Biosensors and Bioelectronics 35 (2012) 401–406

Contents lists available at SciVerse ScienceDirect

Biosensors and Bioelectronics

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

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

Jiangtao Ren[a,b], Jiahai Wang[a,*], Jin Wang[a,c,*], Nathan W. Luedtke[d], Erkang Wang[a,*]

[a] State Key Laboratory of Electroanalytical Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun, Jilin 130022, China
[b] Graduate School of the Chinese Academy of Sciences, Beijing 100039, China
[c] Department of Chemistry, Physics and Applied Mathematics, State University of New York at Stony Brook, Stony Brook, NY 11794, USA
[d] Institute of Organic Chemistry, University of Zürich, Winterthurerstrasse 190, Zürich CH-8057, Switzerland, United Kingdom

ARTICLE INFO

Article history:
Received 29 December 2011
Received in revised form 6 March 2012
Accepted 15 March 2012
Available online 23 March 2012

Keywords:
Chiral discrimination
Enantiomer
DNA aptamer
Label-free
Fluorescent indicator

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

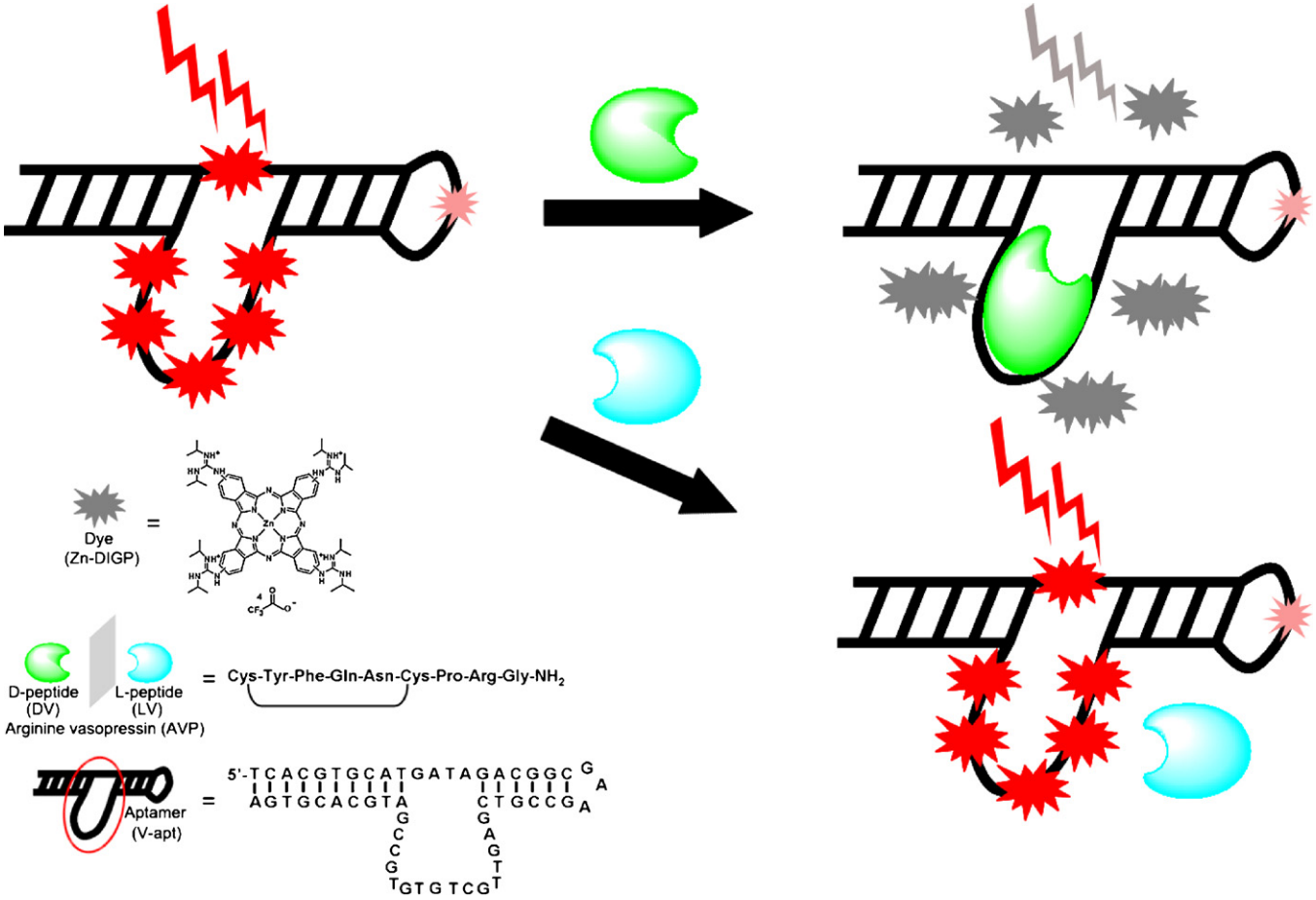
Chiral discrimination is always a research focus. Most chrial molecules exist in the form of racemic mixture. However, a single enantiomer probably naturally occurs in organism, such as D-ribose, D-2-deoxyribose and L-amino acids, and only a single enantiomer is non-toxic and medicable for human. For 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

