Biochimie 145 (2018) 137–144

Contents lists available at ScienceDirect

Biochimie

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

Research paper

Development of a thermal-stable structure-switching cocaine-binding aptamer

Aron A. Shoara, Oren Reinstein, Okty Abbasi Borhani, Taylor R. Martin, Sladjana Slavkovic, Zachary R. Churcher, Philip E. Johnson*

Department of Chemistry and Centre for Research on Biomolecular Interactions, York University, Toronto, Ontario, M3J 1P3, Canada

ARTICLE INFO

Article history:
Received 23 June 2017
Accepted 18 August 2017
Available online 21 August 2017

Keywords:
Aptamer design
DNA melts
NMR spectroscopy
Fluorescence spectroscopy
Isothermal titration calorimetry
DNA-Small molecule interactions

ABSTRACT

We have developed a new cocaine-binding aptamer variant that has a significantly higher melt temperature when bound to a ligand than the currently used sequence. Retained in this new construct is the ligand-induced structure-switching binding mechanism that is important in biosensing applications of the cocaine-binding aptamer. Isothermal titration calorimetry methods show that the binding affinity of this new sequence is slightly tighter than the existing cocaine-binding aptamer. The improved thermal performance, a T_m increase of 4 °C for the cocaine-bound aptamer and 9 °C for the quinine-bound aptamer, was achieved by optimizing the DNA sequence in stem 2 of the aptamer to have the highest stability based on the nearest neighbor thermodynamic parameters and confirmed by UV and fluorescence spectroscopy. The sequences in stem 1 and stem 3 were unchanged in order to retain the structure switching and ligand binding functions. The more favorable thermal stability characteristics of the OR3 aptamer should make it a useful construct for sensing applications employing the cocaine-binding aptamer system.

1. Introduction

The cocaine-binding aptamer has become a model system for the development of aptamer-based biosensors. The secondary structure of the aptamer is composed of three stems arranged into a three-way junction with both tandem AG base pairs and a dinucleotide bulge located at or adjacent to the three-way junction (Fig. 1) [1]. When stem 1 is six base pairs long, the aptamer folds in the free state and retains the same secondary structure when ligand-bound [1]. However, if stem 1 is shortened to be three base pairs in length, as shown in Fig. 1, the aptamer undergoes a ligand-induced structure switching binding mechanism. In the ligand-free state, the short stem 1 aptamer is loosely or poorly structured. Upon ligand binding, the aptamer folds or dynamically tightens into the secondary structure shown in Fig. 1 [1–5]. It is this structure-switching binding mechanism that has been exploited in many of the applications of the cocaine-binding aptamer in biosensor development, and consequently, the short stem 1 version of the cocaine-binding aptamer is a widely utilized version of the cocaine-binding aptamer [2,6–22].

The structure switching nature of the short stem 1 construct of the cocaine-binding aptamer must arise from the interplay between the destabilising reduction in the length of stem 1 and the stabilizing nature of ligand binding. This is demonstrated by the observation that tighter binding ligands for the cocaine-binding aptamer such as quinine [4,23,24], result in aptamer-ligand pairings with a higher melt temperature than the cocaine-bound aptamer [25]. Additionally, the steroid-binding aptamer, a three-way junction aptamer based on the cocaine-binding aptamer sequence, binds deoxycholic acid very weakly. This interaction is not tight enough to fold the version of the aptamer that contains a three-base-pair-long stem 1. Instead, deoxycholic acid binds and folds a version of this aptamer with a stem 1 four base pairs long [26].

Despite the widespread adoption of the short stem 1 version of the cocaine-binding aptamer (Fig. 1; MN19) in biotechnology, this sequence is not optimal, as it is only marginally stable at room temperature. This is demonstrated by our published temperature-dependent NMR studies [1], temperature-dependent ITC-based

* Corresponding author. Department of Chemistry, York University, 4700 Keele St., Toronto, Ontario, M3J 1P3, Canada.
E-mail address: pjohnson@yorku.ca (P.E. Johnson).

