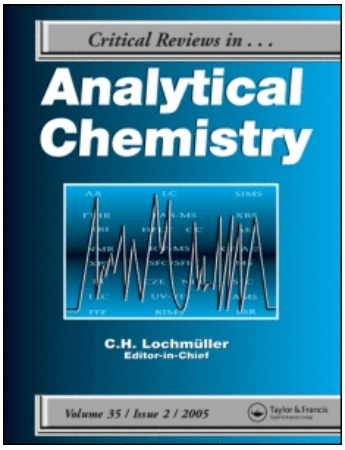

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Prospects of Ligand-Induced Aptamers

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Prospects of Ligand-Induced Aptamers

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Aptamers are rare functional nucleic acid ligands that bind with high affinity and specificity to their target ligands. Selected aptamers have been shown to inhibit the functions of their cognate targets both *in vitro* and *in vivo*. As a consequence, the first aptamer-based drug to treat age-related macular degeneration has been developed. On another front, aptamers have also successfully shown potential use in diagnostics and imaging technology. The next wave of aptamer applications involves the development of ligand-induced aptamers. These aptamers rely on the principles commonly observed in many ribonucleic acid (RNA)-ligand interactions. In the present review, we describe the various strategies for designing ligand-induced aptamers and their applications, including monitoring different ligands, regulating gene expression and expanding microarray analyses.

Keywords Aptamer, microarray, molecular beacon, RNA, signalling

INTRODUCTION

Aptamers are rare functional nucleic acid motifs derived from libraries of nucleic acids by iterative rounds of selection and amplification, in a process called "SELEX", systematic evolution of ligands and exponential enrichment. As the name implies, the aptamers are evolved for specific binding and high affinity from combinatorial libraries of nucleic acid motifs. In the aptamer selection process, the oligonucleotide library is incubated with the target of interest and the buffer of choice at a given temperature. The bound oligonucleotides are then separated from the unbound oligonucleotides, either by filtration on nitrocellulose filters or by an affinity process. By exploiting the SELEX method, several aptamers have been selected for a wide variety of targets, including simple ions, small molecules, peptides, proteins, organelles, viruses and even entire cells (1–3). Interestingly, the isolated aptamers displayed very high specificity (molecular discrimination as much as 10,000 fold) and affinity to targets (K_ds at sub-nanomolar levels), which are comparable to the affinities achieved by antibodies for antigens. Several applications of aptamers have been proposed, and they are presently in different stages of development.

Since the inception of aptamer technology more than decade ago, several aptamers are now poised for various applications in medical diagnostics and therapeutics. On the therapeutic front, aptamers have been successfully used to sequester viral or defective proteins in cells as decoys. Interestingly, among the various nucleic acid-based strategies for inhibiting gene functions, aptamers are the most promising. The ambassador of this kind of molecule is "Macugen" (Pegaptanib), developed by EyTech Pharmaceuticals, USA, for the treatment of age-related macular degeneration. As of last year, there were about 11 aptamers undergoing clinical trials (4). On the diagnostic front, aptamers are now widely accepted as substitutes for antibodies in immunodiagnostics. The affinities displayed by the aptamers (nucleic acids) are comparable to the affinities of antibodies for antigens; therefore, their utility as a fundamental molecular recognition element in biosensors has been realized. In contrast to antibodies, however, aptamers are smaller and less complex and consequently are easier to manufacture and modify.

Several applications of aptamers in medical diagnosis and immunodiagnostics have also been reviewed (5–7), suggesting that aptamer ligands could be valuable in diagnostic imaging. While *in vitro* diagnostic applications use aptamers for quickly detecting various analytes, aptamer applications are simultaneously evolving for *in vivo* imaging and diagnosis. Charlton et al. (8) reported an aptamer that binds specifically to the human as well as the rat neutrophil elastase, which is released near the sites of infection and inflammation. When injected intravenously, the labeled aptamer became localized near the sites of inflammation, and thus it identified these sites within the entire body when an imaging analysis was performed. The aptamer yielded a higher peak-to-background ratio in a shorter time, as compared to the clinically used immunoglobulin G (IgG) molecule.

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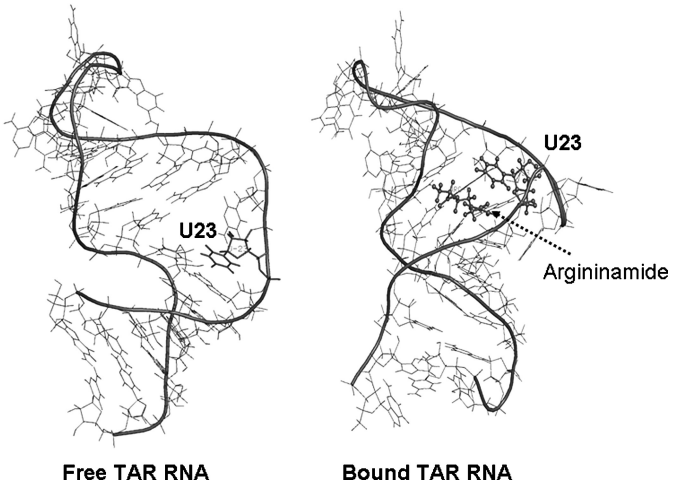


FIG. 1. Structures of free TAR RNA and TAR RNA bound with argininamide.

To date, diverse analytical formats have been reported for detecting various analytes by aptamers, including flow cytometry, affinity probe capillary electrophoresis, and enzyme-linked immunosorbent assay (ELISA)-like assays. An enzyme-linked oligonucleotide assay (ELONA) was developed to detect human vascular endothelial growth factor (hVEGF) levels in sera. The ELONA yielded results equivalent to those from an ELISA with similar accuracy, specificity and interference. Therefore, it appears that the *in vitro* evolved aptamers could potentially substitute for antibody use in clinical research and diagnostics. Several previous reviews have described the wide range of applications of aptamers in therapy and diagnosis. Although the aforementioned studies have been encouraging and promising, the application of these aptamers will require modifications of their cognate ligands for their analysis.

As an alternative to these aptamers, we and others previously showed that ligand-induced aptamers are advantageous for monitoring the choice of ligands in a real-time fashion, without significantly affecting the affinity and specificity to their ligands. In this communication, we review the various strategies used to design ligand-induced aptamers for their remarkable ability to detect and analyze diverse ligands *in vitro,* and their future applications in high-throughput analyses for drug candidate screening, gene regulation and *in vivo* applications.

APTAMER-LIGAND INTERACTIONS

The aptamer and its cognate ligand undergo conformational changes, which are generally referred to as "induced fit" as also observed in many RNA-protein interactions. For example, the trans-activation responsive region (TAR) and rev-responsive element (RRE) RNAs of human immunodeficiency virus undergo significant conformational changes when interacting with their cognate proteins mimics, Tat and Rev, respectively (Figs. 1 and 2). Similarly, based on structural studies of aptamer-ligand complexes, it is evident that upon ligand binding, either the aptamer alone or together with the ligand undergoes a conformational change to recognize the target specifically, as in Tat-aptamer-bound to the argininamide complex (9) (Fig. 3). By this induced fit process, the aptamers not only are able to discriminate cognate versus non-cognate ligands but also to dissociate slowly, as compared to antibodies (Gopinath et al. unpublished data). Aptamers discriminate closely related ligands from their cognate one in various ways, including stacking, shape complementarity, electrostatic interactions and hydrogen bonding. Since many of the known aptamers undergo conformational changes, it is very useful to exploit these phenomena to monitor the binding events as they occur and to explore their use in diagnostics. In this respect, we and others engineered selected aptamers to observe these conformational changes within the aptamer upon ligand