* Corresponding authors at: State Key Laboratory of Electroanalytical Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun, Jilin 130022, China. Tel.: +86 431 85262003; fax: +86 431 85689711.
E-mail addresses: jhwang@ciac.jl.cn (J. Wang), jin.wang.1@stonybrook.edu (J. Wang), ekwang@ciac.jl.cn (E. Wang).

0956-5663/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.bios.2012.03.028



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

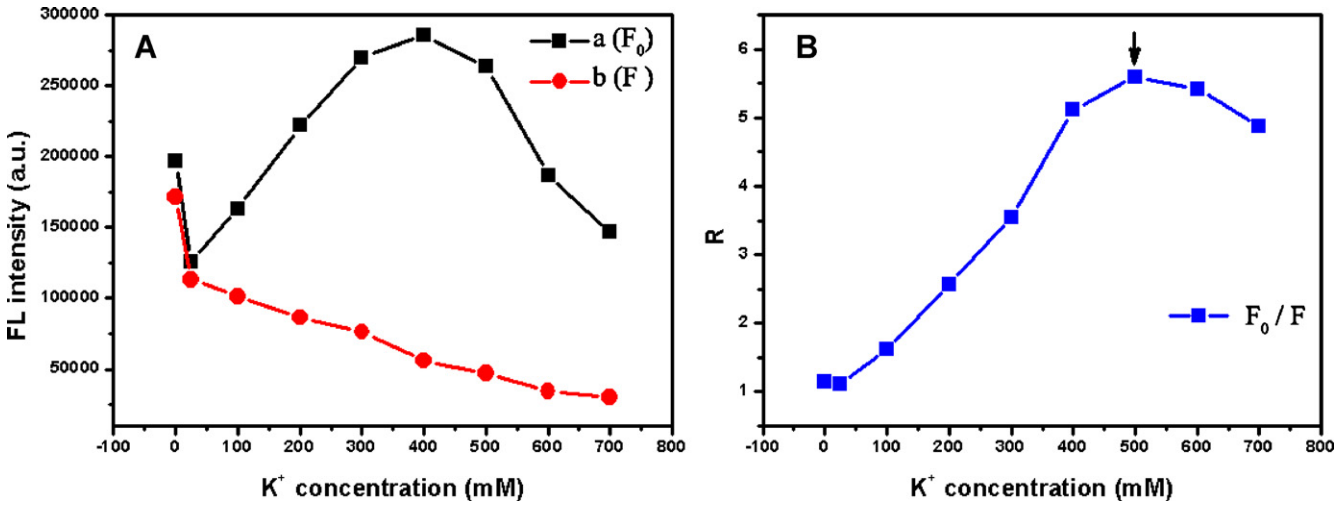


Fig. 1. (A) Potassium-dependent fluorescence intensity of Zn-DIGP plus V-apt at 696 nm without (a) or with (b) DV. (B) Potassium-dependent sensitivity (**R**, $\mathbf{F_0/F}$) of the FID technique. The concentrations of Zn-DIGP, V-apt and DV were 1 μM, 200 nM and 10 μM, respectively in PBS buffer (5 mM Na_2HPO_4, pH = 6).

prepared in DMSO, stored in the dark at −20 °C, and diluted to the required concentration with PBS buffer. Double distilled water was used throughout.

2.2. Optimization of metal cation (K^+ and Mg^{2+}) concentration

Samples in Fig. 1 and Figure S1 were prepared using the general procedures as follows: 50 μl of V-apt (2 μM), 50 μl of DV (100 μM), 50 μl of Zn-DIGP (10 μM) and the certain amount of metal cation (K^+ or Mg^{2+}) were mixed successively. The mixtures were adjusted to 500 μl with PBS buffer, incubated at room temperature for an hour and measured on a Fluoromax-4 spectrofluorometer (Horiba Jobin Yvon Inc., France), with slits of 5/5 nm, excitation at 620 nm, and emission at 696 nm. Final concentrations of V-apt, DV and Zn-DIGP were 200 nM, 10 μM and 1 μM, respectively. The concentrations of K^+ (0, 25, 100, 200, 300, and 400, 500, 600 and 700 mM) and Mg^{2+} (0, 1, 5, 10, 20 and 30 mM) were investigated.

