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Colorimetric Enantiorecognition of Oligopeptide and Logic Gate Construction Based on DNA Aptamer–Ligand–Gold Nanoparticle Interactions

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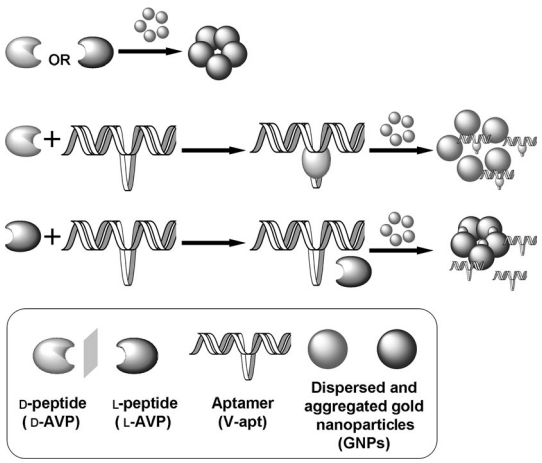
Enantiomers of chiral biomolecules and drugs have distinct pharmacological and toxicological activities.[1]. Consequently, separation and determination of enantiomers are vital in pharmaceutical and chemical industries.[2] It is well-known that the L-enantiomer of oligopeptides naturally exists in the organism; however, D-oligopeptides are regarded as potential therapeutic agents because of their resistance to protease degradation.[3] Thus, chiral discrimination of oligopeptides has been a research focus,[4] and will be required prospectively. In recent years, many methods based on fluorescence,[5] UV/Vis[6] and electrochemistry[7] have been constructed for chiral recognition of biomolecules. However, colorimetric enantiorecognition of oligopeptides has not been reported. Herein, we have utilized unmodified gold nanoparticles (GNPs) as indicator and DNA aptamer as chiral selector, and have realized colorimetric and enantioselective recognition of one oligopeptide, arginine vasopressin (AVP). In addition, because of their convenient visualization, colorimetric molecular logic gates have attracted researchers' attention[8] and they have been constructed for biosensors,[9] drug release,[10] and self-powered biofuel cells.[11] Therefore, we constructed two kinds of colorimetric logic gates (OR and INHIBIT) in combination with colorimetric enantiorecognition of AVP.

AVP is a nine-amino acid cyclic hormone peptide, that exists in most mammals including humans, and acts as a modulator of neuronal function in the brain and regulates water absorption and urine production in the kidneys.[12] Its primary structure is Cys^1-Tyr^2-Phe^3-Gln^4-Asn^5-Cys^6-Pro^7-Arg^8-Gly^9-NH_2, and has a disulfide bridge between Cys^1 and Cys^6 residues. The disulfide group can adsorb onto gold surfaces.[13] It is speculated that AVP can induce aggregation of citrate-coated GNPs, due to its two main functional groups, disulfide and guanidinium groups derived from cystine and arginine residues.[14]

Aptamers are selected in vitro by systematic evolution of ligands by exponential enrichment (SELEX), and are single-stranded DNA or RNA molecules that can bind various target ligands (i.e., small-molecule drugs, peptides, proteins and cells) with high affinity and specificity.[15] Analogous to cyclodextrins and their derivatives, macrocyclic antibiotics, and so on,[16] varieties of aptamers have been exploited and utilized as chiral selectors for chiral separation and detection of target ligands.[5c,17] Williams et al. selected a nucleic acid aptamer that binds the D-enantiomer of AVP (D-AVP).[18] In this study, the D-enantiomer-specific aptamer (V-apt) was utilized as masking agent to impede GNP aggregation via binding interactions between D-AVP and V-apt, and as chiral selector to discriminate enantiomers of AVP. The sequence of V-apt is 5′-TCACG TGCAT GATAG ACGGC GAAGC CGTCG AGTTG CTGTG TGCCG ATGCA CGTGA-3′. Based on the interactions among DNA aptamer, oligopeptide ligand and gold nanoparticle, chiral recognition of AVP was realized (Scheme 1 and Scheme S1 in the Supporting Information).



Scheme 1. Colorimetric and enantioselective recognition of oligopeptide (arginine vasopressin, AVP) based on DNA aptamer–ligand–gold nanoparticle interactions.

