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Determination of free tryptophan in serum with aptamer—Comparison of two aptasensors

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ABSTRACT

Two aptasensors based on graphene oxide (GO) and molecular beacon were designed for the detection of L-tryptophan (L-Trp) using L-Trp aptamer (Trp3a-1). The fluorescein (FAM) labeled Trp3a-1 was absorbed by GO, which resulted in the fluorescence quenching, and exhibiting minimal background fluorescence. Upon the addition of L-Trp, Trp3a-1 was not absorbed quickly. This effect allows for a quantitative assay of L-Trp over the concentration range of 10–500 μM and with a detection limit of 6.84 μM. However, due to the unspecific adsorption of GO, the GO based aptasensor can't be applied in complex matrixes. In respect of molecular beacon based aptasensor, FRET between Trp3a-1 labeled with FAM and CS-Trp3a-1 labeled with BHQ-1(black hole quencher-1) which is partially complementary with the aptamer was used to detect L-Trp. L-Trp binding could induce the disassociation of CS-Trp3a-1, resulted in the enhancement of fluorescence in solution. With an excellent linear relationship in 10–500 μM and a detection limit of 6.97 μM in 25% serum, the aptasensor is expected to be improved for the detection of free L-Trp in other complex samples.

1. Introduction

Selective fluorescent sensing of free amino acids is a very important task in biochemistry and molecular biology, since the concentration of free amino acids is closely related to the metabolism of peptides and proteins in life and various physiological processes. L-Trp is an essential amino acid for many animals and human body. It also serves as a precursor for serotonin and melatonin [1,2] which is related to schizophrenia [3], hallucinations and delusions [4]. The concentration of free tryptophan in blood plasma of the liver cancer patients [5], autistic patients [6], depressive patients [7], patients with Kawasaki disease [8], colorectal cancer patients [9] and patients suffering from chronic uremia [10] is significantly different from the normal control. Besides, free tryptophan could also been taken as the nutritional value of yoghurt [11] and an index of soy sauce adulteration [12].

Several protocols have been proposed to determine L-Trp in biological fluids, such as spectrophotometric measurement after chemical derivation [13] and the HPLC with fluorescence detection method without derivation [14–16]. In addition to the poor sensitivity, the spectrophotometric measurement cannot distinguish the free tryptophan from protein tryptophan either. So, HPLC-based analyses are the commonly used methods. However, the high cost, complicated operation procedure, the multi-step sample pretreatment and the time consuming approach about getting optimal chromatographic conditions prevented the practical application of HPLC-based analyses of L-Trp. Therefore, the development of simple, sensitive and low-cost molecular ligand to L-Trp is of great significance and would be helpful for the determination of L-Trp and the study of L-Trp associated molecular pathway.

Aptamers are artificial DNA or RNA molecules evolved by an *in vitro* selection technique named SELEX (systematic evolution of ligands by exponential enrichment) [17]. As the nucleic acid equivalent of antibodies, not only the binding affinity and specificity of aptamers can rival that of antibodies [18], but also aptamers possess a number of competitive advantages over antibodies for sensing application. Firstly, aptamers can be selected to bind essentially any target of choice by folding into well-defined tertiary structures [19]. Secondly, unlike antibodies, aptamers possess higher stability, low molecular weight, fast tissue penetration, and low immunogenesis. Furthermore, aptamers can be synthesized chemically with low cost and labeled easily with various modifications at any position of choice [20,21]. Finally,

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the predictable and tailored structure of aptamers would facilitate the design of signal transduction. Due to these advantages, various aptasensors have been advanced for transducing aptamer-target interaction into electrochemical, colormetric and fluorescent signals [22]. Among these various optical signal transduction methods, fluorescence has been extensively used due to its high signal to noise ratio. In addition, fluorescence can be easily detected with simple instrument. Moreover, the availability of a great number of fluoreophores and quenchers makes it a popular choice. However, most of aptasensors based on fluorescence focused on only a few well-characterized aptamers such as the aptamers against thrombin, ATP and cocaine. Additionally, a large proportion of aptasensors were not applied in the biological fluids.

In this study, based on GO and aptamer beacon, two fluorescence aptasensors for L-Trp were constructed to detect L-Trp in serum. The advantages and limitations of each aptasensor were also discussed, which would be helpful for practical application of aptamer.

