Analytica Chimica Acta 1001 (2018) 143–150

Contents lists available at ScienceDirect

Analytica Chimica Acta

journal homepage: www.elsevier.com/locate/aca

Mirror-image aptamer kissing complex for arginine-vasopressin sensing

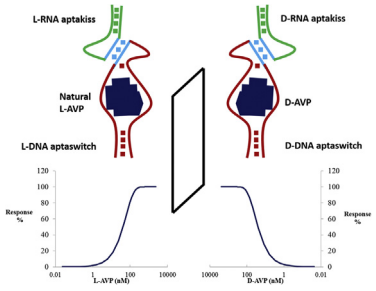
Benoit Chovelon [a, b, c], Emmanuelle Fiore [a, b, c], Patrice Faure [c, d], Eric Peyrin [a, b, *], Corinne Ravelet [a, b, **]

[a] University Grenoble Alpes, DPM UMR 5063, F-38041 Grenoble, France
[b] CNRS, DPM UMR 5063, F-38041 Grenoble, France
[c] Département de Biochimie, Toxicologie et Pharmacologie, CHU de Grenoble Site Nord - Institut de Biologie et de Pathologie, F-38041 Grenoble, France
[d] University Grenoble Alpes, Laboratory of Hypoxy Physiopathology Study Inserm U1042, 38700 La Tronche, France

HIGHLIGHTS

- The mirror-image aptamer kissing complex (AKC) approach was demonstrated.
- The scope of the AKC strategy was extended to peptide targets.
- The all-L AKC improved the fluorescence anisotropy assay robustness for complex matrix analysis.
- The use of methanol as cosolvent enhanced the assay sensitivity.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 15 July 2017
Received in revised form
3 November 2017
Accepted 13 November 2017
Available online 23 November 2017

Keywords:
Aptamer
Kissing complex
Mirror-image assay
Peptide target
Complex matrix

ABSTRACT

The recently reported aptamer kissing complex (AKC) strategy has allowed for the development of a new kind of sandwich-like sensing tools. Currently AKC assays have been only applied to low molecular weight molecules and their functionality in complex matrices remains challenging. The objective of the present study broken down into two sub-aims; exploring the propensity to broaden the scope of detectable analytes and designing a more robust system for potential applications to realistic samples. An all L-configuration aptaswitch module derived from a hairpin spiegelmer specific to a larger target, *i.e.* the arginine-vasopressin (AVP) hormone, was elaborated. The target-induced AKC formation in presence of a specific mirror-image RNA hairpin (L-aptakiss) probe were analyzed by using fluorescence anisotropy. The mirror-image kissing complex was successfully formed when the L-AVP target bound to the engineered L-aptaswitch element. It was also established that the use of methanol as cosolvent significantly improved the assay sensitivity through the stabilization of the ternary complex. Finally, the capability of the mirror-image method to operate in 10-fold diluted, untreated human serum was illustrated. The current work revealed that the AKC concept can be expanded to a wider range of targets and converted to a L-configuration sensing platform especially suitable for bioanalysis purposes.

* Corresponding author. University Grenoble Alpes, DPM UMR 5063, F-38041 Grenoble, France.
** Corresponding author. University Grenoble Alpes, DPM UMR 5063, F-38041 Grenoble, France.
E-mail addresses: eric.peyrin@univ-grenoble-alpes.fr (E. Peyrin), corinne.ravelet@univ-grenoble-alpes.fr (C. Ravelet).

https://doi.org/10.1016/j.aca.2017.11.043
0003-2670/

1. Introduction

During the last two decades, the development of aptamer-based analytical devices has become a research field in constant expansion. Numerous aptamer assays/sensors rely on strategies that commonly depend on the target-induced allosteric switch of the functional nucleic acid [1–5]. Among them, the aptamer kissing complex (AKC) concept constitutes a promising approach to detect small molecules [5]. The principle is based on the ability of an engineered aptamer (aptaswitch) to undergo a ligand-mediated transition from an unstructured to a structured hairpin. A short sequence, able to interact with the loop of a second hairpin (aptakiss), is incorporated into the aptaswitch apical loop. In absence of ligand, the central and apical domains of the switching aptamer are largely unfolded in such a way that no loop-loop (kissing) interactions can occur between the aptakiss and the aptaswitch. Target binding to the central bulge of the aptaswitch stabilizes its overall structure and shapes the stem-loop motif. The apical loop of the folded aptaswitch can then interact with the loop of the aptakiss, signaling the presence of the analyte. The AKC strategy is devoted to hairpin aptamers for which the apical loop is not involved in the target recognition [5]. For analytical applications, one particularly interesting feature of the approach is related to its ability to detect small molecules under a sandwich-like format, potentially providing signal amplification [6].