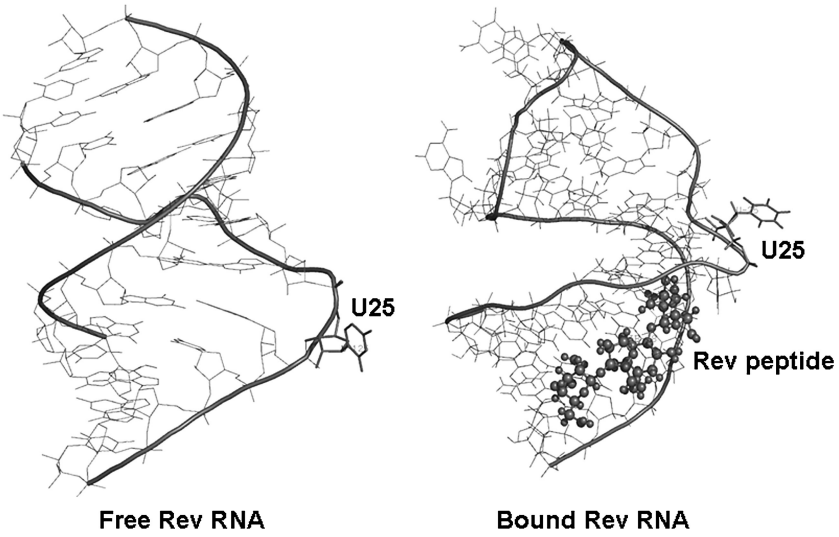


FIG. 2. Structures of free Rev RNA and Rev RNA bound with argininamide.

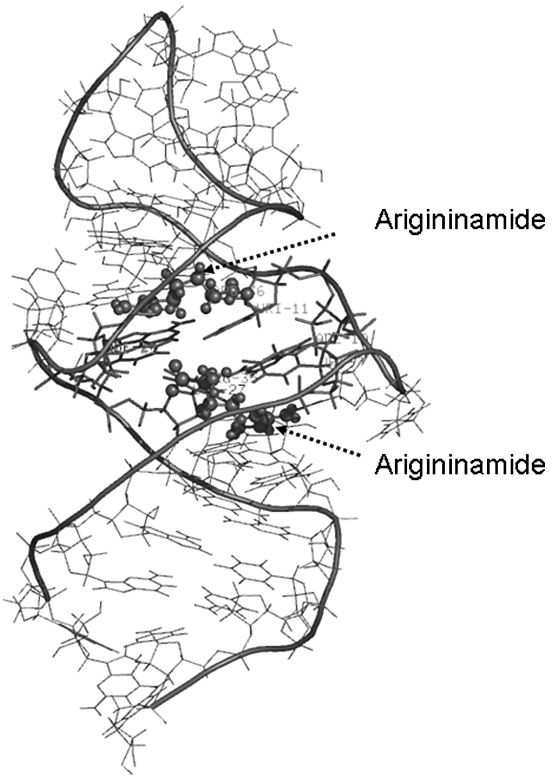


FIG. 3. A high affinity RNA aptamer-argininamide complex structure.

binding. Our engineered aptamers (in this review we refer to these as ligand-induced aptamers), as opposed to unmodified aptamers, yield an output signal (fluorescence or other signals) that can be measured quantitatively. Such ligand-induced aptamers are certainly more versatile than conventional aptamers. In view of these advantages, a number of ligand-induced aptamers have been reported against different ligands in recent years. We have classified these strategies broadly based on whether the ligand-induced aptamers were designed either by post selection or during the aptamer selection process.

POST-SELECTION MODIFICATION AND DESIGN OF LIGAND-INDUCED FLUORESCENT APTAMERS

Aptamer-Derived Splits

A novel RNA-binding motif (aptamer RNA^{Tat}) that binds to the Tat protein of HIV with two orders of magnitude greater affinity relative to the authentic trans-activation response region has been isolated (10, 11). Various biochemical analyses of the Tat-aptamer interactions suggested that the aptamer terminal loop sequences are not essential for binding to the Tat protein of HIV. Based on these results, we hypothesized that the deletion of the terminal loop sequences would convert the aptamer into two oligomers that could reassemble to form the ligand-binding pocket. For this, we designed two oligomers derived from the Tat-aptamer and found that these oligomers indeed bind to the Tat of HIV-1 with a similar affinity as that of the aptamer containing terminal loops. Next, a series of RNA oligomers (splits) with different lengths, derived from the aptamer sequences, were prepared and analyzed for the Tat–dependent reconstitution of the ligand-binding pocket (Fig. 4) (12, 13). Among the various RNA oligos tested that efficiently reconstituted the binding pocket only in the presence of Tat, the DA-5/DA-6 oligos were found to be the best in forming the ternary complex. One of the oligomers, DA-5, was then converted into a hairpin-shaped beacon molecule (after the addition of a few nucleotides to the two ends of the RNA to form the hairpin structure, and the attachment of a fluorophore at one end of the RNA and a quencher at the other end) (Fig. 5) (11). In the absence of Tat, the hairpin beacon molecule and the complementary oligo derived from the aptamer exist independently; the beacon half of the aptamer adopts the hairpin structure, emitting a low level of fluorescence even in the presence of the complementary oligo. When Tat is introduced, under similar conditions, the beacon changes its structure in order to engage the other half of the aptamer for Tat binding. The disruption of the hairpin structure causes the physical separation of the fluorophore from the quencher, which enhances the fluorescence (11). These studies represent the first report on the development of ligand-induced fluorescent aptamers (Fig. 5). Nevertheless, we could design other signaling bipartite aptamers that self-assemble in the presence of their ligands, because the selected RNA aptamer terminal loop regions are not involved in the Tat recognition.

Similar to the above strategy, DNA aptamers that bind to cocaine and adenosine ribonucleotide triphosphate (rATP) were engineered by Stojanovic et al. (14, 15) (Fig. 6). One of the terminal loop regions that connects the domains in these aptamers can be deleted without affecting the affinity to the cognate targets. To monitor the equilibrium shift that favors the assembly of two oligos in the presence of the cognate ligand (cocaine), a fluorescein fluorophore and a universal quencher (Dabcyl, a non-fluorescent dye) were attached at the 5′ and 3′ ends, respectively, to two oligos derived from a single chain aptamer. The two chains behave as self-assembling fluorescent sensors, as the fluorescein emission at 518 nm was quenched by about 65% in the presence of cocaine. The reliabile concentration range of this kind of sensor was between 10 and 1250 μM. With a similar strategy, a previously reported rATP bipartite aptamer was explored to detect rATP levels. In this study, one of the oligos was labeled with rhodamine (instead of fluorescein, as in the above studies) and the other oligo was labeled with Dabcyl. In the presence of rATP, the designed oligos specifically quenched the fluorescence emission at 608 nm, to about 40% of the initial value. Moreover, in the same studies, these two ligand-induced aptamers were combined to detect two targets simultaneously (as multiplex reporting capabilities) to report ligand binding by measuring the absorbances at 518 and 608 nm for cocaine and rATP, respectively. These studies delineated the suitability of the strategy to detect small ligands as well.

