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Aptamer-Based Colorimetric Probe for Cocaine

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In vitro selection and amplification procedures allow isolation of nucleic-acid receptors or aptamers for almost any small molecule or protein target.[1] Similarly, immunization can elicit antibodies against most molecular targets.[2] However, no general method exists to engineer such nucleic acid- or protein-based receptors to permit direct transduction of the recognition event into a change in the visible spectrum.[3,4a,5] As a solution to this problem, we considered that if a macromolecule would bind both a chromophore and the analyte of interest, then the binding of analyte might alter the microenvironment of the chromophore and produce a visible signal of that event. Oligonucleotide receptors were of particular interest to us, because the interactions between oligonucleotides and dyes have been well studied.[4] Accordingly, we sought to convert our leading anti-cocaine aptamer **MNS-4.1**[6] (K_d < 5 μM, Figure 1) into a colorimetric sensor. The low-micromolar dissociation constant and selectivity for cocaine of this aptamer does not allow the determination of picomolar concentrations of cocaine metabolites in urine, but is sufficient for hand-held colorimetric field tests used in drug supply interdiction.

Cocaine (**1**) binds **MNS-4.1** via a hydrophobic pocket formed by a noncanonical three-way junction, with one of the stems (S_3) structured through currently less well-defined non-Watson−Crick interactions. We screened a collection of 35 dyes for changes in visible spectra upon addition of a stock solution of cocaine to a mixture of a given dye and the aptamer. We tested final cocaine concentrations of 20 and 200 μM, and 2 mM. Many dyes complexed with our aptamer but were displaced only at the highest concentration of cocaine with mild shifts in absorption maxima. In contrast, the absorbance of cyanine dyes exhibited substantial inverse dependence on cocaine concentration in the micromolar range. Furthermore, one cyanine dye, diethylthiotricarbocyanine iodide (**2**), displayed both a significant attenuation of absorbance and a change in the ratio of two relative maxima that dominated the visible spectrum. Therefore, we chose **2** to construct a colorimetric molecular sensor for cocaine.

Upon testing various conditions, the following procedure was used to study the concentration-dependent changes in absorption spectra: the mixture of diethylthiotricarbocyanine (c = 7 μM, stock solution in methanol or DMSO, 1 mg/mL) and our aptamer (c = 4 μM) was formed in the binding buffer (50 μL, 20 mM TRIS*HCl, pH = 7.4, 140 mM NaCl, 2 mM $MgCl_2$), and after equilibration for 5 min, cocaine (stock solutions 100, 10, and 1 mM) was added in portions via micropipet, followed by acquisition of the absorption spectra within 1 min. At cocaine concentrations increasing from 2 to 600 μM, the absorption at 760 nm decreased progressively, whereas absorption at 670 nm remained nearly constant. At the higher concentrations of the dye and aptamer, the 670-nm peak actually increased significantly upon addition of cocaine. Incubation

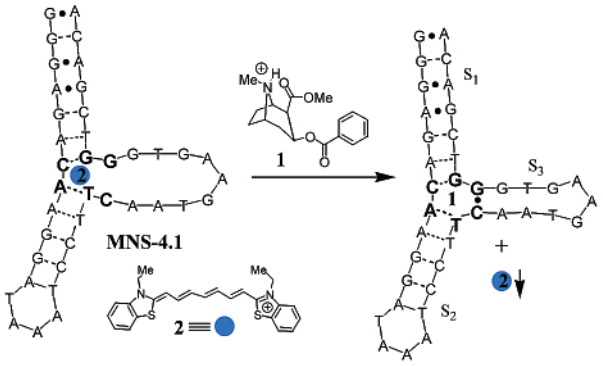

Figure 1. Aptamer **MNS-4.1** shown complexed with a monomeric dye; cocaine (**1**) displaces dye, causing an immediate attenuation of absorbance and eventual precipitation of dye.

of mixtures for 15 min led to an increase in sensitivity (to 0.5 μM with aptamer at 2 μM), although with reduced range (to 64 μM). We did not detect any change in the visible spectra of the aptamer−dye complex upon addition of cocaine metabolites benzoyl ecgonine (**3**) and ecgonine methyl ester (**4**) up to 2 mM final concentration, indicating a highly selective interaction with cocaine. The sensitivity and selectivity of the colorimetric sensor was slightly better than of the corresponding fluorescent sensors, indicating that the noncovalent attachment of the dye is less intrusive than the covalent attachments and modifications of secondary structure required to construct our fluorescent sensors for cocaine.[6]

Interestingly, **2** is poorly soluble in the binding buffer without added aptamer (A < 0.1 after standing for 15 min and addition of cocaine caused no change in visible spectra), whereas addition of the aptamer significantly increased absorbance. On the basis of the literature precedent for indocyanine dyes interacting with DNA sequences[4a] we assigned the two maxima for indocyanine bound to aptamer as monomer (760 nm) and as dimer (670 nm). To confirm this assignment, we progressively increased the concentration of the aptamer in a solution of dye−aptamer complex and observed the expected increase in the absorbance of monomer at 760 nm and a corresponding decrease in the absorbance of dimer at 670 nm. To characterize the structure of the dye−aptamer complex, we assessed the binding of the dye to various domains within the aptamer: the stacked S_1 and S_2 stems connected through a five-nucleotide bulge (GAAAC) in place of S_3, and the isolated S_1 stem (with an added terminal AAAA tetraloop) and the S_2 stem−loop. We observed strong binding of the dye to the aptamer mutant containing both S_1 and S_2 stems, with unchanged positions of the relative maxima. In contrast, we observed no dye−oligonucleotide interactions with the individual stems. As expected, none of the three oligonucleotides showed detectable binding to cocaine. These results indicate that coaxially stacked S_1 and S_2 stems are the minimal binding motif for the dimer and monomer of **2**. The fully

