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Enantioselective and label-free detection of oligopeptide via fluorescent indicator displacement

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ABSTRACT

In this work, a simple and label-free fluorescent method via fluorescent indicator displacement (FID) was proposed for enantioselectively determining D-enantiomer of arginine vasopressin (DV) using DV-specific DNA aptamer (V-apt) and one guanidiniophthalocyanine dye (Zn-DIGP). Zn-DIGP that preferentially binds to single-stranded DNA with fluorescence enhancement rather than duplexes occupies the long internal loop of V-apt and generates intensive fluorescence. Then DV is introduced into the solution containing Zn-DIGP and V-apt, and displaces the Zn-DIGP from the binding site of internal loop, leading to fluorescence decrease. But L-enantiomer cannot induce any fluorescence change due to the selectivity of V-apt. This established FID technique can detect DV with a detection limit of 100 nM and exhibits a broad linear range, and is able to discriminate enantiomers of arginine vasopressin unambiguously. Moreover chiral separation by chromatography, complicated experimental procedures and covalent modification of tags (such as organic dyes, redox-active metal complexes) are avoided in our strategy. This simple and label-free method is promising for fabricating diverse aptasensors to determine other biomolecules and drugs.

1. Introduction

Chiral discrimination is always a research focus. Most chrial molecules exist in the form of racemic mixture. However, a single enantiomer probably naturally occurs in organism, such as D-ribose, D-2-deoxyribose and L-amino acids, and only a single enantiomer is non-toxic and medicable for human. For example, *R*-thalidomide has the sedative function (Höglund et al., 1998), while *S*-thalidomide has the teratogenic and antitumor prosperities (Wnendt et al., 1996); Only the *S*-enantiomer of ibuprofen possesses the curative effect against fever, pain and inflammatory diseases (Hao et al., 2005; Hawel et al., 2003). In addition, apart from L-amino acids, D-amino acids of trace amount in higher order organisms play important biological roles (Fuchs et al., 2005). Due to differences in the pharmacological and toxicological activity, separation and quantitative detection of enantiomers have attracted widespread interests in last decades. Most reported methods for chiral discrimination were based on HPLC (Brumbt et al., 2005), capillary electrophoresis (CE) (Ruta et al., 2006) and capillary electrochromatography (CEC) (Andre et al., 2006; Ruta et al., 2009), coupling with UV–vis, fluorescence and mass spectrometry (Issaq et al., 2009). A common feature among those methods was that separation procedures were essential before quantitative determination of enantiomers, which made analytical processes complex and costly.

Nucleic acid aptamers that are single-stranded oligonucleotides with high and specific affinity to targets (Mairal et al., 2008) have been widely utilized as chiral selectors to discriminate enantiomers of chiral compounds, such as adenosine (Michaud et al., 2004; Null and Lu, 2010), arginine (Null and Lu, 2010; Ruta et al., 2007b), histidine (Ruta et al., 2007a), thalidomide (Shoji et al., 2007), ibuprofen (Kim et al., 2010) and so on. However, separation by HPLC, CE or CEC was still required for most of the aptamer-based applications.

Therefore, it is highly desirable to propose a simple and cost-effective method for determination of a single enantiomer of chiral compounds without separation. Indicator displacement assays (IDA) have been widely applied to design methods for analytical detection due to their simplicity and quickness (Nguyen and Anslyn, 2006). For example, Stojanovic group designed one

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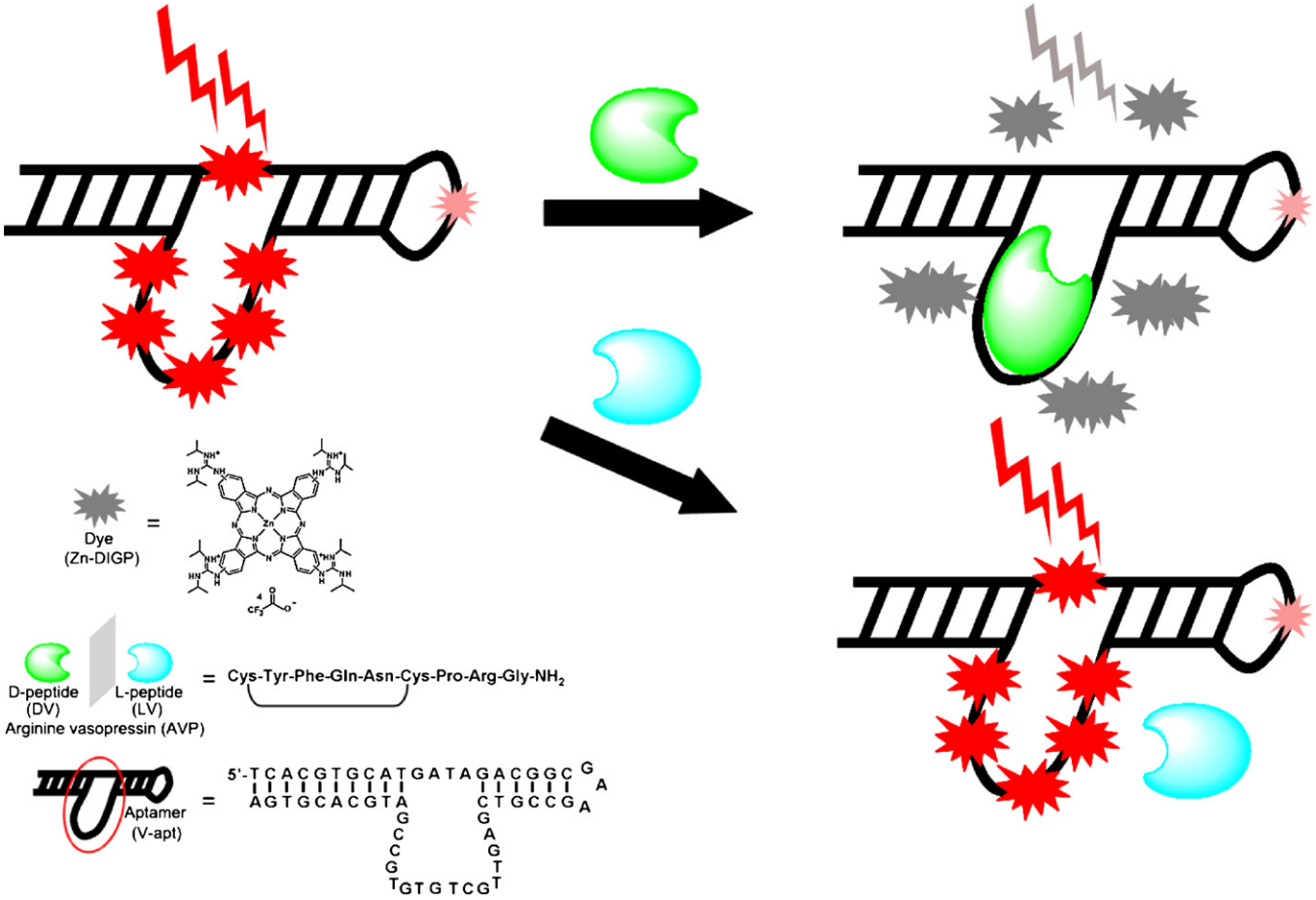