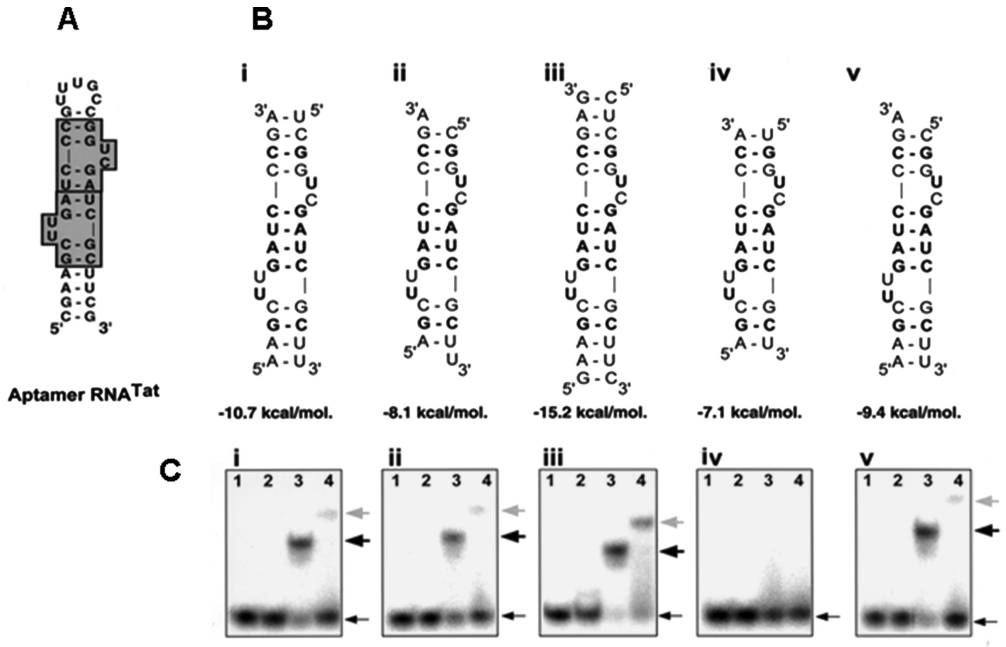


FIG. 4. Secondary structures of aptamer RNA^{Tat}-derived oligo RNAs and gel-shift assays for these modulating oligos. (a) Secondary structure of the aptamer RNA^{Tat}; shadowed and outlined letters indicate core elements that are required for Tat binding. (b) Modulating aptamer RNAs (i, DA-1/DA-2; ii, DA-3/DA-4; iii, DA-5/DA-6; iv, DA-7/DA-8; v, DA-1/DA-4). (c) Representative autoradiograms from gel-shift assays for various modulating aptamers: lane 1, radiolabeled 5′-oligo (10 nM); lane 2, radiolabeled 5′-oligo (10 nM) and unlabeled 3′-oligo (200 nM); lane 3, raidolabeled 5′-oligo (10 nM) and unlabeled 3′-oligo (200 nM) in the presence of CQ (20 nM); lane 4, radiolabeled 5′-oligo (10 nM) and unlabeled 3′-oligo (200 nM) in the presence of 200 nM Tat-1. Shadowed and bold arrows indicate the positions of the ternary complexes. The free 5′-oligonucleotide is indicated by a thin arrow.

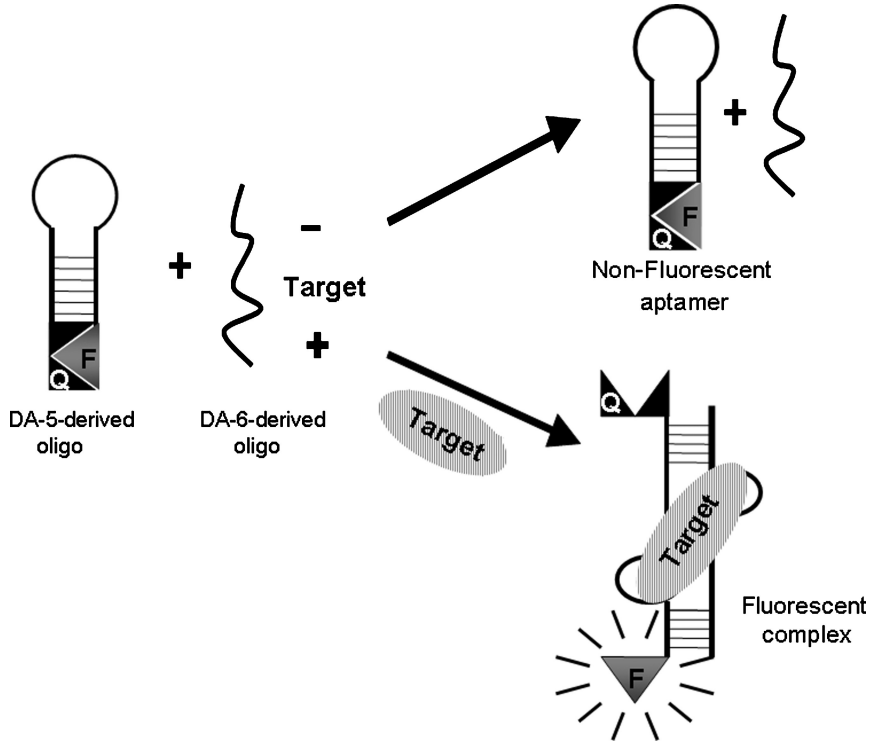


FIG. 5. A schematic representation of the ligand-induced fluorescent aptamer strategy.

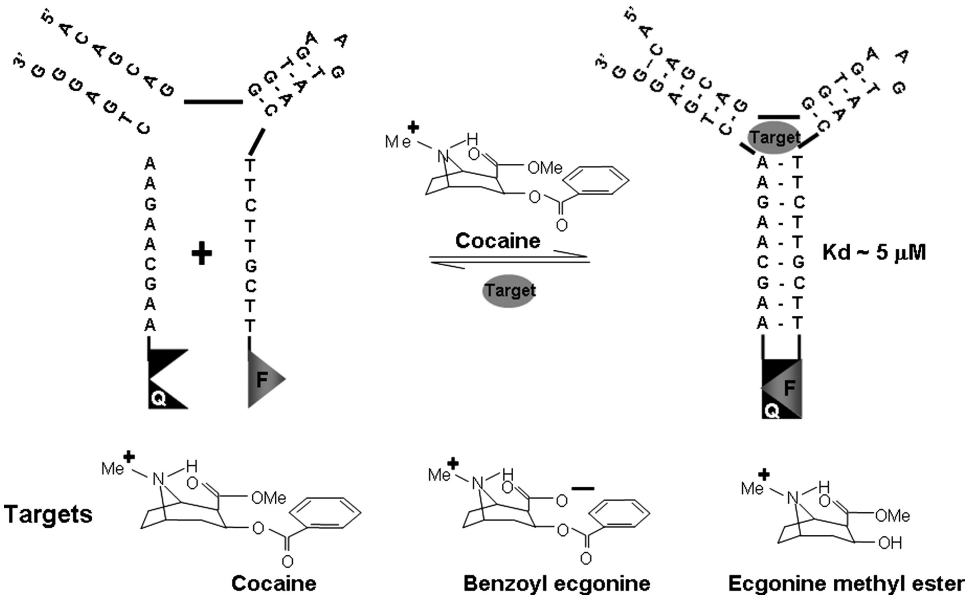


FIG. 6. A scheme showing the ligand induced-fluorescent quenching aptamer strategy.

Incorporation of Fluorescent Dyes Near the Ligand Binding Site

Another kind of ligand-induced fluorescent aptamer was reported for DNA and RNA aptamers. In this study, a single fluorescent reporter, incorporated near the ligand binding site, signals the presence of the cognate ligand. Jhaveri et al. (16) described aptamers labeled with a single fluorophore using an aptamer against ATP with a known tertiary structure (Fig. 7). They succeeded with two aptamers among the series of aptamers tested, which included both DNA and RNA aptamers. The fluorescent dye was placed adjacent to the functional moiety, and the signaling of the prepared chimeras was evaluated in the presence of the cognate ligand, ATP. In this modulated aptamer, since an acridine moiety was present at Residue 7 in the DNA aptamer, they placed the fluorophores at Residue 7 and between Residues 7 and 8. Upon the addition of ATP, a 25–45% increase in fluorescence was observed. Neither the presence of GTP, CTP and UTP nor mutations at important residues caused this ligand-dependent fluorescence (Fig. 7).