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Due to their facile preparation and unique optical property, the 13 nm citrate-capped GNPs were synthesized and used as colorimetric indicator for AVP. Since complexation interaction could occur between guanidinium groups of AVP and carboxylate anions of citrates,[19] GNP stock solution was centrifuged and redispersed in double-distilled water to eliminate interference of free citrate molecules. It has to be noted that a high concentration of GNPs can weaken its sensitivity for AVP. Hence, the final concentration of GNPs in all samples during the experiments was 1 nM, and their color was still evidently visible by the naked eye.

As shown in Figure 1 A, the UV/Vis spectrum of pure GNPs exhibited a surface plasmon resonance peak at 520 nm, and a new absorption peak near 620 nm appeared with increasing L-AVP concentration; this was due to the coupling of the GNPs' plasma mode and dipole–dipole interactions[20] and indicates that the AVP can act as a cross-linking agent and aggregate the GNPs without addition of salt.[21] The absorption ratio (A_{620}/A_{520}, R) of the absorbance of GNPs at 620 nm to the absorbance at 520 nm was adopted as output signal to indicate the aggregation and dispersion state of GNPs. As little as 50 nM L-AVP could induce obvious change of the absorption ratio of GNPs (S/N = 5) and a linear range from 125 to 225 nM was obtained (R = 0.997; Figure 1 B). L-AVP concentrations above 150 nM could be visually detected according to the color change from red to purple or even blue (Figure S1 in the Supporting Information). GNP aggregation upon addition of L-AVP was also characterized by transmission electron microscopy (TEM, Figure S2 in the Supporting Information). In addition, GNP aggregation was not observed with single amino acids at high concentrations of up to 10 μM (Figure S3 in the Supporting Information), illustrating GNP aggregation was ascribed to the multifunctional molecular structure of AVP oligopeptide. Although our detection limit for AVP is higher than the average level (5–10 pM) in mammalian plasma,[22] the potential for colorimetric analysis of AVP could be realized by utilizing enrichment techniques.

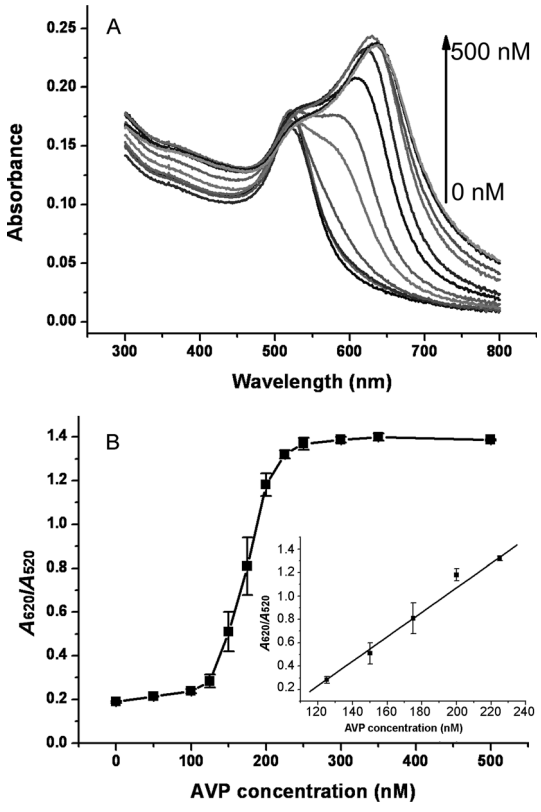


Figure 1. A) UV/Vis spectra, and B) absorption ratio (R, A_{620}/A_{520}) values of samples as a function L-AVP concentration (0, 50, 100, 125, 150, 175, 200, 225, 250, 300, 350 and 500 nM) in the presence of GNPs (1 nM). Inset: linear relationship between the R value and L-AVP concentration (R = 0.997). The error bars indicate the standard deviations of three measurements of independent samples for each concentration of L-AVP.

Furthermore, GNP aggregation induced by AVP at different pH values was investigated (Figure 2). The isoelectric point (pI) of AVP is 10.9,[23] so we increased the solution pH from 7 to 12 to adjust the charge state of AVP; the citrate-coated GNPs are negatively charged and stable in this pH range (a). When the pH was less than the pI value of AVP, the positively charged peptide could interact with the negatively charged surfaces of GNPs and induce GNP aggregation. When the pH was raised above the pI, no GNP flocculation was observed in the presence of negatively charged AVP (b). The results demonstrate that the electrostatic charge of AVP played a significant role in the interaction between GNPs and AVP. In addition, the AVP-induced GNP assembling and disassembling were reversible and the GNP aggregates could be redispersed by ultrasonic treatment in a basic solution the pH of which was raised above the pI of AVP (Figure S4 in the Supporting Information); this indicates the formation of self-assembled and stable AVP layers on GNP surfaces. Lysine vasopressin (LVP), is a hormone peptide found in pigs that contains a lysine residue instead of arginine at position 8, and has the same function as AVP, in vivo.[24] The data (Figure 2 c) show that LVP (pI 10.0)[25] was not able to aggregate the GNPs, illustrating

