imino groups of the TFO cytosines and can therefore be driven dynamically by controlled pH changes.

In order to study the dynamic behavior and the robustness of this transition we designed a DNA structure capable of alternately assuming two conformations by means of the pH-driven intramolecular formation and breakdown of a CT-motif DNA triple helix, then performed static and dynamic characterization of the structure.†

Results and discussion

The structure under examination was generated by self-assembly from stoichiometric quantities of two synthetic DNA single-stranded oligonucleotides A and B (Scheme 1). Oligo A is a homopurinic 16-mer. Oligo B is a 35-mer whose sequence is composed of three sections: a 16-nucleotide (nt) long homopyrimidinic section starting from the 5′ terminus, capable of forming a Watson and Crick duplex with oligo A; a central 'hinge' made of 5 random nucleotides; and a 14-nt long homopyrimidinic portion ending on the 3′ terminus the sequence of which was designed to act as an intramolecular CT-motif TFO targeting the constituted duplex. The two oligonucleotides form an adduct with a 16-bp duplex and a 19-nt long TFO single-stranded overhang. As noted before, the conformation of this bipartite structure is critically dependent on the pH; in slightly acidic conditions (pH 5.0), the TFO section folds back on the duplex, binding parallel to its purine strand in the major groove, forming Hoogsteen-type triplets and bringing the two opposite termini of the adduct in close proximity. In moderately alkaline conditions (pH 9.0), the TFO section of oligo B cannot form a stable CT-motif triplex and assumes a random-coil conformation dangling from one end of the stable duplex (the open state). The two termini of oligo B in the adduct are thus kept further apart than in acidic conditions. The two states of the system were first characterized statically by circular dichroism (CD) spectroscopy, UV spectroscopy and electrophoretic mobility shift assay (EMSA).



Scheme 1 Oligonucleotide sequences and conformations.

CD spectra of the A + B adduct were recorded in both acidic (pH 5.0) and alkaline (pH 9.0) conditions (Fig. 1a). The spectrum recorded at acidic pH shows a negative peak at *ca.* 215 nm that has been typically associated with the proposed triplex structure.[8]

The shift between open and closed conformations was also observed following ascending and descending pH titrations of the A + B adduct by UV spectroscopy (Fig. 1b). A plot of the UV absorbance at 260 nm *vs.* the pH of the sample shows the stability zones of the two conformations, evidenced as zones of markedly different absorption separated by a sharp variation at pH 6.5. This can be explained owing to the different extent of the hypochromic effect associated with the two different structures.[9]

To further confirm the hypothesized structural change of the adduct, a modified version of oligo B was synthesized, with

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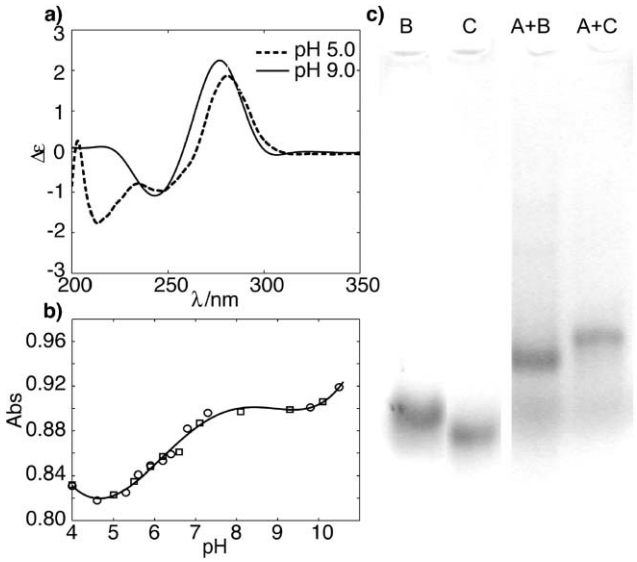

Fig. 1 (a) CD spectra of the open and closed states. Open state: 10 μM A + B in 20 μM tris, 20 mM $MgCl_2$, 50 mM NaCl at pH 9.0. Closed state: 10 μM A + B in 20 mM, 20 mM $MgCl_2$, 50 mM NaCl at pH 5.0. (b) Ascending (squares) and descending (circles) pH titration with 0.1 M HCl or NaOH of a 15 μM A + B, 20 mM $MgCl_2$, 50 mM NaCl solution followed by UV absorption at 260 nm. (c) Electrophoretic mobility of the B and C 35-mer single-stranded oligonucleotides and their adducts A + B and A + C at pH 5.0. Oligo B is slower than oligo C, however its adduct with oligo A is faster than the A + C adduct (see text for discussion). Gel run in a 20 mM acetate, 20 mM $MgCl_2$, 50 mM NaCl buffer at pH 5.0.