2.3. Characterization for Zn-DIGP

Split aptamers (V-sp1 and V-sp2) were designed (Figure S2) to characterize the binding preference of Zn-DIGP for dsDNA and ssDNA. Metal cation effects on fluorescence intensity of solution containing Zn-DIGP, V-sp1, and V-sp2, were investigated firstly. The final concentrations of Zn-DIGP, V-sp1 and V-sp2 were 1 μM, 200 nM and 200 nM in PBS buffer, and the metal cation concentrations were indicated in Fig. 2A and Figure S3. Then, in the presence of 500 mM K^+, Zn-DIGP only, Zn-DIGP plus V-sp1, Zn-DIGP plus V-sp2 and Zn-DIGP plus V-sp1 + 2 (V-sp1 and V-sp2) were studied respectively in PBS buffer (Fig. 2B), The final concentrations of Zn-DIGP, V-sp1 and V-sp2 were 1 μM, 200 nM and 200 nM, respectively.

2.4. Interaction analysis between V-apt and Zn-DIGP

All solutions in PBS/K buffer (5 mM Na_2HPO_4, 500 mM K^+, pH: 6) were prepared using aforementioned general procedures. The concentrations of each component in Fig. 3A were 200 nM for V-apt, 1 μM for Zn-DIGP, and 10 μM for DV or LV. Then adsorption spectra were collected. In addition, distinct orders of addition of components were investigated (Figure S4). Sample of column c was prepared as follows: 350 μl PBS/K buffer, 50 μl of V-apt (2 μM) and 50 μl of Zn-DIGP (10 μM) were mixed and incubated for 1 h. Then 50 μl of DV (100 μM) was added and reacted for 1 h. Finally

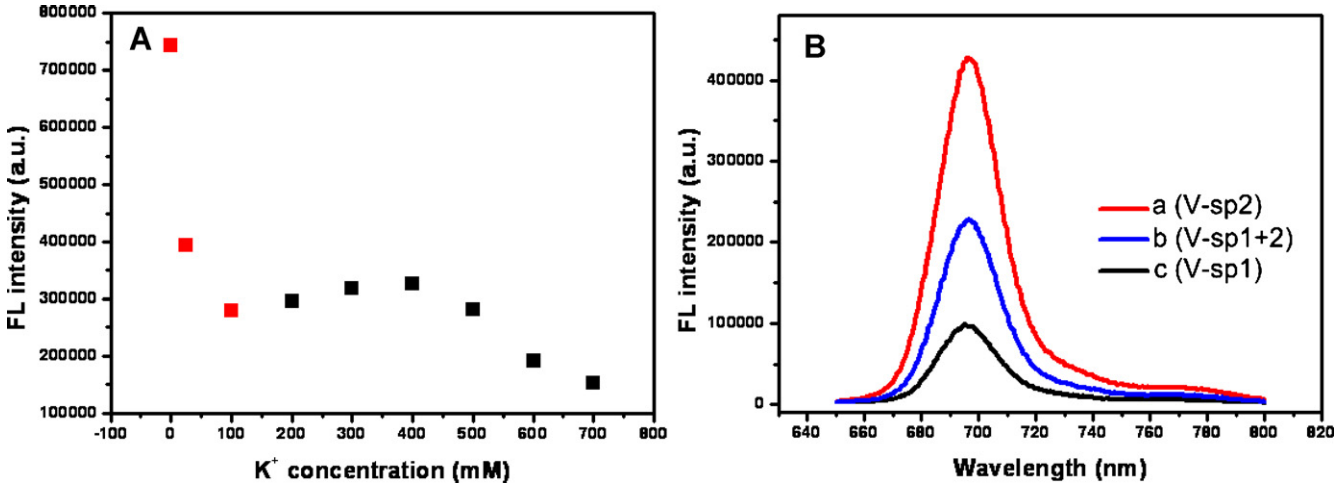


Fig. 2. (A) Potassium-dependent fluorescence intensity of Zn-DIGP at 696 nm with split aptamers (V-sp1 and V-sp2). (B) Fluorescence spectra of Zn-DIGP with each split aptamer (curve a for V-sp2 and curve c for V-sp1) or with two split aptamers (curve b for V-sp1 + 2), at the same K^+ concentration (500 mM). The concentrations of Zn-DIGP, and each split aptamer were 200 nM.