Scheme 1. Enantioselective detection of D-arginine vasopressin (DV) via fluorescent indicator displacement (FID) using DV-specific aptamer (V-apt). Recognition and interaction between V-apt and DV result release of Zn-DIGP and fluorescence decrease, while LV cannot induce any fluorescence change. The internal loop of V-apt is indicated by a red circle.

colorimetric IDA method for cocaine detection, utilizing anti-cocaine aptamer as receptor and one cyanine dye as indicator (Stojanovic and Landry, 2002). However, to the best of our knowledge, IDA technique has not been developed for discriminating enantiomers of oligopeptide.

As mentioned above, two enantiomers of chiral molecule perhaps have distinct physiological behaviors. In addition, although only L-enantiomer of oligopeptide probably naturally occurs in organism, D-oligopeptide drugs have been designed and studied widely due to their resistance to proteolytic degradation (Eckert et al., 1999; Welch et al., 2010; Zhan and Lu, 2011). Therefore, chiral separation of oligopeptide has attracted researchers' interests (Czerwenka et al., 2002; Michaud et al., 2003; Wan and Blomberg, 2000), and effective methods for enantioselective and quantitative detection of oligopeptide will be required in future. Consequently, in this paper, arginine vasopressin (AVP) was chosen as a model system to realize label-free and chiral discrimination of oligopeptide without separation by chromatography. As a nine-amino acid cyclic oligopeptide hormone, AVP is found in most mammals, including humans, and acts as an important modulator of neuronal function, the water adsorption and urine production in the kidney (Thomson and Napier, 2010). Its detailed amino acid sequence is shown in Scheme 1 (Vigneaud et al., 1953). Williams et al. (1997) have in vitro selected a DNA aptamer that specifically binds D-enantiomer of AVP. Based on this aptamer, chiral separation of AVP by HPLC has been realized (Michaud et al., 2003).

Herein, we used the D-arginine vasopressin aptamer (V-apt, detailed sequence shown in Scheme 1) as a chiral selector, and realized enantioselective determination of D-enantiomer of arginine vasopressin in solution via fluorescent indicator displacement (FID) technique. The D-enantiomer can be successfully detected in the presence of L-enantiomer by the fluorescence changes, which is attributed to the specificity of V-apt. Without complex experimental procedures and covalent modification of tags, the strategy is simple and lable-free. In addition, it is much more facile and cost-effective, because labour-intensive chromatographic techniques for chiral separation are avoided.

2. Materials and methods

2.1. Materials

D- and L-arginine vasopressin were purchased from ZiYuPeptides Co., Ltd. (Shanghai, China). D-Enantiomer and L-enantiomer of AVP were abbreviated as DV and LV, respectively. Ultrapage-purified oligonucleotides were obtained from Sangon Biotechnology Co., Ltd. (Shanghai, China). Tetrakis(diisopropylguanidino)-zinc-phthalocyanine·TFA_4 salt (Zn-DIGP, Scheme 1) was synthesized according to published procedures (Alzeer et al., 2009). Potassium chloride was purchased from Sinopharm Group Chemical Reagent Co., Ltd. (Shanghai, China). All oligonucleotides were dissolved in PBS buffer (5 mM Na_2HPO_4, pH: 6) and quantified using UV–vis absorption spectroscopy with a Cary 50 Scan UV–vis spectrophotometer (Varian, USA). All oligonucleotides were stored at −20 °C, heated to 95 °C for 5 min and gradually cooled to room temperature before use. Stock solutions of Zn-DIGP (1 mM) were