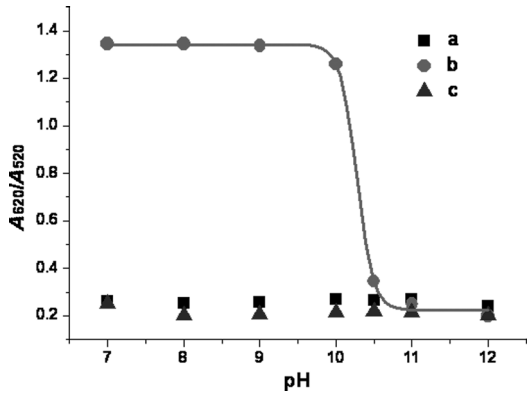


Figure 2. The R values of GNPs (a) in the presence of L-AVP (b) or LVP (c) at different pH values. The concentrations of GNPs, L-AVP and LVP were 1 nM, 1 μM and 1 μM, respectively.

that the positively charged guanidinium group of the arginine residue was critical for AVP-induced aggregation of GNPs.

In previous studies, our group has developed electrochemical and fluorescent methods for chiral discrimination of D-AVP from L-AVP using the D-AVP-specific aptamer (V-apt).[26] Herein, colorimetric recognition of enantiomers of AVP was realized by using V-apt. The dissociation constant (K_d) of V-apt with D-AVP is about 1 μM.[18] To make it fold into the stable secondary structure, V-apt oligonucleotides were heated and annealed in solution containing phosphate buffer and KCl. In addition, it has to be noted that premixing and incubation of V-apt and AVP were vital for discrimination of the peptide enantiomers. Optimization of V-apt concentration was performed in the presence of 250 nM of each AVP enantiomer (Figure 3A). In the absence of V-apt,

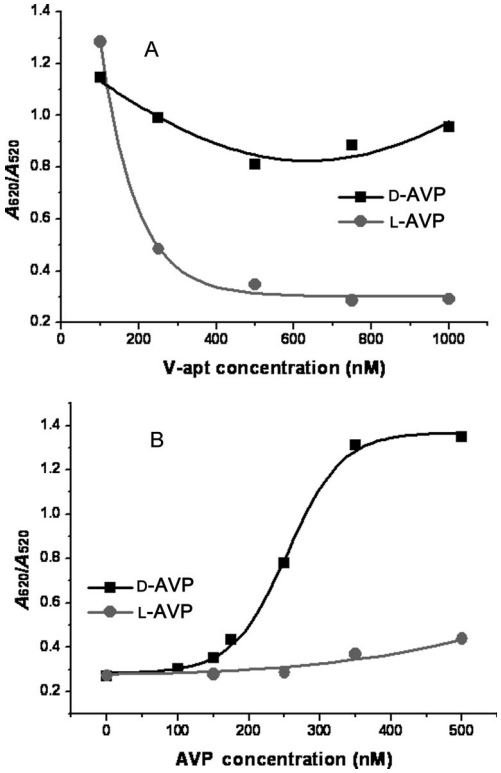


Figure 3. A) The R values of GNPs in the presence of AVP (250 nM) with V-apt at different concentrations. B) The R values of GNPs with AVP at different concentrations in the presence of V-apt (1 μM).