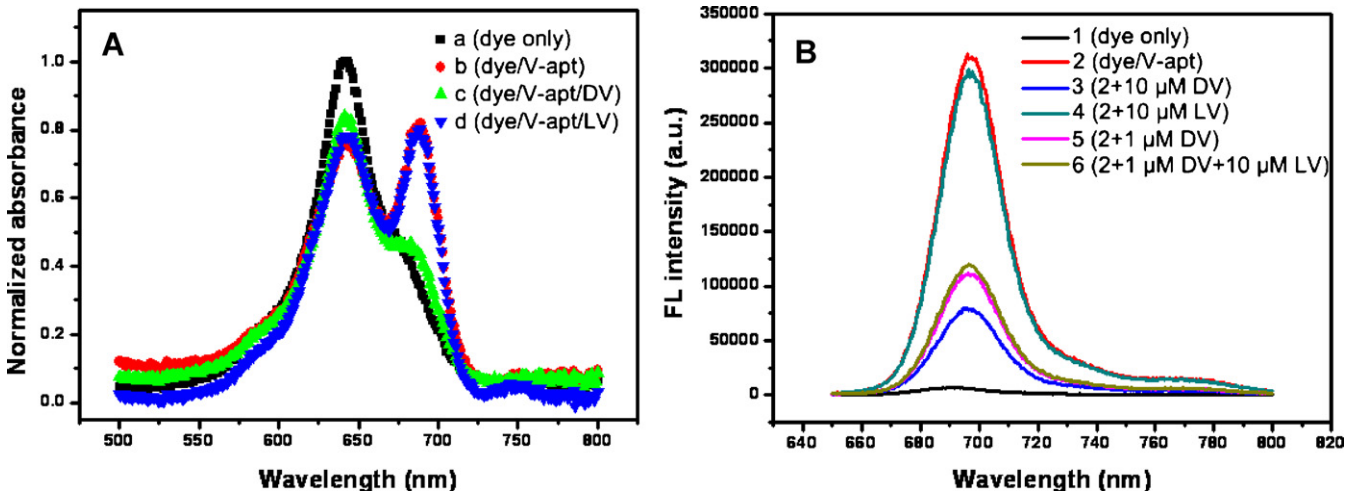


Fig. 3. (A) UV–vis spectroscopic analysis of interaction between Zn-DIGP and V-apt in the presence of DV (c) or LV (d). Curve "a" represents the Zn-DIGP only and curve "b" is Zn-DIGP plus V-apt. The concentrations of Zn-DIGP, V-apt, DV or LV were 1 μM, 200 nM and 10 μM, respectively. (B) Fluorescent spectra of Zn-DIGP with distinct components in PBS buffer. Curves from 1 to 6 represent Zn-DIGP only (1), Zn-DIGP + V-apt (2), Zn-DIGP + V-apt + 10 μM DV (3), Zn-DIGP + V-apt + 10 μM LV (4), Zn-DIGP + V-apt + 1 μM DV (5), and Zn-DIGP + V-apt + 1 μM DV + 10 μM LV (6). The concentrations of Zn-DIGP and V-apt were 1 μM and 200 nM, respectively.

the mixture was measured on the spectrofluorometer. Other samples in Figure S4 were prepared according to the order of addition indicated by arrows.

2.5. Chiral discrimination between D- and L-enantiomers

In Fig. 3B, samples in pure PBS/K buffer were prepared as Fig. 3A. To test applicability of the system in biological fluids, human urine and serum were utilized. The serum and urine were centrifuged at 8000 rpm for 10 min to remove the precipitates, and the supernatants were diluted to 10% using PBS/K buffer. The samples containing 1% of biological fluids (addition of 50 μl of 10% of urine or serum) were prepared using aforementioned general procedures and fluorescence spectra were collected (Figure S5).

2.6. DV titration

In Fig. 4, samples in PBS/K buffer were prepared using aforementioned general procedures. Final concentrations of V-apt and Zn-DIGP were 200 nM and 1 μM, respectively. And the concentrations of DV (0, 0.1, 0.25, 0.5, 1, 5 and 10 μM) were investigated.

