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Small-Molecule-Dependent Split Aptamer Ligation

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Supporting Information

ABSTRACT: Here we describe the first use of small-molecule binding to direct a chemical reaction between two nucleic acid strands. The reported reaction is a ligation between two fragments of a DNA split aptamer using strain-promoted azide−alkyne cycloaddition. Utilizing the split aptamer for cocaine, we demonstrate small-molecule-dependent ligation that is dose-dependent over a wide range of cocaine concentrations and is compatible with complex biological fluids such as human blood serum. Moreover, studies of split aptamer ligation at varying salt concentrations and using structurally similar analogues of cocaine have revealed new insight into the assembly and small-molecule binding properties of the cocaine split aptamer. The ability to translate the presence of a small-molecule target into the output of DNA ligation is anticipated to enable the development of new, broadly applicable small-molecule detection assays.

Molecular recognition between DNA molecules has proven to be a powerful tool for directing and promoting chemical reactions.[1] DNA-templated reactions have been utilized for a wide assortment of tasks including ligating complementary oligonucleotides,[2] generating encoded libraries of complex small molecules,[3] and detecting nucleic acids[4] and proteins.[5] However, in all of these examples, the DNA-templated reactions are dependent upon the sequence-defined affinity of the nucleic acid strands for one another.[6] Herein we report what is, to our knowledge, the first DNA-templated reaction controlled by small-molecule binding rather than inherent Watson−Crick affinity between the DNA strands. The recognition element utilized to accomplish this task is a split aptamer, which is comprised of two nucleic acid strands that only bind to one another in the presence of a specific small-molecule target.[7] Using the reported split aptamer for cocaine,[8] we demonstrate a small-molecule-dependent ligation that is dose-dependent over a wide range of cocaine concentrations and is compatible with complex biological fluids such as human blood serum. The ability of a split aptamer to translate a specific small-molecule signal into the output of DNA ligation is anticipated to enable the development of new, broadly applicable small-molecule sensing platforms.

In choosing a reaction suitable for small-molecule-dependent split aptamer ligation, our goal was to utilize chemistry that would not interfere with the small-molecule target and would be compatible with complex biological fluids. Strain-promoted azide−alkyne cycloaddition appeared to be a promising choice as it is orthogonal to a wide assortment of functional groups and does not require additional reagents.[9] As shown in Figure 1, one cocaine split aptamer fragment was coupled with a cyclooctyne carboxylic acid[10] to give **1**, and the other was synthesized incorporating an azide (**2**). Strand **1** was additionally functionalized with a Cy3 fluorophore, enabling visualization of the ligation reaction by denaturing polyacrylamide gel electrophoresis (PAGE).

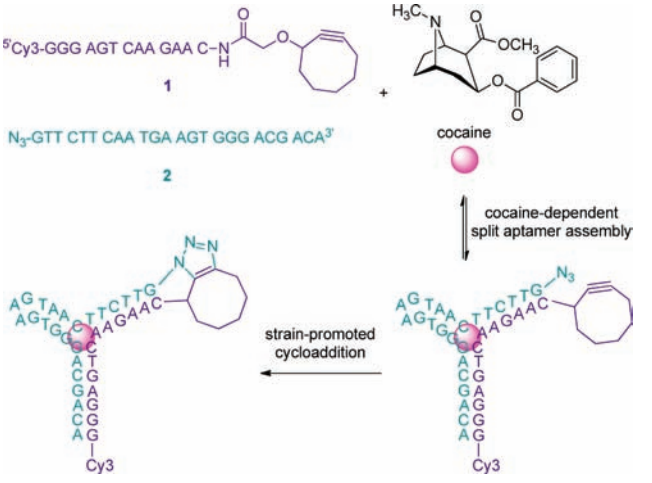

Figure 1. Cocaine-dependent split aptamer ligation using strain-promoted azide−alkyne cycloaddition. Cocaine directs assembly of the split aptamer, bringing the azide and cyclooctyne in close proximity and thus promoting the ligation.

If present in solution, cocaine directs assembly of the split aptamer fragments, placing the azide and cyclooctyne in close proximity to one another. This dramatically enhances the effective molarity of the reactants, accelerating the templated cycloaddition relative to the untemplated background reaction. Split aptamer assembly is an equilibrium process dependent in part upon the concentration of cocaine. Thus, we anticipated that the templated ligation would proceed in a dose-dependent manner with respect to cocaine. Denaturing PAGE was used to monitor reaction progress, as unligated **1** migrates farther on the gel relative to ligated **1**+**2**, and quantification of Cy3 fluorescence reveals the portion of **1** that has been incorporated into ligated product. As shown in Figure 2, the templated ligation does in fact proceed in a dose-dependent manner for cocaine concentrations from 1 μM to 1 mM, providing ligation yields of 5−74%. Moreover, no reaction is observed when the cyclooctyne (**1-NH$_2$**) or azide (**2-OH**) is omitted or one base is mutated in the azide strand (**2-mut**). These controls demonstrate that cocaine-dependent assembly of the split aptamer is sequence-specific and that ligation proceeds via reaction between the azide and cyclooctyne functional groups.

