SHORT COMMUNICATION

Allosteric regulation of small-molecule binding to aptamers*

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Abstract: Modular aptameric sensors for oligonucleotides were constructed by combining molecular beacon stem-loops and malachite green aptamers. These sensors were designed so that oligonucleotides complementary to the stem-loop region accomplish the release of malachite green from its binding module. The oligonucleotides that bound to both loop and stem caused efficient release of the dye. While this system is not suitable for single-mismatch detection, it represents proof-of-concept for drug delivery components of autonomous therapeutic devices on a molecular scale.

Keywords: aptamers, allosteric control, fluorescence, malachite.

INTRODUCTION

Almost three years have passed since our group proposed to use molecular scale logic elements based on nucleic acid catalysts for the construction of autonomous therapeutic devices.1 Since the initial suggestion, we have made continuous progress toward general Boolean computation in solution-phase by deoxyribozyme based logic gates.2,3 The next step in our work is to demonstrate the ability of molecular logic gates to trigger a therapeutic action through the release of drugs. There are several ways to accomplish this goal, and the conceptually simplest would be to build on the precedent set by Sullenger and colleagues,4 who demonstrated that the activity of an RNA aptamer could be reversed in vivo by adding an oligonucleotide antidote. In order to expand this approach for our purposes, we could take an aptamer that binds to a small molecule drug and engineer it to release this drug upon sensing an oligonucleotide formed by a molecular scale logic gate. In this paper, I demonstrate the engineering of malachite green aptamer into a sensor that recognizes oligonucleotides, releases the dye and signals this process by a change in fluorescence.

RESULTS AND DISCUSSION

The choice of malachite green for this demonstration was inspired by the report by Tsien's group5 that malachite green (MG) complex with its aptamer...
MGA is strongly fluorescent, while the dye itself is not. This observation provides us with a simple method to follow the dye release, that is, the loss of fluorescence in the solution. We have recently taken MGA and engineered a series of modular aptamers capable of sensing small molecules (ATP, FMN and theophylline) through cascaded stabilization of the malachite green binding site; it was likely that the principles behind such modular aptamers could be expanded to sensing oligonucleotides in solution. For this purpose, the standard oligonucleotide recognition element, a stem-loop molecular beacon, could be combined with malachite green aptamer. Previously, we used this stem-loop successfully in our work on deoxyribozyme-based logic gates. In this element, when complementary oligonucleotide recognizes the loop, the stem opens, because double helical loop cannot co-exist with a closed stem. Thus, the hypothesis behind the new constructs is that, if this stem is an integral part of the malachite green aptamer, its opening

1 TCT CAA GCC GAA CAC
2 GGAC TCT CAA GCC GAA CAC
3 GAC TCT CAA GCC GAA CAC
4 CTTG TCT CAA GCC GAA CAC CT
5 TTG TCT GAAGC C GAA CAC CT
6 GAC TCT CAA GCC GAA CTC
7 GAC TCT CAA CCC GAA CTC

Fig. 2 Structures of oligonucleotide inputs.
would distort the malachite green binding core. This would in turn trigger the release of malachite green, which could be detected by a decrease in fluorescence.

The malachite green aptamer has two stems, and in order to test this hypothesis, two modular aptamers were constructed, by attaching stem-loops at the both ends of MGA. The resulting MB.1-MGA (from molecular beacon 1 bound to one stem of the malachite green aptamer) and MGA-MB.1 (malachite green aptamer bound by the other stem to the molecular beacon 1) are shown in Fig. 1. In principle, both of these constructs should release malachite green if the stem is opened through the binding of an oligonucleotide complementary to the loop. Accordingly, I proceeded to test the ability of these constructs to signal the presence of oligonucleotides complementary to the loop region, as is standard for molecular beacons. Somewhat surprisingly, the addition of excess of oligonucleotide containing the stretch complementary only to the loop to the solution of beacon-aptamer constructs (0.3 μM and MG (2 μM) in buffer (Tris-HCl 20 mM, pH 7.4, KCl 140 mM, NaCl 5 mM, MgCl₂ 5 mM) did not lead to a significant change of solution fluorescence (λ_exc = 610 nm, λ_em = 650 nm). The likely explanation for this observation is that the complementary oligonucleotide is bound to the loop, but then either failed to completely open the stem or relieved the strain by not base-pairing at all 15 complementary positions. In any case, it was clear that malachite green binding was interfering with the stem-opening and that we had to extend the binding oligonucleotide into the stem to achieve MG release. And, as long as the oligonucleotide could be extended only into one part of the stem, this would not interfere with our ability to recognize generic oligonucleotide sequences in solution.

Next, the ability of two oligonucleotides to affect the release of MG from MB.1-MGA was tested: oligonucleotide 2 (Fig. 2), which extended into the complete one side of the loop, and oligonucleotide 3 which extended only three-base pairs into the loop; furthermore, similar tests were performed for oligonucleotides 4 and 5 that bound to MGA-MB.1. The first conclusion of these experiments is that MB.1-MGA is more suitable for our purposes, because it yielded a better signal (Fig. 3); so we decided to focus on this construct. A somewhat unexpected result was that 2 and 3 behaved nearly identically; specifically, even when present in great excess, they affected only incomplete release of MG from its aptamer complex. This shows how strong the stabilizing effect of the MG binding is on the stem structure within aptamer, and it argues that to achieve complete MG release we would have to add an oligonucleotide complementary to part of binding pocket.

Importantly, the observation that we need longer oligonucleotides to achieve the complete release of MG does not reveal any limitation for its eventual applications in molecular devices. However, the somewhat reduced signal for the detection of shorter oligonucleotides implies a reduced sensitivity in applications related to the general detection of oligonucleotides.

In order to test the ability of MB.1-MGA to detect mismatches we tested two oligonucleotides 6 and 7, which both had a secondary, so called auxiliary mutation
While the sensor was able to distinguish the same concentrations of these oligonucleotides in solution, at higher concentrations both analytes were able to affect equally the release of malachite green (Fig. 4). While similar results could be interpreted as the ability to detect mismatches (at least at certain concentrations), within the context of near perfect mismatch detection by some of the other systems, we are reluctant to put forward that claim.

In conclusion, the results presented herein clearly demonstrate that stem-loop control could be expanded to the release of small molecules from their complexes with aptamers. However, small molecule binding stabilizes the connecting stem, which requires us to use either longer loops (this option was not tested here) or to extend the complementary region into the stem, as was demonstrated here. While aptamers attached to the stem-loop seem not to be perfect tools for mismatch detection, they suffice as small-molecule releasing elements that could accept oligonucleotides formed by upstream computation elements.
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ИЗВОД

АЛОСТЕРНА РЕГУЛАЦИЈА ВЕЗИВАЊА МАЛИХ МОЛЕКУЛА ЗА АПТАМЕРЕ

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У циљу да се демонстрира могућност да олигонуклеотидни рецептор (или аптамер) отпусти мали молекул у зависности од присуства било ког олигонуклеотида, конструкцисан су модуларни аптамерни сензори. У овим сензорима, комбинован је аптамер који везује малахит-зелено рецептор за олигонуклеотиде. Низом експеримената, демонстрирано је да само олигонуклеотиди који се везују за део основне структуре аптамера могу ефикасно да ослободе везану боју. Мада овај систем неће бити погодан да се препознају у раствору олигонуклеотида са једном неспареном Вотсон-Крик базом они су важан корак ка конструкцији аутономних терапеутских направа на молекулском нивоу.

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REFERENCES