3. Results and discussion

3.1. Fluorescent indicator displacement (FID) technique

One guanidiniophthalocyanine dye, tetrakis(diisopropylguanidino)-zinc-phthalocyanine (Zn-DIGP), was utilized as fluorescent indicator to monitor the recognition event between V-apt and DV in solution (Scheme 1). Firstly, the Zn-DIGP binds to the internal loop (indicated by a red circle) of V-apt with fluorescence enhancement. Then, upon addition of DV into the system, strong interaction between DV and the internal loop of V-apt compels the Zn-DIGP to be released from the V-apt with fluorescence decreasing. Decreased fluorescent signal from the direct fluorophore displacement provides a sensitive and label-free quantitative determination of DV, while LV is not able to induce any fluorescence change.

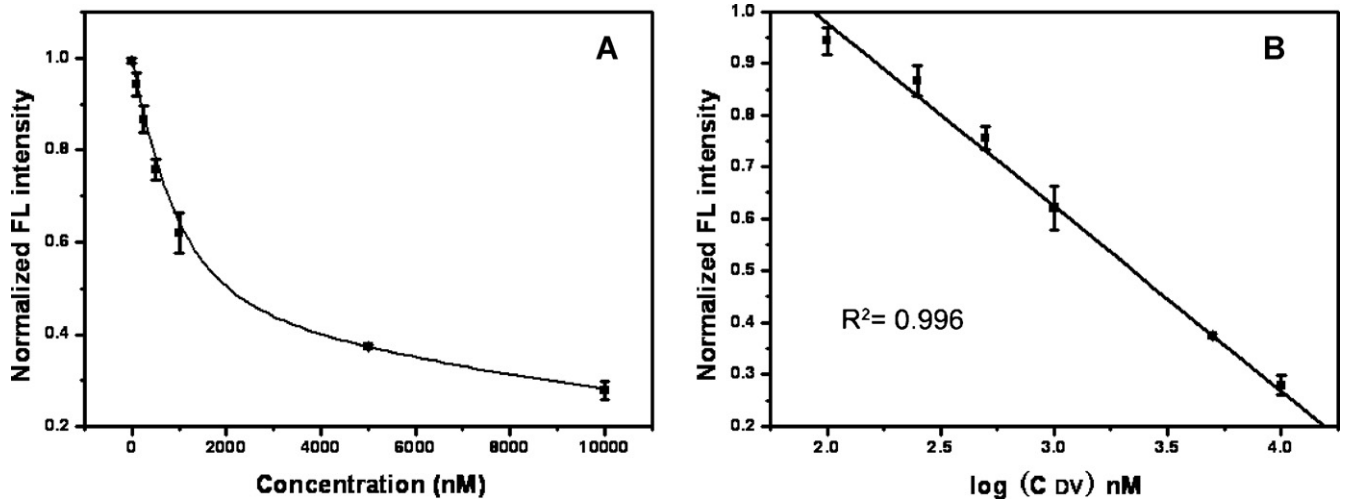


Fig. 4. (A) Normalized fluorescence intensity of Zn-DIGP plus V-apt at 696 nm as function of DV concentration (0, 100, 250 and 500 nM, 1, 5 and 10 μM). (B) A linear relationship between the normalized fluorescence intensity and the logarithm of DV concentration from 100 nM to 10 μM. The error bars indicates the standard deviation of three repeated measurements for each concentration of DV. The concentrations of Zn-DIGP and V-apt were 1 μM, and 200 nM.

The salt concentration in solution is a key-determinant factor, which significantly affects the recognition between V-apt and DV (Michaud et al., 2003) and sensitivity of our system. Therefore, optimizations of potassium ion (K^+) and magnesium ion (Mg^{2+}) concentration in the system were performed. For convenience, we use a ratio, **R** (**F_0/F**) to evaluate the system sensitivity, where **F_0** and **F** are the fluorescence intensity of Zn-DIGP with V-apt in the absence and presence of DV. As shown in Fig. 1A, with the K^+ concentration increasing, **F_0** exhibits a maximal value at 400 mM K^+ (curve a) which means the optimal interaction between V-apt and Zn-DIGP; meanwhile, decrease of **F** over the whole range (curve b) suggests high amount of K^+ conduces to interaction between DV and V-apt, DV competes with the Zn-DIGP and compels it to be released from V-apt. Fig. 1B shows that **R** reaches the maximal value of 5.6 at 500 mM K^+ (indicated by an arrow). In addition, Mg^{2+} has an analogous effect on the **F_0**, **F** and **R** at the concentration from 0 to 30 mM, and maximal **R** of 4.4 can be obtained at 20 mM concentration (indicated in Figure S1). To achieve best sensitivity for DV, 500 mM K^+ was utilized in our system.