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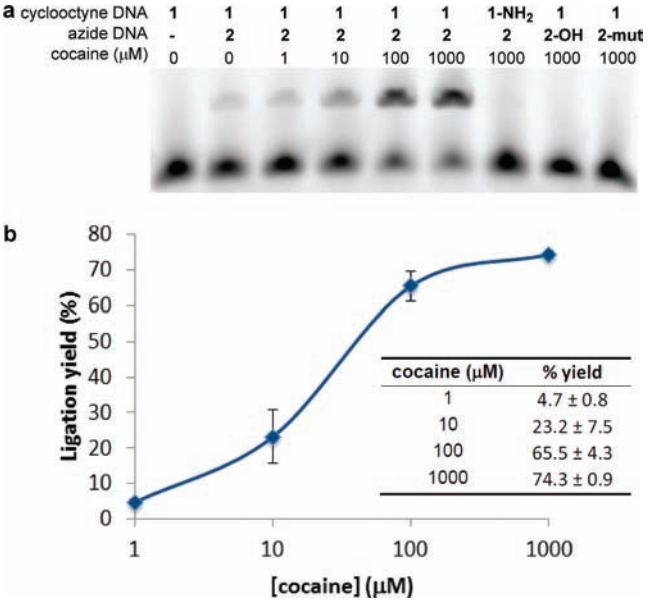

Figure 2. (a) Denaturing PAGE of ligation reactions. Lower bands represent unreacted strand **1** and upper bands represent **1**+**2** ligated product. Conditions: 0.5 μM **1**, 2.0 μM **2**, 25 mM Tris, pH 8.2, 5 mM NaCl, 4 h. (b) Yield of ligated product as a function of cocaine concentration. Errors represent standard deviation of three independent trials.

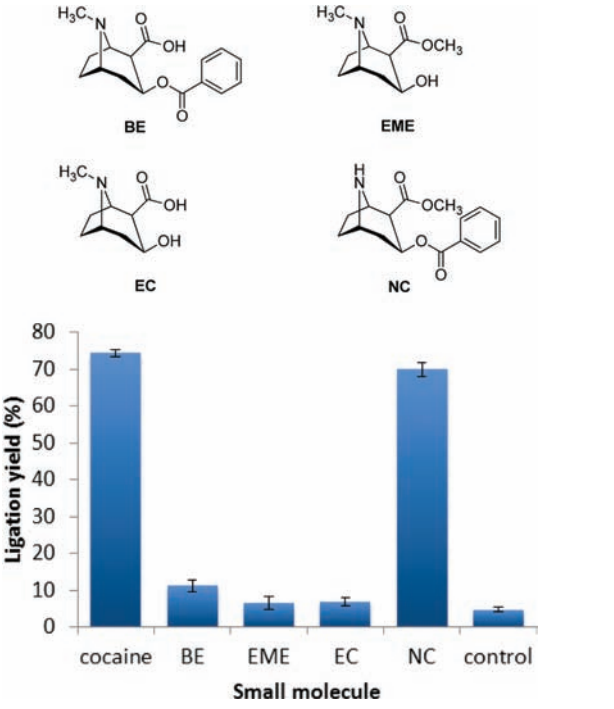

Figure 3. Selectivity of split aptamer ligation for cocaine versus metabolites. Conditions: 0.5 μM **1**, 2.0 μM **2**, 25 mM Tris, pH 8.2, 5 mM NaCl, 1 mM metabolite, 4 h. Control is same conditions as above but no cocaine or metabolite. Error bars represent standard deviation of three independent trials.

We next investigated the selectivity of the split aptamer ligation for cocaine versus its structurally similar metabolites. Previous studies using the cocaine split aptamer have demonstrated that aptamer assembly is not significantly induced by benzoylecgonine (BE) or ecgonine methyl ester (EME).[8] Binding of ecgonine (EC) by the split aptamer has not been studied, but the regular cocaine aptamer has been shown to selectively bind cocaine over EC.[11] Thus, we anticipated that our split aptamer ligation would not be strongly promoted by BE, EME, or EC. We were surprised to find no reports on the selectivity of the cocaine aptamer or split aptamer for the metabolite norcocaine (NC), in which the bridge nitrogen is demethylated. As shown in Figure 3, reactions using 1 mM BE, EME, or EC result in nominal ligation yields of 11, 7, and 7%, respectively. This indicates that both the methyl ester and the benzoyl group are crucial for binding of the small molecule to the split aptamer. Interestingly, we found that 1 mM NC did in fact promote the ligation reaction, giving a yield of 70%, only 4% less than the yield observed with cocaine. This result suggests that the methyl group of the bridge nitrogen does not play a significant role in recognition of cocaine by the split aptamer. Curiously, the overall tolerance of the split aptamer to cocaine modification is similar to the tolerance of the biological receptors that cocaine targets *in vivo*, as norcocaine is the only cocaine metabolite shown to be pharmacologically active.[12]