To date, several aptaswitch-aptakiss couples have been identified. Natural RNA-RNA kissing complexes have been initially mimicked by designing RNA aptaswitches that detect the GTP and theophylline targets in combination with an RNA aptakiss [5–7]. We have also engineered a chimera by inserting an RNA kissing motif into a DNA aptamer specific to adenosine [5]. More recently, an all-DNA adenosine aptaswitch able to bind to a novel RNA aptakiss has been reported [8]. These diverse hairpin couples have been successfully used in a variety of AKC assays (AKCA) based on SPR, fluorescence, fluorescence anisotropy, or colorimetry signaling techniques [5–10]. Although the analytical capabilities of the AKC approach have been established, there remains some major challenges notably in terms of analyte range and assay robustness.

The range of target-aptamer couples potentially amenable to AKCA represents a key questioning. At the present time, AKCA have been only demonstrated for aptamers specific to purine bases and derivatives [5–10]. These targets are planar aromatic compounds of low molecular weight (M_W in the 180–500 Da range) that all shape the hairpin through intercalation between purine base platforms of the internal bulge [11–13]. The spatial confinement of these small-size ligands within the closely delimited binding central core can preserve both the topology and accessibility of the apical loop-junction motif of the bound aptaswitches. These structural features enable the efficient formation of the kissing complex in presence of the aptakiss. However, the aptness of the switch-kiss system to accommodate larger targets with different structures and binding modes needs to be established.

Another issue of the AKCA is related to the use of the RNA aptakiss as signaling element (*i.e.* the probe), constituting a limitation in biological contexts due to its inherent (bio)chemical instability [14]. Furthermore, the non-specific binding of matrix proteins to nucleic acids and the off-target effects complicate its analytical applications in real conditions [15]. Moreover, laborious sample ultrafiltration pre-treatment steps (eventually associated to a very high dilution factor of matrices) are required to achieve reliable AKC-based sensing in complex media [6,8]. One elegant and powerful way to circumvent these difficulties relies on the use of mirror-image analogs (referred to as L-nucleic acids or sometimes spiegelmers) that are both highly resistant to the nuclease digestion and much less sensitive to the binding interferences due to the biopolymer enantiopecificity [15–17]. Although the mirror-image approach has been previously exploited for designing more practical L-nucleic acid-based assays/sensors [15,18–21], the demonstration of functional mirror-image kissing complex has not yet been reported.

The aim of the present study was to (i) determine if the AKC strategy can be extended to a wider range of ligand structures and (ii) elaborate a new robust sensing system by using opposite chirality concept. Our work focused on a previously isolated imperfect hairpin aptamer specific to the arginine-vasopressin (AVP) [22], a mammalian hormone that plays a crucial role in the regulation of renal water excretion. This choice was initially motivated by the fact that AVP is a cyclic ~1000 Da nonapeptide. When compared with a flat small ligand, the binding of peptides to functional nucleic acids obviously implies a larger interfacial surface and causes on a stronger structural distortion of aptamers [11]. Moreover, previous thermodynamic data have suggested that a significant adaptative transition occurs upon the AVP-aptamer complex formation [23]. Thus, this model system appeared well suited to explore the potential of the AKC scope. In addition, the AVP aptamer provided the platform to use the spiegelmer strategy [22]. By exploiting the mirror-image approach, we expected that the aptakiss in the L-series coupled to the L-aptaswitch specific to the natural hormone could provide an all-L configuration AKC assay with improved robustness (see the schematic representation of the approach in Fig. 1). Finally, AVP is a clinically relevant biomarker for diagnosis of several pathological conditions [24].

The present work was carried out by using the recently described DNA-RNA kissing sequence pair [8]. The fluorescence anisotropy technique was employed as signaling method (the fluorescently labeled aptakiss was used as probe) for allowing facile monitoring of both kissing complex formation and resistance potential toward biological interferences. The AVP aptaswitch was first engineered and the experimental binding conditions for the AKC were optimized to improve the assay response. The ability of the mirror-image AKCA to function under optimal conditions was established and its enhanced capabilities to operate in a biofluid context (10-fold diluted serum) were demonstrated.

2. Experimental section

2.1. Reagents

Both D-Arginine Vasopressin trifluoroacetate salt and L-Arginine

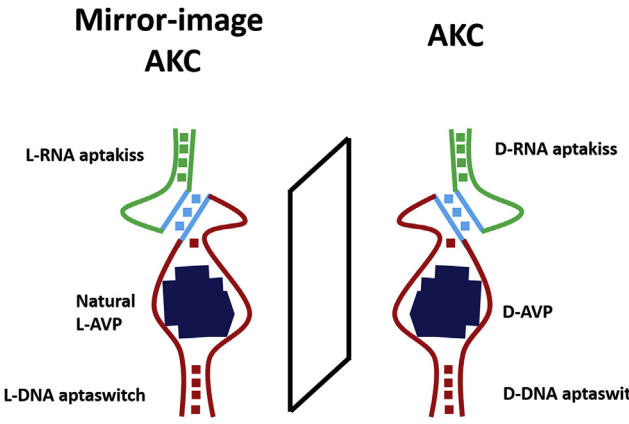


Fig. 1. Schematic illustration of the mirror-image AKC approach for creating an all-L configuration sensing system specific to the natural L-AVP target. The loop-loop interaction is depicted in light blue. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)