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A novel fluorescent aptasensor based on silica nanoparticles, PicoGreen and exonuclease III as a signal amplification method for ultrasensitive detection of myoglobin

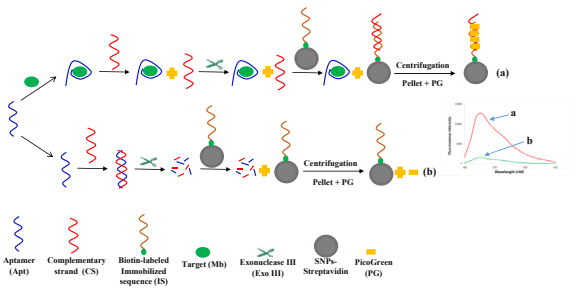
Khalil Abnous [a, b, 1], Noor Mohammad Danesh [c, d, 1], Ahmad Sarreshtehdar Emrani [e], Mohammad Ramezani [c], Seyed Mohammad Taghdisi [f, *]

[a] Academic Center for Education, Culture and Research (ACECR)-Mashhad Branch, Mashhad, Iran
[b] Pharmaceutical Research Center, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran
[c] Nanotechnology Research Center, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran
[d] Research Institute of Sciences and New Technology, Mashhad, Iran
[e] Cardiovascular Research Center, Ghaem Hospital, Mashhad University of Medical Sciences, Mashhad, Iran
[f] Targeted Drug Delivery Research Center, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran

HIGHLIGHTS

- Measurement of myoglobin (Mb) is of great interest for quick diagnosis of acute myocardial infarction (AMI).
- In this study, a novel fluorescent aptasensor was designed for ultrasensitive and selective detection of Mb.
- The fabricated fluorescent aptasensor showed good selectivity toward Mb with a limit of detection as low as 52 pM.
- Moreover, the designed aptasensor was successfully used to detect Mb in serum.

GRAPHICAL ABSTRACT



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ABSTRACT

Measurement of myoglobin (Mb) in human blood serum is of great interest for quick diagnosis of acute myocardial infarction (AMI). In this study, a novel fluorescent aptasensor was designed for ultrasensitive and selective detection of Mb, based on target-induced high fluorescence intensity, complementary strand of aptamer (CS), PicoGreen (PG) dye, exonuclease III (Exo III) and silica nanoparticles coated with streptavidin (SNPs-Streptavidin). The developed aptasensor obtains characteristics of SNPs as enhancers of fluorescence intensity, Exo III as an enzyme which selectively digests the 3'-end of double-stranded DNA (dsDNA), PG as a fluorescent dye which could selectively bind to dsDNA and high selectivity and sensitivity of aptamer (Apt) toward its target. In the absence of Mb, no free CS remains in the environment of SNPs-Streptavidin, resulting in a weak fluorescence emission. In the present of Mb, dsDNA-modified SNPs-Streptavidin complex forms, leading to a very strong fluorescence emission. The developed fluorescent aptasensor exhibited high specificity toward Mb with a limit of detection (LOD) as low as 52 pM. In addition, the designed fluorescent aptasensor was efficiently used to detect Mb in human serum.

* Corresponding author.
E-mail addresses: abnouskh@mums.ac.ir (K. Abnous), taghdisihm@mums.ac.ir (S.M. Taghdisi).
[1] These authors contributed equally to the work.

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1. Introduction

Acute myocardial infarction (AMI) is one of the leading causes of morbidity and mortality worldwide [1,2]. The sensitive detection of cardiac markers is important as a helpful diagnostic tool to access accurately the patients with AMI symptoms [3,4]. One of the best markers of AMI is myoglobin (Mb) [3,5,6]. In AMI the concentration of Mb could elevate up to 600 ng/mL compared to its normal range (6–100 ng/mL) [2,7,8].

Liquid chromatography, mass spectrometry, surface plasmon resonance (SPR), colorimetric methods, luminescence and fluorescent approaches have been utilized for detection of Mb. Most of these analytical methods are laborious and require trained operators and expensive instruments [2–4].