3.2. Binding preference of Zn-DIGP for ssDNA over dsDNA

It was reported that Zn-DIGP exhibited intensified fluorescence when binding nucleic acid G-quadruplex (Alzeer et al., 2009). Most nucleic acid stains preferentially bind to double-stranded DNA (dsDNA) with fluorescence enhancement, compared to single-stranded DNA (ssDNA), such as Ethidium bromide (Waring, 1965), SYBR green I (Zipper et al., 2004), PicoGreen (Dragan et al., 2010) and so on. Interestingly, we found that Zn-DIGP showed a preference for binding to ssDNA over dsDNA. In this study, we split the integral V-apt into two segments (V-sp1 and V-sp2, Figure S2) and adopted two approaches to prove the binding preference of Zn-DIGP.

Firstly, we investigated the salt effect on interaction of V-sp1 and V-sp2 with Zn-DIGP (Fig. 2A). It is well-known that, metal ions can stabilize the nucleic acid duplex (Owczarzy et al., 2008; Tan and Chen, 2006). In the absence of K^+, complementary segments of V-sp1 and V-sp2 could not form intermolecular duplex secondary structure, and the quite high fluorescence intensity of Zn-DIGP plus V-sp1 and V-sp2 suggested that Zn-DIGP can bind to single-stranded V-sp1 and V-sp2 effectively. After addition of K^+ into solution, the fluorescence intensity dramatically decreased due to generation of stable duplexes between V-sp1 and V-sp2, indicating Zn-DIGP was released from the duplex segments of resulting V-sp1 + 2 duplex-loops structure. Analogous phenomenon was observed with Mg^{2+} (Figure S3). Secondly, in the presence of 500 mM K^+, fluorescence of Zn-DIGP plus V-sp2 decreased after addition of V-sp1 (Fig. 2B, curve b) and formation of duplex structure between V-sp1 and V-sp2.

Therefore, it is reasonable to postulate that Zn-DIGP preferentially binds to ssDNA over dsDNA. As compared with split aptamers (V-sp1 and V-sp2), integral aptamer (V-apt) is more inclined to fold into the duplex-loops structure, thus there is no obvious fluorescence decreasing after addition of metal ions in Fig. 1A and Figure S1A (curve a) as V-sp1 + 2. Analogously, Zhou group has reported a zinc phthalocyanine (ZnPc) that selectively binds to guanine-residues in the bulges and loops of DNA in a non-intercalative mode (Zhang et al., 2007). So, Zn-DIGP mostly binds to the ssDNA loop (mainly the long internal loop) of V-apt rather than its duplex segments in the presence of 500 mM K^+.

3.3. Chiral discrimination via FID

Previous studies have indicated that the internal loop is essential for the specific DV binding (Michaud et al., 2003). Therefore, DV can bind to the loop segment of V-apt and occupies the binding site and shield the V-apt away from the Zn-DIGP. We investigated the order of addition of DV and Zn-DIGP (Figure S4). The data (column c, d and e) reveals that order of addition does not impact the sensitivity for DV, and DV is competent to displace the Zn-DIGP from the internal loop of V-apt upon addition into the premixed and incubated solution of Zn-DIGP and V-apt. In addition, DV cannot induce any fluorescence change of Zn-DIGP in the absence of V-apt (column a and f).

To characterize interaction between V-apt and Zn-DIGP, UV–vis measurements were conducted (Fig. 3A). The Q adsorption band of phthalocyanine can be utilized as the indicator that provides the information what happened to Zn-DIGP. Dissolved Zn-DIGP in the PBS buffer existed in the form of dimer which exhibited 641 nm Q adsorption band (curve a); upon addition of V-apt, one adsorption band at longer wavelength of 689 nm emerged (curve b), which was assigned to the Q band of the Zn-DIGP monomer (De Filippis et al., 2000; Dummin et al., 1997), illustrating that Zn-DIGP can bind to the internal loop of V-apt in the form of monomer. When DV was added into the mixture of Zn-DIGP and V-apt, the adsorption peak at 689 nm disappeared (curve c). The results disclosed that DV was capable to bind to the internal loop of V-apt and shield V-apt away from Zn-DIGP, which dimerized in the buffer solution. While the adsorption intensity at 689 nm of solution containing Zn-DIGP plus V-apt (curve b) did not change at all upon addition of LV (curve d), which demonstrated that V-apt was specific for DV. Consequently, chiral discrimination of DV and LV via FID is well-founded.