the TFO sequence portion replaced by a random sequence of the same length (oligo C). Stoichiometric adducts A + B and A + C were prepared, and then analyzed by nondenaturing polyacrylamide gel electrophoresis, both in basic (pH 9.0) and acidic (pH 5.0) buffer conditions along with their single-stranded constituents. At pH 9.0, the B and C oligonucleotides exhibit the same electrophoretic mobility. Similarly, the A + B and the A + C adducts also have the same mobility at this pH (data not shown). At pH 5.0, however, oligo C runs faster than oligo B, while the A + C adduct is slower than the A + B adduct (Fig. 1c). Oligo C includes 12 cytosines, whereas oligo B includes 16. Since these are protonated at acidic pH, the overall negative charge density of oligo C is expected to be higher than that of oligo B, explaining the observed mobility difference. The higher mobility of the A + B adduct with respect to the A + C can be rationalized by the increase in compactness upon triple helix formation that overrides the aforementioned charge density effect.

The repeated switching back and forth between the open and closed states of the system was monitored in real time with fluorescence spectrophotometry. Following an established approach,[2] a modified version of oligo B was synthesized, bearing a Rhodamine Green fluorophore at the 5′ end and a Dabcyl quencher moiety at the 3′ end (oligo B*). Rhodamine Green shows a strong fluorescence emission at *ca.* 530 nm when excited at 500 nm, and its fluorescence yield is pH-insensitive between pH 4.0 and 9.0. The fluorescence emission intensity of the A + B* system was measured while the pH of the sample was repeatedly cycled between 5.0 and 9.0 with controlled additions of concentrated HCl and NaOH. This evidenced dramatic shifts in fluorescence synchronized with the induced pH changes (Fig. 2a).

The fluorescence emission changes are indicative of the system alternately assuming the open and closed state, because the fluorescent dye and the quencher on the two termini of the adduct are kept in close proximity in the closed state (thus allowing energy transfer from emitter to quencher)[10] and further apart in the open state. The switching between the two states of the structure is completed within a few seconds from the acid or base addition and is therefore comparable with the switching speeds shown by published DNA transition-based molecular devices.[3–6] The fluorescence intensity consistently oscillates between two clearly defined states upon repeated exposure of the construct to the input stimuli, thus demonstrating the robustness of the observed transition. The slight gradual reduction observed in the overall fluorescence intensity over the cycles is due to, and quantitatively correlates with, the dilution of the construct solution upon acid or base addition. This demonstrates that the molecular mechanism of this transition is not hampered (over the observed cycles) by the accumulating 'waste product' NaCl. The gradual increase of ionic strength due to the accumulating salt is not expected to significantly change the electrostatic potential near the surface of the DNA structure, and consequently its performance, at least until the salt reaches molar concentration.[11]

The cycling of the examined structure does not entail any binding event involving two macromolecules, and should not therefore be influenced by the local concentration of the structure itself. The speed and the robustness of the cycling were proved to be concentration-independent by diluting the A + B adduct from micromolar (Fig. 2a) to less than nanomolar (Fig. 2b) and successfully repeating the cycling experiment, evidencing the same overall behavior.

From a publicly available 3D structure of a CT-motif triplex,[12] we could estimate the distance between the two termini of oligo B to be approximately 2 nm in the closed state. Employing a polymer statistics model developed for chains with sections of different flexibilities,[13] we estimated that the root-mean-square distance between the two termini of oligo B is around 8 nm in the open state. This implies that the described repeated cycling of the pH generates a fully controllable relative motion of two termini that brings them to span on average a 6 nm long segment.