Similar to the above studies, Yamana et al. (17) also reported a method using a bis-pyrene fluorophore as a fluorescent non-nucleoside linker in internal or terminal positions. The bis-pyrene fluorophore is well known for its sensitivity to local structural changes caused by base-pairing and/or nucleotide sequence variations near the bis-pyrene site. The bis-pyrene fluorophore was incorporated into different nucleoside positions of an anti-ATP aptamer, and the ATP binding was analyzed. It was previously reported that the ATP aptamer binds to ATP molecules in a non-canonical manner (18). The ratio of excimer fluorescence intensity at 480 nm versus monomer intensity at

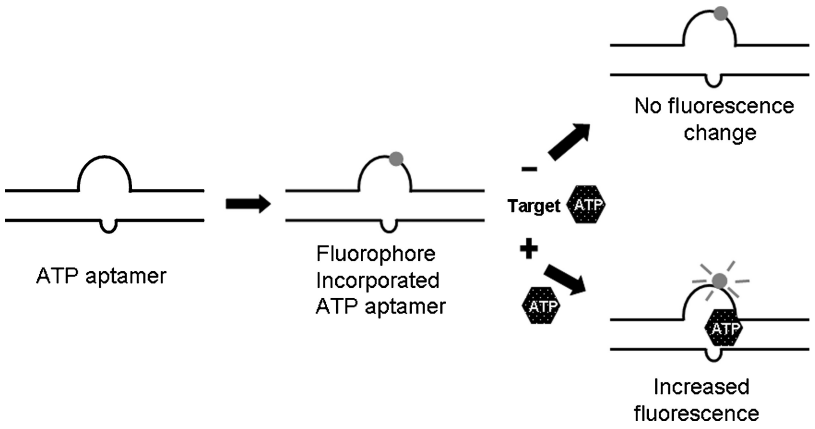


FIG. 7. A scheme that shows the incorporation of a single fluorescent molecule near the ligand-binding site, which reports the changes in fluorescence.

380 nm was used to evaluate the aptamer with the excimer label as a sensor for detecting the ATP ligand. Specifically, in the presence of 3 mM of ATP, the aptamer containing the bis-pyrene fluorophore near the ATP binding site showed a 3.3-fold higher fluorescent signal. In addition, these analyses allowed the detection of the successive binding of two ATP molecules by monitoring the responses of the pyrene excimer and monomer fluorescences. Incorporating these excimers near the binding site did not alter either the specificity or affinity of the aptamer for ATP.

Altering the Structure of the Full-Length Aptamer

Nutiu and Li (19) reported a general approach for solution-based signaling aptamers, which function by a coupled structure-switching/fluorescence dequenching mechanism. This strategy is based on a DNA aptamer that can adopt two distinct structures, a duplex DNA with a complementary DNA sequence, and a tertiary complex with a cognate target. This modulation operates by target-induced switching between a DNA/DNA duplex and a DNA/target complex (Fig. 8). They used an ATP-binding aptamer as a model to demonstrate the strategy, and designed three oligonucleotides, based on the sequence of the aptamer. One of the oligos was labeled with a fluorophore at the 5′-end (fluorescent oligo, FDNA) and another oligo was labeled with a quencher at the 3′-end (quencher oligo, QDNA). The QDNA was complementary to the aptamer and bound near the FDNA oligo. Upon QDNA binding to the aptamer the fluorophore and the quencher were brought into close proximity, resulting in highly efficient quenching in the absence of the target. In the presence of the target, the aptamer tended to favor complex formation rather than aptamer-QDNA duplex formation, inducing the release of the QDNA from the fluorophore-labeled aptamer. In the presence of the target (ATP), they observed about 90% fluorescence, whereas no fluorescence was observed when CTP, UTP and GTP were tested. This strategy can be generalized for many aptamers without prior knowledge of secondary or tertiary structure, and is suited for the development of aptamer-based reporters for real-time sensing applications (Fig. 8).

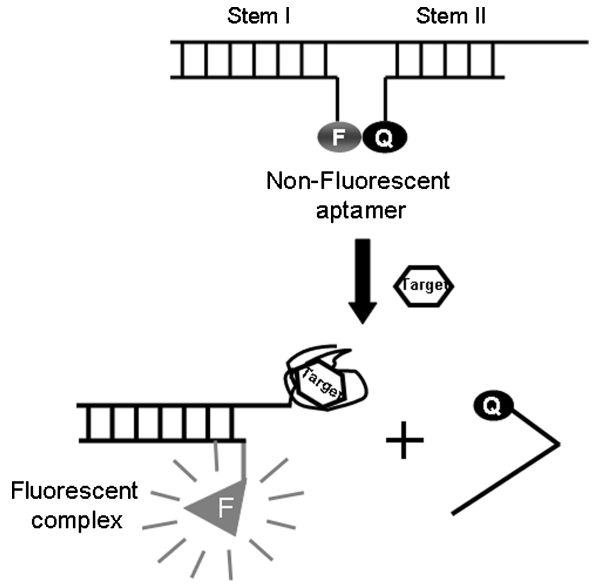


FIG. 8. A scheme showing the design of structure switching ligand-induced aptamers.

A modular aptameric sensor that transduces recognition events into fluorescence changes through conformational changes, mediated by non-covalent interactions with a fluorophore, was reported by Stojanovic and Kolpashchikov (20). This modulated aptamer consists of three domains: a signaling domain (malachite green aptamer), a recognition domain (analyte specific aptamer) and a connecting stem (communication module) (Fig. 9). In this system, ligand binding to the detector module stabilizes the communication module. This allows the reporter module to create a binding site for malachite green within the reporter module. For example, when an ATP aptamer was used as a detector module with two other modules, specifically in the presence of ATP, the aptamer was able to bind malachite green. Therefore, the authors were able to correlate the ATP binding with malachite binding, but not the other way around. Using a similar strategy, but with substitutions of only the detector module with other aptamers, such as flavine mononucleotide (FMN) and theophylline, allowed the detection of these ligands (Fig. 9). This kind of ligand-induced aptamer is not only suitable for *in vitro* diagnostics but also useful for detecting ligands *in vivo*.

Another strategy involving an aptamer with a conformational transition from an unfolded to a folded structure was proposed by Ho and Leclerc (21). In this strategy, a conformational change was used for detection, with the help of a water-soluble, cationic polythiophene derivative, which produced a new complex (Fig. 10). The optical signal thus obtained can be detected by colorimetric or fluorometric means without any requirement for target labeling, at a femtomole level. This polymeric stain specifically yields a clear optical signal and does not require any chemical modifications of either the aptamer or analyte. The strategy is based on the conformational changes in the backbone of the cationic polythiophene derivative when complexed with anionic single-stranded DNA. The cationic polymer yielded different optical changes when complexed with single stranded deoxyribonucleic acid (ssDNA) and double stranded deoxyribonucleic acid (dsDNA). Using this approach, the conformational changes in an α-thrombin-binding ssDNA aptamer were evaluated using two ligands, K^+ ions and human α-thrombin. It is interesting that these two ligands yielded similar UV-visible absorption spectra, suggesting that both ligands generated a specific conformation within the ssDNA aptamer (a G-quartet). Although G-quartet conformational change in ssDNA were detectable using this cationic stain, its suitability for probing a subtle conformational change occurring locally in some aptamers upon ligand binding has not yet been demonstrated.