Our long-term goal is the application of small-molecule-dependent split aptamer ligation toward the development of new assays for sensing drug molecules and metabolites in biological samples. Thus, we sought to establish whether the templated ligation would be compatible with human blood serum. DNA **1** and **2** were incubated in the same buffered solution used in the experiments above (25 mM Tris, pH 8.2, 5 mM NaCl), but having 20% added serum. PAGE analysis revealed significant ligation even in the absence of cocaine (Figure 4a, lane 1). Our earlier studies of the ligation in buffer had revealed that increasing the concentration of NaCl in the reaction mixture led to increasing degrees of background ligation (see Supporting Information). We hypothesize that higher salt concentrations increase the affinity of the DNA strands for one another such that they can anneal, and subsequently react, even in the absence of the small-molecule target.[13] Given this observation and the fact that sodium ion concentration in normal human serum is 137−147 mM,[14,15] it was not entirely surprising to observe significant background reaction in 20% serum.

Rather than further dilute the blood serum, we hypothesized that we could re-engineer the split aptamer to be more "salt tolerant" by converting one or more base pairs to mismatches. This would lower the inherent affinity of the DNA strands, presumably compensating for the increased driving force for annealing that is imparted by the higher salt concentration. We screened eight azide strands having varying levels of mutation[16] and found that DNA **3** was sufficiently mutated to drastically reduce the undesired background reactivity yet retain the ability to assemble and react with **1** in the presence of cocaine (Figure 4). In strand **3**, one GC base pair is mutated to a CC mismatch. However, this negative effect is partially compensated for by mutating a GT wobble pair to a GC base pair. These results speak to the tunability of split aptamer assembly, as only a subtle change to the level of base-pairing was necessary to dramatically alter the binding properties of the DNA strands.

Using mutated DNA **3**, we were able to demonstrate cocaine dose-dependent ligation with **1** in a sample containing 20% human blood serum (Figure 4a, lanes 2−6).[17] Interestingly, PAGE does not show any evidence of nuclease degradation of the DNA strands. However, we do observe lower yields for the cocaine-dependent ligation in the buffer−serum mixture

compared with buffer alone. This is likely a result of cocaine hydrolysis by serum esterases, as cocaine is known to be rapidly metabolized *in vivo* to BE and EME,[18] neither of which is capable of promoting the ligation reaction.[19]

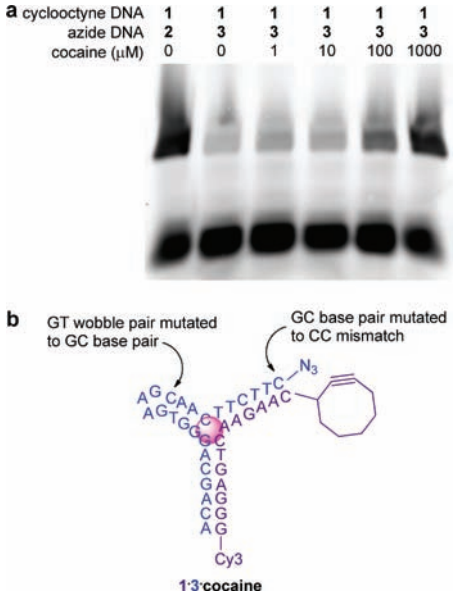

Figure 4. (a) Denaturing PAGE of ligation reactions in human blood serum. Conditions: 0.5 μM **1**, 2.0 μM **2** or **3**, 25 mM Tris, pH 8.2, 5 mM NaCl, 20% serum, 8 h. (b) Mutant sequence **3** has two mutated bases, which impart a net negative effect on duplex formation with **1**.

Using the cocaine split aptamer, we report here the first example of a DNA-templated reaction that is dependent upon small-molecule binding rather than inherent Watson−Crick affinity. This templated ligation is dose-dependent for cocaine concentrations from 1 μM to 1 mM in buffer and from 10 μM to 1 mM in human blood serum. Studies of the templated reaction under varying conditions have revealed the salt sensitivity of split aptamer assembly and have enabled the re-engineering of a more "salt-tolerant" cocaine split aptamer sequence. Additionally, studies of the split aptamer ligation using cocaine metabolites in which the main functional groups are systematically modified have revealed new insights into the interaction of the split aptamer with cocaine.

The ability to transduce a small-molecule signal into the output of DNA ligation is anticipated to serve as an enabling technology for the development of new small-molecule biosensing platforms. Work is currently underway in our laboratory to explore alternative ligation chemistry, engineer split aptamers for new small-molecule targets, and use split aptamer ligation for the development of enzyme-linked small-molecule detection assays.

■ ASSOCIATED CONTENT

Supporting Information. Complete DNA sequences, experimental procedures, and results for salt-dependent background ligation. This material is available free of charge via the Internet at http://pubs.acs.org.

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