http://dx.doi.org/10.1016/j.biochi.2017.08.010
0300-9084/

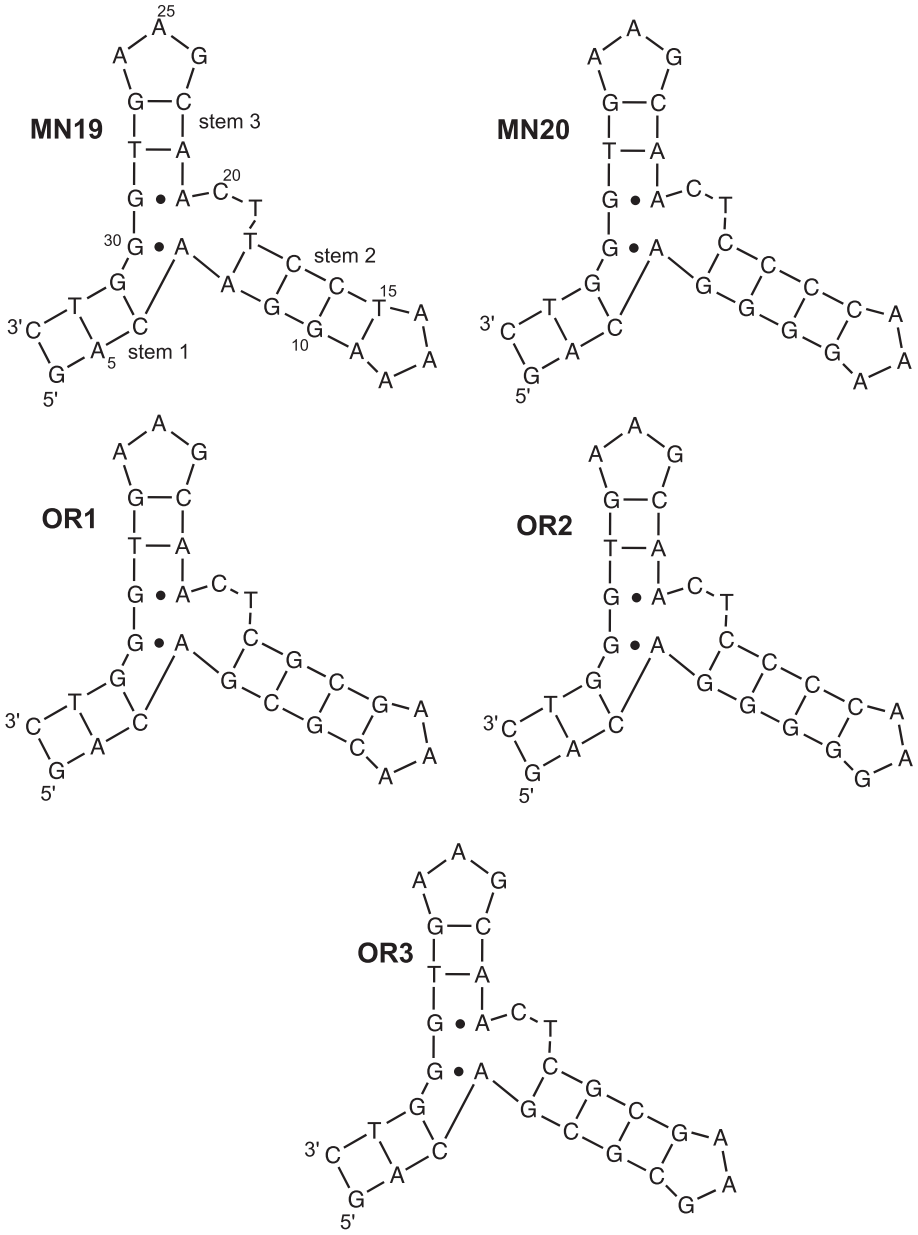

Fig. 1. Sequence and secondary structures of aptamers used in this study. For ease of comparison the numbering system is the same as used for the 36-nucleotide version of the aptamer and starts at 4 for these constructs.

binding studies [1,4], and DSC analysis [25]. The goal of this study is to develop a more thermally stable version of the cocaine-binding aptamer that preserves the functionally important ligand-induced structure-switching binding mechanism. To achieve this, we retained the three base pair-long stem 1 and the sequence of stem 3, as these stems are important for the structure-switching and ligand binding functions. We modify the sequence of stem 2 to obtain a cocaine-binding aptamer variant with as high a melt temperature as possible. We demonstrate that the resulting sequence, OR3 (Fig. 1), has these characteristics.

2. Materials and methods

2.1. Materials

DNA was obtained from Integrated DNA Technologies (IDT). DNA aptamer samples were dissolved in distilled deionized H_2O (ddH_2O) and then exchanged three times using a 3-kDa molecular weight cut-off concentrator with sterilized 1 M NaCl followed by three exchanges into ddH_2O. Aptamer samples were heated in a boiling water bath for 3 min and cooled in an ice bath prior to use to allow the DNA aptamer to anneal. Final aptamer and ligand concentrations were determined by UV–Vis spectroscopy using the extinction coefficients provided by suppliers.

2.2. Isothermal titration calorimetry

Isothermal titration calorimetry (ITC) experiments were performed using a MicroCal VP-ITC instrument. Samples were degassed at 4 °C for 5 min prior to use with the MicroCal Thermo Vac unit. All experiments were corrected for the heat of dilution of the titrant. Cocaine binding experiments were performed at low-c