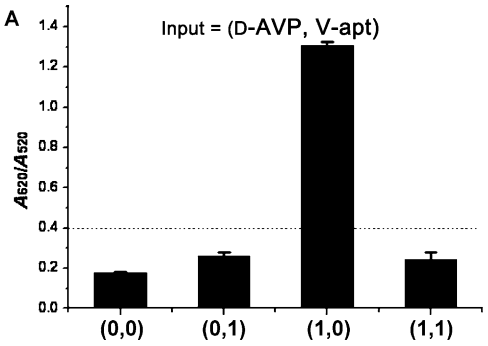
D-AVP and L-AVP both increased the R value obviously in 10 min. When V-apt was introduced into the system, the R value for D-AVP decreased drastically until the concentration of V-apt reached 750 nM. It has been reported that the arginine guanidinium group of D-AVP engages in specific interaction with V-apt,[27] and the guanidinium group participates in the interaction with GNPs as demonstrated above. Therefore, the aptamer for D-AVP can bind with D-AVP, mask the guanidinium groups, impede the interaction between D-AVP and gold surfaces and inhibit GNP aggregation. Since there was a much weaker interaction between V-apt and L-AVP, the R value for L-AVP in the presence of aptamer did not change obviously compared to that for D-AVP. As shown in Figure 3B, response signals for L-AVP were remarkably higher than that for D-AVP in the presence of 1 μM V-apt, and chiral selectivity (R_L/R_D) of the colorimetric system toward L-AVP was up to 3.6 at the same concentration of 350 nM, whereas no enantioselectivity (R_L/R_D: 0.98) for AVP oligopeptide was observed without V-apt (Figure S5 in the Supporting Information). Thus, by virtue of V-apt as chiral selector, the enantiomers of AVP can be discriminated enantioselectively and colorimetrically.

Molecular logic gates are widely constructed based on molecular recognition and assembly, and are anticipated to overcome the limitations of classical semiconductor devices.[28] Herein, by the use of unmodified GNPs and based on the mechanism of chiral recognition of oligopeptide, two kinds of colorimetric logic gates (OR and INHIBIT) were constructed with AVP enantiomers and DNA aptamer as inputs (Scheme S2 in the Supporting Information). The presence and absence of inputs were defined as "1" and "0". The outputs of absorption ratios above and below the threshold value (0.4) were defined as "1" and "0", and the aggregated (blue) and non-aggregated (red) states of GNPs were defined as "1" and "0", respectively. As mentioned above, due to the structural characteristic of AVP, which has disulfide and guanidinium groups, both enantiomers of AVP can induce GNP aggregation with color change from red to blue. Hence, a very simple colorimetric "OR" logic gate can be fabricated with D-AVP and L-AVP as inputs, and solution color (A_{620}/A_{520}) as output (Scheme S2A). Only under the condition of (0, 0) its output was "0", and the outputs of other combinations (0, 1), (1, 0) and (1, 1) were all "1" (Figures S6 and S7A in the Supporting Information).

Moreover, a colorimetric "INHIBIT" logic gate was designed based on the shielding effect of V-apt, which can bind D-AVP and inhibit GNP aggregation (Scheme S2B). D-AVP and the annealed V-apt were utilized as inputs and solution color (A_{620}/A_{520}) as output. Figure S8 in the Supporting Information shows that the color of combinations (0, 0) and (0, 1) were red (output = 0). In the presence of D-AVP (1, 0), the color of GNPs turned blue and the output was "1". When V-apt and D-AVP were premixed, incubated and introduced into the GNPs (1, 1), the color of GNPs remained red (output = 0). Therefore, the input of V-apt can logically disable the whole system and it had a veto. The data of absorption ratios and UV/Vis spectra are shown in Figure 4A and Figure S7B, respectively. The truth table of the "INHIBIT" logic gate is in Figure 4B. However, L-AVP with V-apt cannot implement the "INHIBIT" logic and was still able to induce GNP aggregation in 10 min.

To the best of our knowledge, most previous logic systems that use fluorescent signals as outputs always suffer problems, such as trivial experimental procedures, photobleaching and lack of portability. By contrast, colorimetric signals can be observed by the naked eye directly, and expensive or complicated instruments are not required. In addition, our

A



B

Inputs		Output
D-AVP	V-apt	(A_{620}/A_{520})
0	0	0
0	1	0
1	0	1
1	1	0

Figure 4. The colorimetric "INHIBIT" logic gate shown by: A) the absorption ratios (A_{620}/A_{520}), and B) the truth table. The error bars indicate the standard deviations of three measurements.

logic gates eliminate complex modification and reaction procedures and are more cost-effective and fast-responding. Moreover, it is promising that the colorimetric "OR" and "INHIBIT" logic gates can be visually implemented for enantiorecognition of AVP in practical samples in future.