Fig. 3B shows the distinct fluorescent spectra of Zn-DIGP plus V-apt in the presence of LV or DV, at excitation wavelength of 620 nm, confirming the specificity of the fluorescence readout. After addition of LV, the fluorescence intensity of Zn-DIGP (curve 4) dose not change obviously as compared to solution containing Zn-DIGP and V-apt (curve 2), while DV decreases the intensity significantly (curve 3). Moreover, excess LV in the solution can scarcely impact the fluorescent signal induced by DV (curve 5 and 6). It demonstrates that enantioselective detection of DV without separation has been realized with our system. In addition, whether in pure PBS buffer, or in diluted biological fluids (1% urine and 1% serum in PBS buffer), DV still could be discriminated from the LV clearly (Figure S5).

Titrations of DV were conducted using V-apt and Zn-DIGP in the presence of 500 mM K^+ (Fig. 4). With increasing DV concentration, fluorescence intensity of Zn-DIGP decreased (Fig. 4A). A broad linear range between the normalized fluorescence intensity and the logarithm of DV concentration from 100 nM to 10 μM (R^2 = 0.996, Fig. 4B) and limit of detection of 100 nM (S/N = 3) were obtained. The RSD (relative standard deviation) was less than 7.0%. In previous studies, Erickson group fabricated an optofluidic surface enhanced Raman spectroscopy (SERS) device for AVP detection based on SERS-active metal nanotube substrates (Huh and Erickson, 2010), and our group has developed two electrochemical methods for detection of DV using graphene nanomaterials (Du et al., 2012; Qin et al., 2012). Compared to those reported methods, the FID is much simple, label-free and cost-effective. The FID strategy does not need any substrate and complicated substrate pretreatment and avoids expensive and tedious covalent modification of tags, such as organic dyes, redox-active metal complexes, onto recognition elements and substrates. Therefore, as a "mix-to-signal" sensing platform, our FID strategy is effective and interesting, and promising for construction of diverse aptasensors.

4. Conclusions

In summary, a simple, cost-effective and label-free fluorescent method was successfully established for enantioselective detection

of D-oligopeptide based on fluorescent indicator displacement (FID). Without separation by chromatography, this method can discriminate D-enantiomer of arginine vasopressin in the presence of excess L-enantiomer unambiguously. It can detect DV with a detection limit of 100 nM and exhibits a broad linear range from 100 nM to 10 μM. Moreover, the strategy is anticipated to be utilized to construct diverse aptasensors for detecting other biomolecules and drugs.

Acknowledgements

This work was supported by National Natural Science Foundation of China (nos. 21190040 and 21075210) and the 973 Project (2009CB930100 and 2010CB933600).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bios.2012.03.028.