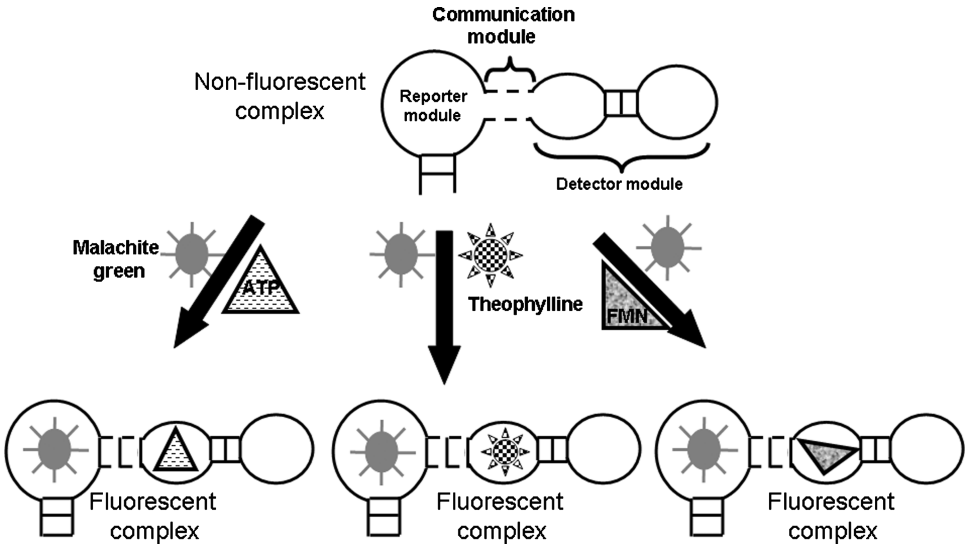


FIG. 9. A modular aptameric sensor.

A molecular light switching complex, $[Ru(phen)_2\text{-}(dppz)]^{2+}$, has been developed by taking advantage of the sensitive luminescence signal (22) (Fig. 11). This newly developed rudium complex has no luminescence in solution, and its excited state is effectively quenched by hydrogen bonding to water and the phenazine nitrogen of the ligand. However, when it intercalates in dsDNA, the phenazine nitrogen is protected from water, resulting in an immense increase in luminescence. Using this principle, a DNA aptamer against immunoglobulin E (IgE) was tested as a model system, in which the luminescence of the $[Ru(phen)_2\text{-}(dppz)]^{2+}$/IgE aptamer decreased with the increased binding of IgE. By monitoring the luminescence change, the binding events between the aptamer and target were detected. The strategy has also been tested with onco-proteins.

SELECTION OF LIGAND-INDUCED FLUORESCENT APTAMERS

As an alternative to the post-selection modifications of ligand-induced fluorescent aptamers described above, a selection strategy has been developed to select ligand-induced fluorescent aptamers (16, 23, 24). Jhaveri et al. (16) reported the direct selection of a ligand-induced fluorescent aptamer from a random RNA pool. In this study, initially, the DNA pool was skewed during chemical synthesis (A, C, G, T in a molar ratio

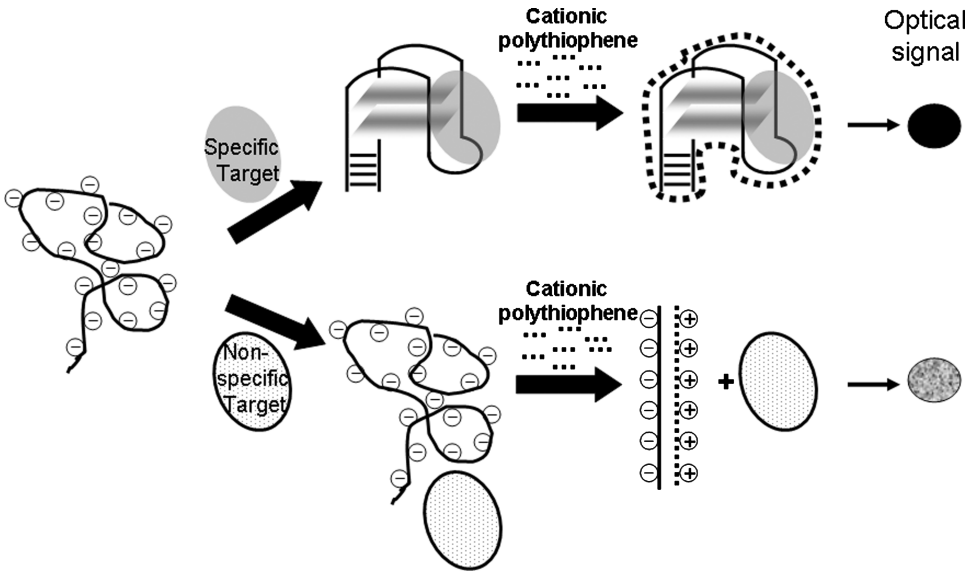


FIG. 10. An optical sensor based on a hybrid aptamer-conjugated polymer complex.

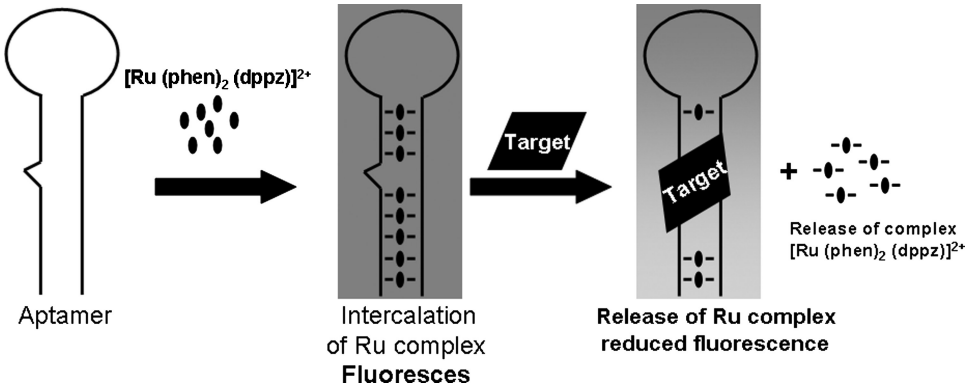


FIG. 11. A scheme showing ligand-induced aptamers intercalating with the Ru complex.

of 3:3:2:0.38), in order to represent only three to four uridines per molecule upon transcription. The fluorescent RNA pool was transcribed in the presence of F-12-UTP, and was used for selections to find ATP-binding aptamers. After 11 rounds of selection and amplification, the pool showed a slight increase of fluorescence in the presence of ATP. The sequences from Cycle 11 were classified and found to have an average of 2.25 uridines per molecule. Among the six classified families, Family I showed the best ligand-induced fluorescence, and the best aptamer in this family was the clone raf17. This aptamer has two uridine residues at positions 52 (U52) and 61 (U61). As compared to U52, the U61 residue clearly shows ATP-dependent changes (about 56%) in fluorescence. Other adenosine derivatives also generated these changes, other nucleotides (CTP, GTP and UTP) did not. These studies show that ligand-induced aptamers can be developed during the selection process.