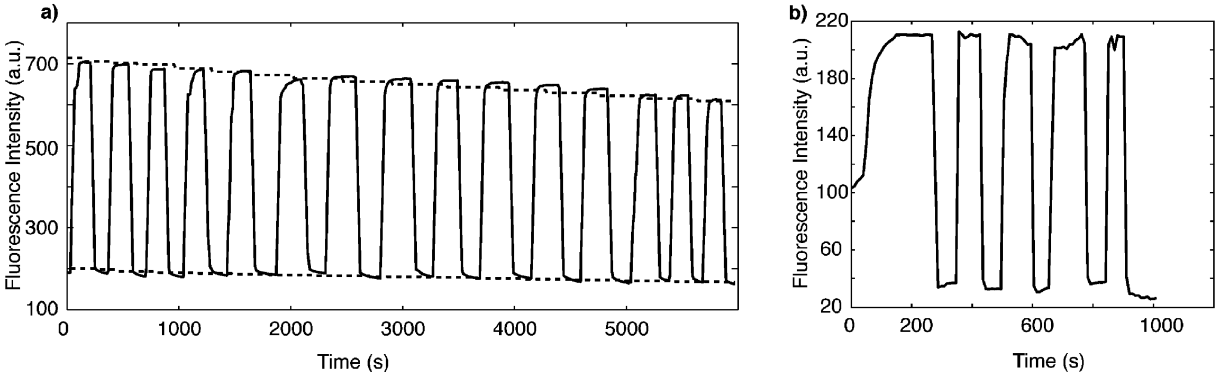

Fig. 2 (a) Cycling of the A + B* nanomotor observed by fluorescence spectroscopy. Excitation was set at 500 nm and emission monitored at 530 nm. The initial A + B concentration was 0.33 μM in a 20 mM $MgCl_2$, 50 mM NaCl solution. The pH was cycled between 5 and 9 by alternated addition of 0.1 M HCl or NaOH. Dashed lines show a plot of the expected peak intensity decrease resulting from dilution calculations. Switching between states is complete within a few seconds after the addition of acid or base. (b) Repetition of the experiment with an initial A + B concentration of 0.33 nM.

The collected evidence on the dynamic properties of the investigated transition permits us to add the duplex–triplex conformational transition to the repertoire of the possible DNA nanodevice mechanisms. A structure capable of performing the transition we discussed could easily be coupled with a variety of nanoobjects to dynamically and precisely control their relative distance. Recent literature reports several examples of DNA nanomotors constituted by a DNA actuator capable of dynamic response to external stimuli, coupled to a rigid DNA structure.[3–6,14]

All the DNA nanoscale devices published so far include in their cycles one or more events involving two macromolecules, usually two complementary oligonucleotides. This implies that their cycling is concentration dependent, and could suffer worsening if performed in the proximity of an obstacle capable of diminishing the access of macromolecules, such as a surface of some sort. Devices powered by the duplex–triplex transition we studied would have the advantage of not producing significant waste products and not being influenced by their concentration or the surroundings in which they would operate. The transmission of the device opening and closing signals would be directly dependent on the mobility of very small and fast-diffusing species such as H^+ and OH^-. These characteristics would make the smooth functioning of the nanodevice possible in conditions that could hamper the performance of the DNA nanomotors reported so far, such as in nanosized pores, or in systems where the mobility of the species involved in the cycling is critical.

Experimental

Oligonucleotides were purchased from MWG (Ebersberg, Germany), HPLC purified and lyophilized by the supplier, and suspended in MilliQ H_2O (Millipore Simplicity) prior to use. Adducts in the desired conformations were assembled by mixing oligonucleotides in the appropriate buffer.

CD studies were conducted on a J-710 spectropolarimeter (Jasco, Easton, USA) in a 300 µl quartz cuvette with a 0.1 cm path length, containing a 10 µM solution of the nanomotor in either 20 mM acetate buffer at pH 5.0 or 20 mM Tris buffer at pH 9.0. Both samples included 50 mM NaCl and 20 mM $MgCl_2$.

UV spectra were recorded on a SmartSpec3000 UV-vis spectrophotometer (BioRad, Hercules, USA) in a 500 µl quartz cuvette with a 1 cm path length, loaded with a 15 µM A + B adduct, 20 mM $MgCl_2$, 50 mM NaCl solution.

FRET experiments were performed on a LSB-50 fluorescence spectrometer (Perkin Elmer, Boston, USA) in a 3 ml quartz cuvette equipped with a magnetic microstirrer and loaded with 2 ml of a 0.33 µM or 0.33 nM A + B* adduct, 50 mM NaCl and 20 mM $MgCl_2$ solution. The pH of the sample was cycled between 5 and 9 alternately adding 6 µl of 0.1 M HCl or 0.1 M NaOH aqueous solutions directly into the cuvette with a syringe micro-pump.

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Notes and references

† During the publishing of this manuscript, the group of Professor C. Mao presented similar experimental results obtained independently (Y. Chen, S.-H. Lee and C. Mao, *Angew. Chem., Int. Ed*., 2004, **43**, 5335–5338).

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