References

Alzeer, J., Vummidi Balayeshwanth, R., Roth Phillipe, J.C., Luedtke Nathan, W., 2009. Angew. Chem. Int. Ed. 48, 9362–9365.
Andre, C., Berthelot, A., Thomassin, M., Guillaume, Y.C., 2006. Electrophoresis 27, 3254–3262.
Brumbt, A., Ravelet, C., Grosset, C., Ravel, A., Villet, A., Peyrin, E., 2005. Anal. Chem. 77, 1993–1998.
Czerwenka, C., Lämmerhofer, M., Maier, N.M., Rissanen, K., Lindner, W., 2002. Anal. Chem. 74, 5658–5666.
De Filippis, M.P., Dei, D., Fantetti, L., Roncucci, G., 2000. Tetrahedron Lett. 41, 9143–9147.
Dragan, A.I., Casas-Finet, J.R., Bishop, E.S., Strouse, R.J., Schenerman, M.A., Geddes, C.D., 2010. Biophys. J. 99, 3010–3019.
Du, Y., Guo, S., Qin, H., Dong, S., Wang, E., 2012. Chem. Commun. 48, 799–801.
Dummin, H., Cernay, T., Zimmermann, H.W., 1997. J. Photochem. Photobiol. B: Biol. 37, 219–229.
Eckert, D.M., Malashkevich, V.N., Hong, L.H., Carr, P.A., Kim, P.S., 1999. Cell 99, 103–115.
Fuchs, S.A., Berger, R., Klomp, L.W.J., de Koning, T.J., 2005. Mol. Genet. Metab. 85, 168–180.
Höglund, P., Eriksson, T., Björkman, S., 1998. J. Pharmacokinet. Pharmacodyn. 26, 363–383.
Hao, H.P., Wang, G.J., Sun, J.G., 2005. Drug Metab. Rev. 37, 215–234.
Hawel, R., Klein, G., Singer, F., Mayrhofer, F., Kahler, S.T., 2003. Int. J. Clin. Pharmacol. Ther. 41, 153–164.
Huh, Y.S., Erickson, D., 2010. Biosens. Bioelectron. 25, 1240–1243.
Issaq, H.J., Chan, K.C., Blonder, J., Ye, X., Veenstra, T.D., 2009. J. Chromatogr. A 1216, 1825–1837.
Kim, Y.S., Hyun, C.J., Kim, I.A., Gu, M.B., 2010. Bioorg. Med. Chem. 18, 3467–3473.
Mairal, T., Ozalp, V.C., Sanchez, P.L., Mir, M., Katakis, I., O'Sullivan, C.K., 2008. Anal. Bioanal. Chem. 390, 989–1007.
Michaud, M., Jourdan, E., Ravelet, C., Villet, A., Ravel, A., Grosset, C., Peyrin, E., 2004. Anal. Chem. 76, 1015–1020.
Michaud, M., Jourdan, E., Villet, A., Ravel, A., Grosset, C., Peyrin, E., 2003. J. Am. Chem. Soc. 125, 8672–8679.
Nguyen, B.T., Anslyn, E.V., 2006. Coord. Chem. Rev. 250, 3118–3127.
Null, E.L., Lu, Y., 2010. Analyst 135, 419–422.
Owczarzy, R., Moreira, B.G., You, Y., Behlke, M.A., Walder, J.A., 2008. Biochemistry 47, 5336–5353.
Qin, H., Liu, J., Chen, C., Wang, J., Wang, E., 2012. Anal. Chim. Acta 712, 127–131.
Ruta, J., Grosset, C., Ravelet, C., Fize, J., Villet, A., Ravel, A., Peyrin, E., 2007a. J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 845, 186–190.
Ruta, J., Perrier, S., Ravelet, C., Roy, B., Perigaud, C., Peyrin, E., 2009. Anal. Chem. 81, 1169–1176.
Ruta, J., Ravelet, C., Baussanne, I., Decout, J.L., Peyrin, E., 2007b. Anal. Chem. 79, 4716–4719.
Ruta, J., Ravelet, C., Grosset, C., Fize, J., Ravel, A., Villet, A., Peyrin, E., 2006. Anal. Chem. 78, 3032–3039.
Shoji, A., Kuwahara, M., Ozaki, H., Sawai, H., 2007. J. Am. Chem. Soc. 129, 1456–1464.
Stojanovic, M.N., Landry, D.W., 2002. J. Am. Chem. Soc. 124, 9678–9679.
Tan, Z.J., Chen, S.J., 2006. Biophys. J. 90, 1175–1190.
Thomson, F., Napier, S., 2010. In: Stolerman, I.P. (Ed.), Encyclopedia of Psychopharmacology. Springer, Berlin/Heidelberg, pp. 151–157.
Vigneaud, V., Lawler, d.H.C., Popenoe, E.A., 1953. J. Am. Chem. Soc. 75, 4880–4881.
Wan, H., Blomberg, L.G., 2000. J. Chromatogr. A 875, 43–88.
Waring, M.J., 1965. J. Mol. Biol. 13, 269–282.
Welch, B.D., Francis, J.N., Redman, J.S., Paul, S., Weinstock, M.T., Reeves, J.D., Lie, Y.S., Whitby, F.G., Eckert, D.M., Hill, C.P., Root, M.J., Kay, M.S., 2010. J. Virol. 84, 11235–11244.
Williams, K.P., Liu, X.-H., Schumacher, T.N.M., Lin, H.Y., Ausiello, D.A., Kim, P.S., Bartel, D.P., 1997. Proc. Natl. Acad. Sci. U. S. A. 94, 11285–11290.
Wnendt, S., Finkam, M., Winter, W., Ossig, J., Raabe, G., Zwingenberger, K., 1996. Chirality 8, 390–396.
Zhan, C.Y., Lu, W.Y., 2011. Curr. Pharm. Des. 17, 603–609.
Zhang, A.M., Huang, J., Weng, X.C., Li, J.X., Ren, L.G., Song, Z.B., Xiong, X.Q., Zhou, X., Cao, X.P., Zhou, Y., 2007. Chem. Biodivers. 4, 215–223.
Zipper, H., Brunner, H., Bernhagen, J., Vitzthum, F., 2004. Nucleic Acids Res. 32, 103.