An alternative to the above procedure has been developed (24). Initially, a DNA pool containing 20 randomized positions was synthesized and used in the selection (Fig. 12). A fluorescent reporter was introduced into the pool, using a 5′-primer with a fluorescent probe conjugated to Position 5 of the thymidine nucleobase at Position 11. In each selection round, the fluorescent

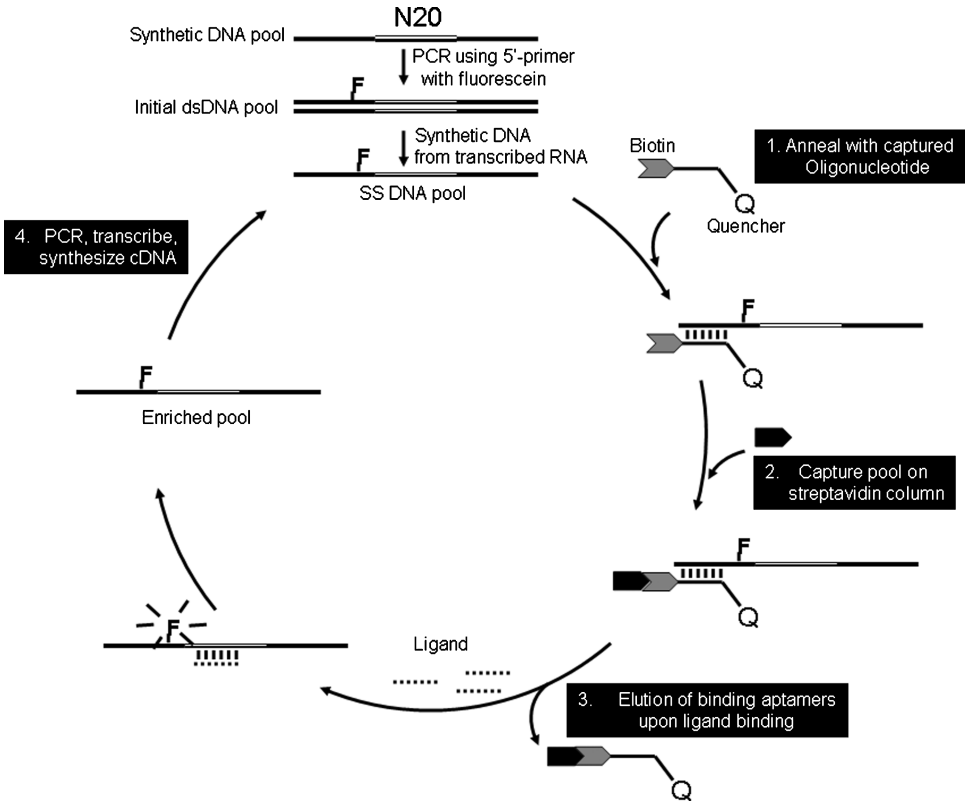


FIG. 12. A selection scheme for a ligand-induced aptamer.

ssDNA pool was annealed to the 12-mer captured oligonucleotide. The resulting duplex was trapped on a streptavidine-agarose column. The oligonucelotide thus obtained also had a fluorescent quencher (Dabcyl) at its 5-foot end, and thus the Dabcyl was in the proximity of the fluorescein. This duplex was washed several times with buffer to remove the unbound and poorly bound species. The pool was then eluted with two different sets of RNA ligands (OT1 and OT2), which had no similarity with the 5′ and 3′ constant regions of the pool. The eluted products presumably have to undergo a conformational change, because it leads to their release from the captured oligonucleotides. The eluted products were amplified and subjected to the next round of selection and amplification. A total of 9 selection cycles were carried out with small modifications, including the use of different kinds of ligands (14a and 16c). An analysis of the sequences of the selected aptamers, by the above procedure, revealed that the fluorescent reporter was located near the quencher. When the cognate target ligand was added to these aptamers, the aptamers efficiently released the captured oligo, resulting in a 17-fold increase in the fluorescence of the oligo. The ligand-dependent fluorescence was specific for the cognate target, and the mechanism was similar to that reported by Nutiu and Li (19).

FLUORESCENCE POLARIZATION ANISOTROPY

Aptamer-based biosensor arrays for the detection and quantification of biological macromolecules were proposed by McCauley et al. (25), who used an aptamer-based biosensor to detect a cancer-associated target by fluorescence polarization data acquisition and analysis. This kind of biosensor can simultaneously detect and quantify the levels of individual proteins in a complex biological mixture by utilizing immobilized DNA and RNA aptamers selected against different targets. Each fluorescently labeled aptamer was immobilized on a glass plate and fluorescence polarization anisotropy was used to recapitulate the solid and solution phase measurements of target protein binding. This imaging system can function in both the epi and total internal reflection (TIR) illumination modes. It utilizes electromagnetic energy that propagates into the lower index of the reflection medium, when an electromagnetic wave is completely reflected internally at the interface between materials with differing refractive indices. When the angle of incidence exceeds the critical angle of TIR for the given materials and the wavelength of light used, this effect occurs and is detected by directing light into a suitable prism. The glass biosensor substrate is optically coupled via an index-matching fluid to the upper surface of the prism, and TIR occurs at the substrate/solution interface on which the biosensor is immobilized. The emitted fluorescence from the bound sensor target complexes can detect the optical signal. The detected signal can be spatially encoded through the use of a pixilated detector, and the images can be acquired and stored by custom software.

APPLICATIONS OF LIGAND-INDUCED APTAMERS

In addition to their applications in real-time monitoring of the choice of a ligand, as mentioned above, these ligand-induced aptamers are now poised for other important applications, as described below.

Cis-Acting Aptamers to Control Gene Expression

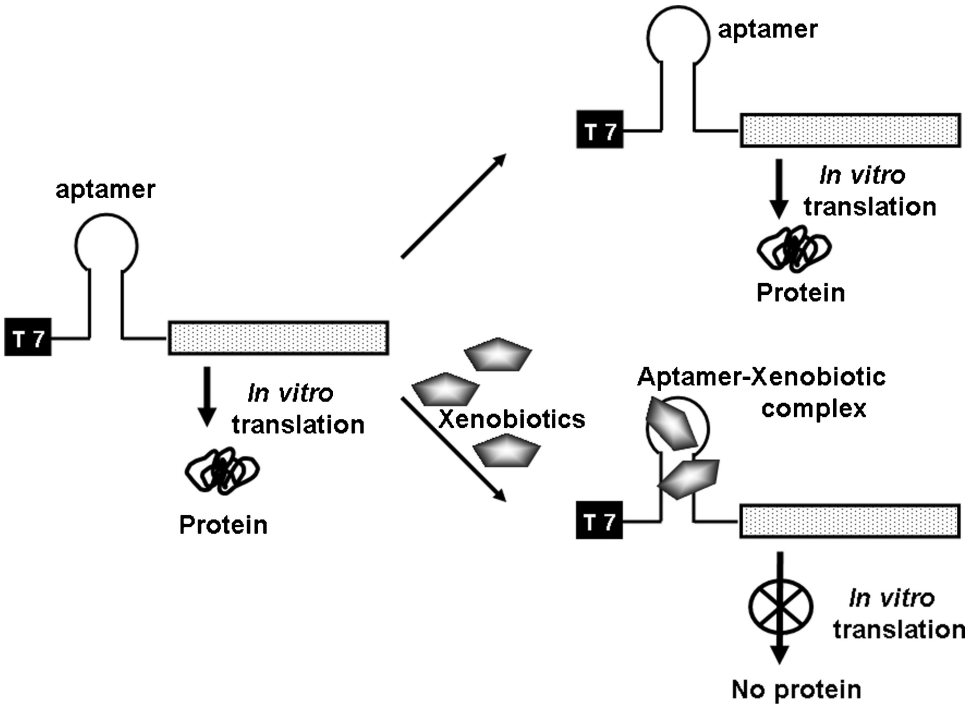
The ability of RNA to interact with small molecules, such as antibiotics and metabolites, has been well documented in the literature (26). Furthermore, high affinity RNA aptamers that bind to antibiotics and small molecules have been reported. To exploit these high affinity aptamers in gene regulation, a novel approach has been reported in which for the first time, *cis*-acting aptamers that control gene expression have been designed (27). Upon binding to the ligand, the aptamer undergoes a conformational change, and the ligand becomes an integral part of the aptamer structure. If an aptamer that recognizes a small molecule is appended into the 5′ untranslated region of a messenger RNA, then its translation can be repressed by ligand addition (Fig. 13a). Reports of aptamer-based translational control *in vitro* and *in vivo* (27) have shown that this type of control can be imposed on translation. The inhibition of the translation product by these small molecules in this assay was attributed to their ability to interfere with ribosome binding. Using this assay, several small ligands that bind to RNAs have been evaluated and explored for controlling gene expression in living cells (28–32).