In summary, it was demonstrated that one hormone oligopeptide (arginine vasopressin) can aggregate unmodified gold nanoparticles with color change from red to blue through its disulfide and positive guanidinium groups, and colorimetric recognition of enantiomers of the oligopeptide was realized with high chiral selectivity by using its D-enantiomer specific DNA aptamer as chiral selector. Based on the mechanism of colorimetric enantiorecognition, for the first time, very simple, label-free and colorimetric "OR" and "INHIBIT" logic gates were also constructed with enantiomers of oligopeptide as inputs. By virtue of colorimetric combinational logic systems, enantioselective analysis of oligopeptides has much potential to be realized in the future. Although such elementary logic gates are insufficient for serious computing applications and numerous kinds of materials (e.g., DNA, protein and other molecules) have been employed for Boolean logic operations,[29] the introduction of enantiomers of chiral molecules may lead to a better understanding of chiral recognition for information processing in natural systems.

Experimental Section

Materials: The D- and L-enantiomers of arginine vasopressin (AVP) were purchased from ZiYuPeptides, Co., Ltd. (Shanghai, China), and are abbreviated as D-AVP and L-AVP, respectively. Lysine vasopressin (LVP) and ultraPAGE-purified oligonucleotides were purchased from Sangon Biotechnology, Co., Ltd. (Shanghai, China). The oligonucleotides (V-apt) were dissolved in PBS buffer (5 mM Na_2HPO_4, pH 6) and stored at −20 °C. $HAuCl_4{\cdot}4H_2O$ was purchased from Beijing Chemical Factory (Beijing, China). Sodium citrate was obtained from Shanghai Chemical Reagent Co. (Shanghai, China). Potassium chloride was purchased from Sinopharm Group Chemical Reagent Co., Ltd. (Shanghai, China). Double distilled water was used throughout.

Synthesis and characterization of GNPs: Gold nanoparticles (GNPs, ~13 nm) were synthesized according to the Frens method.[30] An aqueous solution (100 mL) containing $HAuCl_4$ (0.25 mM) in a conical flask (250 mL) was stirred and boiled. Then 1% sodium citrate (3 mL) was added quickly. After being stirred and boiled for 10 min, the mixture was stirred and cooled to room temperature. The final product was stored at 4 °C. The GNPs solution was centrifuged and washed with double distilled water, and then concentrated before use. The size and concentration of GNPs were obtained from UV/Vis data (A_{spr}/A_{450}, $A_{450}/\varepsilon_{450}$) according to ref. [31].

GNPs aggregation induced by AVP: Samples in the Figure 1 and Figure S5 were prepared as follows: GNPs (40 μL, 10 nM) and different amounts of AVP were mixed and adjusted to 400 μL with water. The final concentration of GNPs was 1 nM. The mixtures were incubated for 30 min, and measured on a Cary 50 UV–visible spectrophotometer (Varian, USA). Photographs of the samples were taken by using a digital camera. A Hitachi H-800 transmission electron microscope with a maximum accelerating voltage of 200 kV was used to observe the change of GNPs state after addition of L-AVP (Figure S2).

Effect of pH on the oligopeptide-induced GNPs aggregation: Borate buffer (5 mM $Na_2B_4O_7$, pH 7–9), carbonate buffer (10 mM K_2CO_3, pH 10–11) and 10 mM NaOH (pH ~12) were used for sample preparation. GNPs (10 nM, 40 μL) were quickly added to the oligopeptide solutions (L-AVP or LVP) at different pH values. The mixtures were incubated for 10 min and were then analyzed. Final concentrations of oligopeptide and GNPs were 1 μM and 1 nM, respectively.

Chiral recognition of AVP enantiomers by D-enantiomer specific aptamer (V-apt): PBS buffer (40 μL), KCl (20 μL, 500 mM) and a certain amount of V-apt (20 μM) were mixed and adjusted to 260 μL with water. Then, the mixtures were heated to 95 °C for 5 min and annealed, overnight, at 4 °C, in order to allow V-apt to form the most stable secondary structure.

The samples in Figure 3A were prepared as follows: AVP (100 μL, 1 μM) and the annealed V-apt solution were mixed and incubated for 30 min. Then, GNPs (40 μL, 10 nM) were quickly added. These mixtures were incubated for 10 min and were then analyzed. Final concentrations of GNPs and AVP were 1 nM and 250 nM, respectively. The samples in Figure 3B containing V-apt (1 μM), GNPs (1 nM) and various concentrations of AVP were prepared according to the aforementioned procedures.

Colorimetric "INHIBIT" logic operations were implemented with D-AVP and V-apt as inputs according to the procedures mentioned above. The final concentrations of D-AVP and V-apt in samples (Figure 4) were 250 nM and 1 μM, respectively. Final concentration of GNPs was 1 nM.

Acknowledgements

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Keywords: aptamers · enantioselectivity · gold nanoparticles · logic gates · oligopeptides

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