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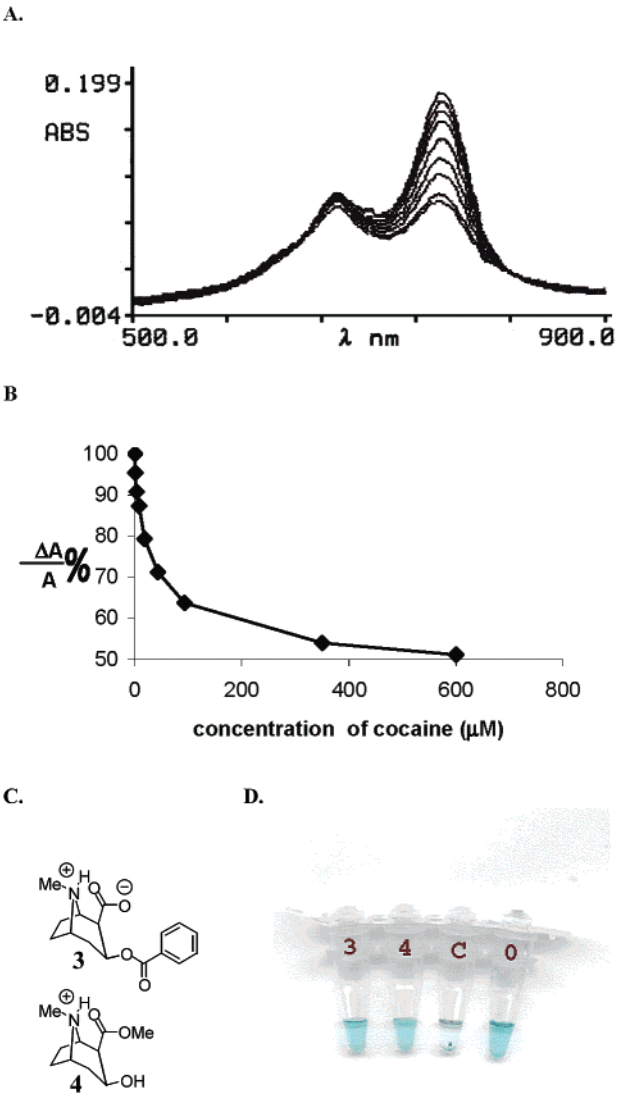

Figure 2. (A) Changes in absorbance upon complexation with cocaine (0.5−600 μM). (B) Calibration curve of changes in absorbance at 760 nm vs concentration of cocaine. (C) Structures of cocaine metabolites benzoyl ecgonine (**3**) and ecgonine methyl ester (**4**). (D) The dye−aptamer complex in the presence, from left to right, of benzoyl ecgonine (**3**), ecgonine methyl ester (**4**), cocaine (**C**), and blank control (**0**).

matched three-way junction[7] also binds to this dye, but with an increased relative proportion of the bound dimer. This complex retains the capacity to sense cocaine in solution, albeit with a lesser sensitivity. These experiments are consistent with the proposal that the dye binds as both monomer and dimer in the region of the hydrophobic pocket,[4a] and that for this binding, two surfaces of the coaxially stacked S_1 and S_2 stems suffice. Cocaine interacts rapidly with the monomeric dye−aptamer complex, and release[8] of dye would explain reduction of the 760-nm absorbance within seconds. The increase in absorption at 670 nm observed at the higher concentrations of the dye and aptamers is consistent with competition of released dye with cocaine for the binding to remaining monomeric dye−aptamer complex.

Finally, we tested whether it was possible to induce a visibly apparent color change through the use of higher dye−aptamer concentrations. Mixing our aptamer with cocaine, cocaine metabolites, or blank, followed by the addition of the dye (c(aptamer) = 20 and c(dye) = 40 μM, c(analytes) = 500 μM, adding dye first, and then analytes gave the same results) resulted in a strongly colored solution ($A_{760} > 1.5$, and $A_{670}/A_{760} \approx 1$). Displacement of the dye was readily detected by spectrophotometer, and the faintly cocaine-containing sample resembled the control solution without aptamer. However, after 12 h we were able to distinguish visually these solutions through the appearance of a blue precipitate and partial decolorization of the solution in samples containing cocaine (Figure 2D). Prolonged incubation (over 48 h) led to the decolorizing of all solutions, apparently due to hydrolysis of the dye in the slightly basic buffer, but the blue precipitate (identified as dye **2** through comparison with an authentic sample by TLC and VIS spectra) remained in the cocaine-containing tube. These results fully support a mechanism in which cocaine displaces dye from aptamer—within seconds for the monomeric dye and within hours for the dimeric dye—and precipitation of dye follows.

The ability of receptors to undergo visible changes upon recognition of their ligands in "mix and measure" assays could result in simpler and less expensive colorimetric analytical procedures, including spot tests for small molecules. The fact that we could screen only 35 dyes and identify more than one able to interact productively with our aptamer underscores the likelihood that for the majority of oligonucleotide receptors the proper dye−aptamer combination could be similarly found. From another perspective, the three representative[1b] oligoribonucleotides that bind small molecules, an anti-ATP aptamer, an anti-theophiline aptamer, and an anti-argininamide HIV TAR, all contain coaxially stacked nonconserved helical stems connected through bulges (similar to the structure of our aptamer), and we anticipate that they would be good candidates for this approach to colorimetric sensing.

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