Trans-Acting Aptamers to Control Gene Expression

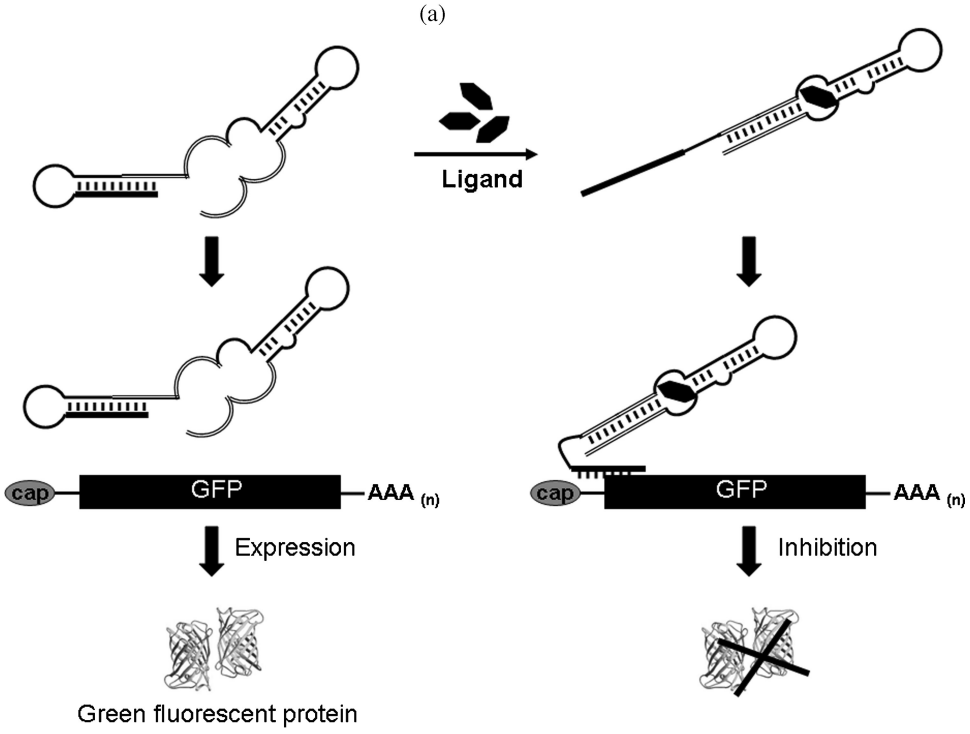
Recently, a *trans*-acting aptamer that directly regulates gene expression in a ligand-dependent manner was reported (33). In these studies, two independent RNA motifs were linked into one RNA motif: a theophylline aptamer and a complementary RNA domain. The latter RNA motif (antisense portion) was designed to base-pair with a 15-nucleotide region around the start codon of a target mRNA encoding green fluorescent protein (GFP). The stem of the theophylline was redesigned so that the complementary RNA domain pairing switches selectively between the theophylline aptamer and the target GFP messenger ribonucleic acid (mRNA), in the absence and presence of ligand (theophylline), respectively (Fig. 13b). Also, when considering the stability of these paring stems, the stability of the antisense stem was designed to be slightly higher than that of aptamer stem. This kind of ligand-induced *trans*-acting aptamer can be tailor-made to regulate the expression of target transcripts in response to different cellular effectors.

High-Throughput Screening

Most inhibitors or modulators of biological or biochemical functions are small organic molecules (34). Screening compound libraries for drugs is a long and tedious process, which is hampered by the lack of functional assays for most targets (35, 36). Many proteins exert their biological roles as components of complexes, and their functions rely entirely on specific



(a)



(b)

FIG. 13. (a) A scheme showing *cis*-acting aptamers to control gene expression. (b) A scheme showing *trans*-acting aptamers to control gene expression.

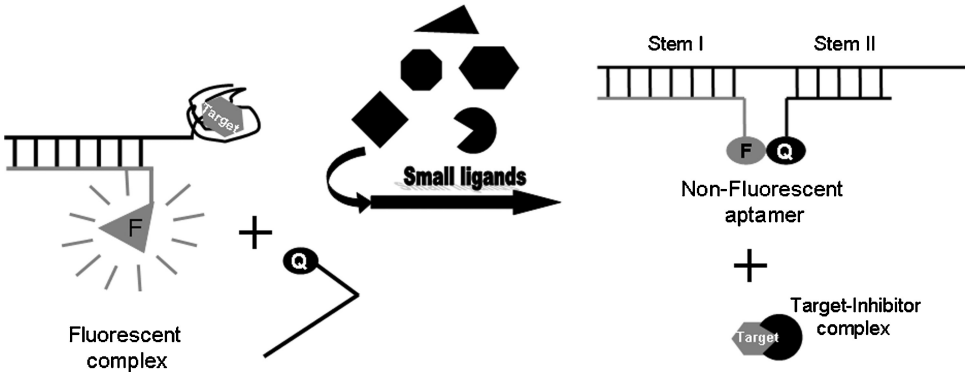


FIG. 14. A strategy for the identification of high affinity small ligand binders against commercially important targets, using ligand-induced aptamers.

interactions with other macromolecules (37). Compounds that modulate the complexes to dissociate in a competitive manner may have immense potential to become potential drugs to block the required interactions of that target molecule. For this purpose, native complexes were used in high-throughput assays to find suitable inhibitory molecules. Since the native complexes are not optimized for high affinity alone, the inhibitors competing for binding may not be able to yield high affinity ligands (or decoys). Hence, it is important to screen for small ligands against high affinity complexes to yield affinity decoys in a competitive assay. From this aspect, information stored in the aptamer can be transferred to a small molecule, in a competitive assay, to find efficient inhibitors. In order to screen the library in a high-throughput manner, it can be adapted from ligand-induced aptamers, as they offer the best screening ability. This would allow us to monitor the interactions in a real-time fashion and to analyze the interactions in a quantitative manner. In addition, these studies can be carried out in solution. The ligand-induced aptamers have the remarkable ability to elicit responses only when they interact with the target molecule in solution.

Although the potential application of ligand-induced aptamers in identifying small ligands from a chemical library for drug-leads has not been shown yet, it would be possible to use an aptamer-target complex to identify drug-leads, as outlined in Fig. 14. A compound that interferes with the formation of a ligand-induced-target complex (that fluoresces) can be identified from a library of small ligands, by monitoring the fluorescence in the presence of various compounds. The compounds that interfere with the ligand binding, resulting in a loss of fluorescence, could be potential drug-leads. Using this strategy, it would be possible to identify small ligand drug candidates.

