METHOD FOR ISOLATING CROSS-REACTIVE APTAMER AND USE THEREOF

GOVERNMENT SUPPORT

This invention was made with government support under 2016-DN-BX-0167 awarded by the National Institute of Justice. The government has certain rights in the invention.

SEQUENCE LISTING

The Sequence Listing for this application is labeled "SeqList-28Jun19-ST25.txt," which was created on Jun. 28, 2019, and is 4-5 KB. The Sequence Listing is incorporated herein by reference in its entirety.

BACKGROUND OF THE INVENTION

Small molecules are important targets with the potential of clinical or commercial applications such as medical diagnostics, environmental monitoring, and forensic science. Thus, efforts to develop methods for portable, low-cost, point-of-care and quantitative detection of a broad range of small molecules are gaining momentum.

Synthetic cathinones (also known as bath salts) are designer drugs sharing a similar core structure with amphetamines and 3, 4-methylenedioxy-methamphetamine (MDMA). They are highly addictive central nervous system stimulants, and are associated with many negative health consequences, including even death. Although these drugs have emerged only recently, abuse of bath salts has become a threat to public health and safety due to their severe toxicity, increasingly broad availability, and difficulty of regulation. More importantly, there is currently no reliable presumptive test for any synthetic cathinone. Chemical spot tests used to detect conventional drugs such as cocaine, methamphetamine, and opioids show no cross-reactivity to synthetic cathinones.

Screening for small molecules such as synthetic cathinones requires cross-reactive assays that can broadly detect small molecules based on their shared molecular framework. Such assays are more efficient and cost-effective than the tandem use of multiple highly specific assays that detect a single analyte.

Antibody-based immunoassays have dominated the field of on-site small-molecule detection, and while numerous assays have been developed for a variety of individual targets, the development of cross-reactive immunoassays has proven difficult. This is in part because the process of antibody generation, which is entirely in vivo, provides no control over the cross-reactivity of the generated antibody.

Nucleic acid-based bioaffinity elements known as aptamers hold much promise in overcoming many of the shortcomings associated with immunoassays. Aptamers are isolated through a process known as systematic evolution of ligands by exponential enrichment (SELEX) to bind targets of interest with high affinity and specificity. Aptamers can be isolated for essentially any target, including metal ions, small molecules, proteins, or whole cells.

Unlike antibodies, aptamers can be isolated relatively quickly and chemically synthesized in an inexpensive manner with no batch-to-batch variation. Aptamers are chemically stable and have shelf-lives of a few years at room temperature. Moreover, aptamers can be engineered to have tunable target-binding affinities or various functionalities. These advantages make aptamers ideal for use in biosensors.

Because SELEX is an in vitro process, it should be possible to isolate a cross-reactive aptamer through precise control of the selection strategy and conditions. Ideally, such aptamer should bind to the core structure of a given class of targets while being insensitive to peripheral substituents, thereby capable of recognizing the whole target family. However, little work has been done to demonstrate the capability of SELEX to achieve such goal.

Therefore, there is a need for developing a novel SELEX strategy to isolate cross-reactive aptamers for structurally-similar compounds. There are also needs for methods and materials for rapid, sensitive, on-site, and naked-eye detection of small molecules such as synthetic cathinones.

BRIEF SUMMARY OF THE INVENTION

The subject invention provides a novel SELEX strategy for isolating cross-reactive aptamers that recognize a core structure of a small-molecule family and bind each structurally-similar molecule in said family.

In one embodiment, the method employs a parallel-and-serial SELEX strategy, comprising at least one step of a parallel selection and at least one step of a serial selection. In another embodiment, the method for isolating an aptamer for a family of structurally-similar small molecules comprises mixing the small molecules in said family with a nucleic acid library, binding the small molecules to one or more aptamers in the nucleic acid library, separating the aptamer bound to the small molecules in said family from at least a portion of the unbound nucleic acid molecules, isolating the aptamer and optionally, amplifying the isolated aptamer.

In one embodiment, the aptamer isolated by the method according to the subject invention is a cross-reactive aptamer that recognizes and binds to the core structure of synthetic cathinones. The synthetic cathinones include, but are not limited to MDPV, ethylone, naphyrone, penthylone, methylone, buthylone, MPHP, 4-MMC, methedrone, pyrovalenrone, MDPBP, α-PVP, MEPBP, 4-FMC, and methcathinone. The aptamer does not cross-react with common cutting agents found in seized samples such as pseudoephedrine, promazine, procaine, ephedrine, acetaminophen, methamphetamine, lidocaine, amphetamine, cocaine, sucrose, and caffeine.

In one embodiment, the cross-reactive aptamer, according to the subjection, is a DNA aptamer comprising at least 46 nucleotides. The target-binding domain of the cross-reactive aptamer comprises a nucleotide sequence selected from SEQ ID Nos: 7-17 and sequences sharing at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identity with SEQ ID Nos: 7-17. In a specific embodiment, the cross-reactive aptamer comprises a nucleotide sequence of SCA2.1 (SEQ ID NO: 6). In a preferred embodiment, the cross-reactive aptamer is SCA2.1 (SEQ ID NO: 6) and sequences sharing at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identity with SCA2.1 (SEQ ID NO: 6).

The subject invention provides methods, assays, and products for rapid, naked-eye detection of small molecules in a sample, in particular, in both clinical and field settings. The subject invention is based on an aptamer sensor that reports the presence of small-molecule targets such as synthetic cathinones via a sensitive colorimetric signal for naked-eye detection. Specifically, exemplified herein is a method for detecting synthetic cathinones in bodily fluids, drinks, and/or seized substances.