2. Materials and methods

2.1. Chemicals and equipments

All oligonucleotides were synthesized by Shanghai Sangon Biotechnology Co., Ltd (Shanghai, China). L-Trp, L-Tyr, L-Phe were purchased from Aladdin Reagent Co., Ltd (Shanghai, China). D-Trp was obtained from Shanghai Sangon Biotechnology Co., Ltd (Shanghai, China), indole acetic(IAA), indole and L-Gly were obtained from sinopharm Chemical Reagent Co., Ltd. Fetal bovine serum (FBS) were purchased from Rong Ye biotechnology Co., Ltd (LanZhou, China). The Tris–HCl buffer (20 mM, pH 7.4) used in the experiments consisted of 100 mM NaCl, 5 mM $MgCl_2$. All of the chemicals were of at least analytical grade. The GO was synthesized from natural graphite powder based on modified Hummers method [23]. Fluorescent emission spectra were obtained with an F-7000 Fluorescence Spectrophotometer, Hitachi High-Technology Co., Ltd. The emission spectra were recorded in the wavelength of 495–630 nm upon excitation at 485 nm with slit widths of 5 nm. Varian Cary 100 UV/vis spectrophotometer was used to quantify the oligonucleotides

2.2. Determination of L-Trp basd on GO in clean buffer and FBS

In a typical L-Trp detection assay, 50 nM FAM labeled Trp3a-1 (5′–FAM-AGCACGTTGGTTAGGTCAGGTTTGGGTTTCGTGC-3′) dissolved in 700 μl Tris–HCl solution were denatured at 95 °C for 5 min, rapidly cooled on ice and kept at room temperature for 5 min prior to use, then incubated with a series concentration of L-Trp at 10–15 °C for 1 h. Then 14 μl of graphene oxide (0.5 mg/ml) was added, the final concentration of GO is 10 μg/ml. The fluorescence spectra were recorded after 30 s or 1 min in a 1 cm path-length quartz cell. For specificity assay, Trp3a-1 was incubated with 200 μM other amino acids and indole analogs in Tris–HCl buffer at 10–15 °C for 1 h. Then 10 μg/ml GO was added. The fluorescence spectra were recorded after 15 s in a 1 cm path-length quartz cell. The titration experiments in 10% FBS were same with that in clean buffer. In order to exclude the unspecific binding between GO and ssDNA, a DNA library containing 34 nucleotides of random sequence was used as control. The concentration of control sequence is same with Trp3a-1 in every protocol.

2.3. Determination of L-Trp by aptamer beacon in clean buffer and 25% FBS

For titration experiment of L-Trp by aptamer beacon, 0.3 μM Trp3a-1 and 0.7 μM CS-Trp3a-1(5′–CAACGTGCT-3′) in 300 μl Tris–HCl buffer were denatured at 95 °C for 5 min, rapidly cooled on ice for 15 min, then were incubated for 1 hour and different concentration of L-Trp was added. After 2 h incubation at 10–15 °C, the fluorescence spectra were collected in a 1 cm path-length quartz cell. For specificity assay, 200 μM other amino acids and indole analogs were used. Other assay procedures were identical to the L-Trp titration experiment. The L-Trp detection and specificity assay protocol by aptamer beacon in 25%

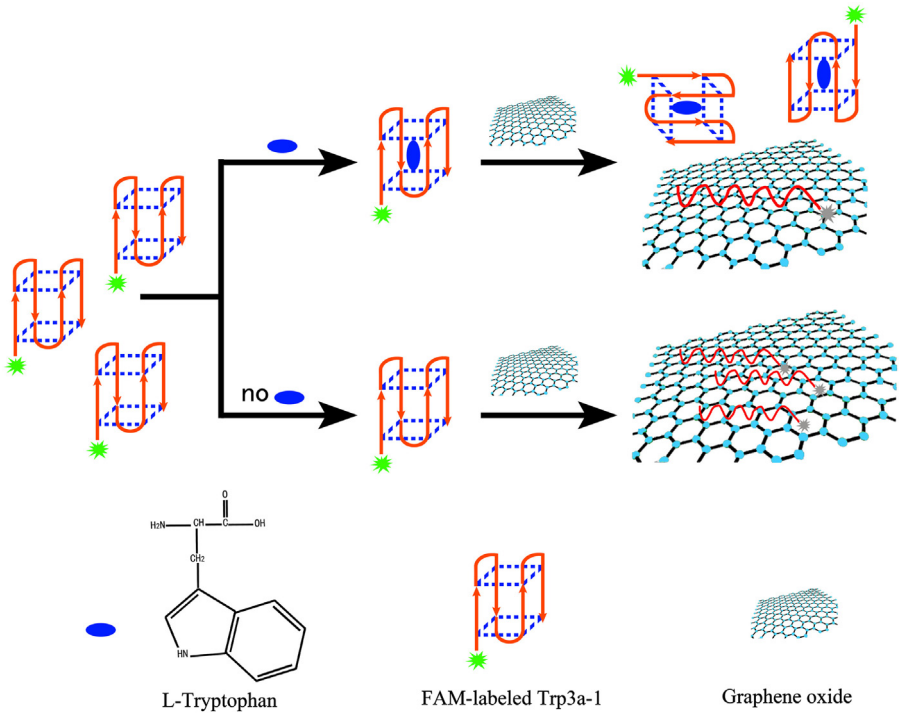

Fig. 1. Illustration of the principle of GO-based aptasensor.