Microarray

In recent years, among the various multiplex and high-throughput assays, microarray technology has been growing most rapidly. These immobilized nucleic acid arrays have proven to be useful for the rapid detection of mutations and polymorphisms, as well as for discovery and expression monitoring. At present, these arrays are limited to analyzing only complementary nucleic acids and cannot be used for other analytes. To expand the applications of nucleic acid arrays for analyzing various ligands, including proteins and small ligands such as metabolites and nucleotides, we have explored the use of aptamer-derived oligos. To use the aptamers effectively in high-throughput assays in a micro-array format, and to analyze various analytes, we developed a strategy in which the aptamer was split into two non-functional units and reassembled into the functional aptamer by interacting with the cognate ligand (Fig. 15a). We have named this method "Analyte Dependent OligoNucleotide Modulation Assay" (ADONMA) (Figs. 15a and b). As proof-of-principle, we used oligonucleotides derived from the aptamer RNA against HIV-1 Tat and demonstrated, with both titer plates and plastic slide chips, that specifically in the presence of Tat or its peptides, the two oligos reconstituted the core binding regions of Tat. The use of aptamer-derived oligos to detect specific analytes, as described here, has many advantages over methods that use the full-length aptamer sequence, including the following: 1) shorter RNA oligonucleotides can be synthesized with higher efficiency than longer molecules; 2) no modification of the analyte is required; 3) modifications to stabilize the nucleic acid are necessary for only a portion of the aptamer; 4) proper folding of the modulating aptamer is facilitated by the analyte; 5) lower cost. The results presented above suggest that the modulating aptamer method has the potential to detect analytes of interest. Moreover, further improvements will be achieved when the RNA aptamers can be fully protected from ribonucleases. Even though the method described here is for a protein that recognizes the bulged regions between the two stems, it can be expanded to proteins or small molecules that recognize stem-loops or other alternative RNA structures. This approach can be generalized for the detection of proteins other than HIV Tat by selecting appropriate non-modulating and/or modulating species from combinatorial libraries. This approach is currently underway in our lab for several viral proteins as well as for small molecules. Thus, these results suggest that ADONMA can potentially be used in nucleic acid micro-arrays for detecting various ligands.

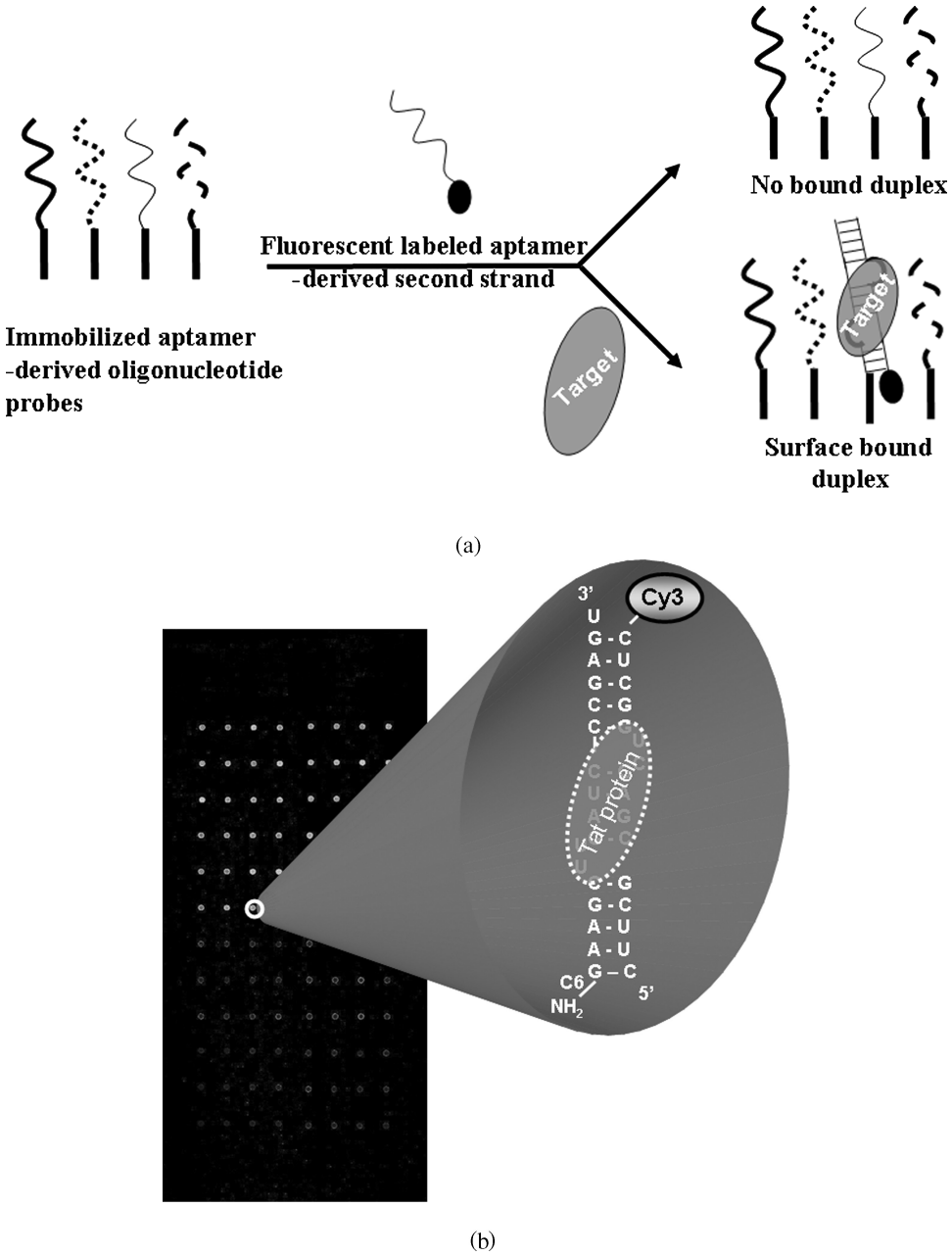


FIG. 15. Ligand-dependent oligonucleotide modulation of aptamer structure. (a) A schematic representation of analyte-dependent duplex formation by oligo-derived from the aptamer. (b) Close-up view of a microarray slide on which a duplex forms in the presence of the cognate liagnd.

EPILOGUE

The ligand-induced aptamers described above can be modified for various applications other than those reported previously, including their use in detecting disease marker proteins *in vivo*. The potential *in vivo* applications of one such aptamer have been reported (20), in which a sensing domain composed of two aptamers (reporter and detector aptamers) is linked through a communication module (Fig. 9). The ligand-induced aptamers have great potential and elicit better signal-to-noise ratios as compared to conventional aptamers, and their applications will certainly increase in the near future.

ABBREVIATIONS

A	Adenosine
ADONMA	Analyte dependent oligonucleotide modulation assay
C	Cytidine
Dabcyl	4-(dimethylaminoazo)benzene-4-carboxylic acid

dsDNA	double stranded Deoxyribonucleic acid
ELISA	Enzyme-Linked immunosorbent assay
ELONA	Enzyme-Linked oligonucleotide assay
Fluorescein	resorcinolphthalein
G	Guanosine
GFP	Green fluorescent protein
HIV	Human immunodeficiency virus
hVEGF	human vascular endothelial growth factor
IgG	Immunoglobulin G
mRNA	Messenger Ribonucleic Acid
rATP	adenosine ribonucleotide triphosphate
Rev	Rev protein of HIV
RNA	Ribonucleic acid
RRE	Rev Responsive Element
ssDNA	single stranded Deoxyribonucleic acid
T	Thymidine
TAR	*trans*-activation responsive region
Tat	trans-activator protein of HIV

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