Application of aptamers in analytical methods is growing so fast. Aptamers are artificial single-chain DNA or RNA oligonucleotides, generated by an in vitro process, termed as the systematic evolution of ligands by exponential enrichment (SELEX) [9,10]. They are capable of interacting with a wide range of targets from small organic molecules to proteins and even whole cells with high specificity and affinity [11,12]. Aptamers present promising features over antibodies, such as ease of synthesis and modification, low cost, small size, no or low toxicity and immunogenicity and high thermal stability [12–15]. Owing to these unique characteristics, aptamers have been extensively applied for the construction of a variety of sensing platforms [16–18].

Newly, silica nanoparticles have received remarkable interest for biomedical applications, due to their significant properties, including good stability and biocompatibility, low cost, ease of production and no toxicity. Besides, these nanoparticles could amplify fluorescence intensity by the principle of optical interference [19–21].

Among the different sensing methods, fluorescence has been extensively used for analytical techniques, because of its high sensitivity and ease of recognition [22,23].

So, in this study, a novel upconversion fluorescent aptasensor was designed for detection of Mb, based on complementary strand of aptamer (CS), silica nanoparticles coated with streptavidin (SNPs-Streptavidin), PicoGreen (PG) dye and exonuclease III (Exo III). In this work, a ssDNA aptamer that binds to Mb with high specificity and sensitivity [2,4], was used as molecular recognition probe.

2. Materials and methods

2.1. Materials

All the sequences were ordered from Bioneer (Table 1, South Korea). C reactive protein (CRP), human immunoglobulin G (IgG), plasma from human, human serum albumin (HSA) and bovine serum albumin (BSA) were purchased from Sigma–Aldrich (USA). Human myoglobin (Mb) was obtained from ProsPec (Germany). Silica nanoparticles coated with streptavidin (SNPs-Streptavidin) were provided by Micromod (100 nm, Germany). PicoGreen (PG, 200x) was purchased from Invitrogen (USA). Exonuclease III was provided by New England Biolabs (UK).

2.2. Preparation of immobilized strand (IS)-modified SNPs-Streptavidin

4 μL SNPs-Streptavidin (1 mg/mL final concentration) was added to 5 μL IS (8 μM, labeled with biotin) in 20 mM Tris–HCl (pH 7.4, final volume 100 μL) and incubated for 1 h at room temperature.

2.3. Effect of the concentration of Exo III on the fluorescence intensity of the aptasensor

10 μL Apt (0.5 μM) was added to 10 μL CS (0.5 μM) in the phosphate buffer saline (10 mM PBS, pH 7.4) to give a final volume of 100 μL and incubated for 1 h at room temperature. The solutions were mixed with increasing amounts of Exo III (0–25 U) for 30 min at 37 °C. Next, 1.5 μL 10x PG was added to each solution. After incubation for 5 min at room temperature, fluorescence spectra, $\lambda_{Ex} = 480$ nm, were measured on a Synergy H4 microplate reader (BioTek, USA).

2.4. Optimization of incubation time of Exo III

10 μL Apt (0.5 μM) was mixed with 10 μL CS (0.5 μM) in 10 mM PBS (pH 7.4). After incubation for 1 h, 12 U Exo III was added to each solution and incubated at 37 °C from 0 to 1 h, followed by addition of 1.5 μL 10x PG. After incubation for 5 min, the fluorescence spectra were measured.

2.5. Effect of the concentration of IS-modified SNPs-Streptavidin on the fluorescence intensity of the designed assay

30 μL Mb (45 nM) was added to 10 μL Apt (0.5 μM) in 10 mM PBS (pH 7.4) to give a final volume of 90 μL and incubated for 30 min at room temperature. Then, 10 μL CS (0.5 μM) was added to the solutions and incubated for 1 h, followed by addition of 12 U Exo III to the solutions for 30 min at 37 °C. Next, the solutions were treated with increasing concentrations of IS-modified SNPs-Streptavidin (0–100 μg/mL final concentration) for 1 h. After that, the samples were centrifuged at 11000g for 10 min and the supernatants containing free DNA were discarded. The CS-IS-modified SNPs-Streptavidin complex was dispersed in the Tris–HCl buffer (100 μL, pH 7.4). Finally, 1.5 μL 10x PG was added to each sample. After incubation for 5 min the fluorescence spectra were recorded.

2.6. Effect of the concentration of PG on the fluorescence intensity

Increasing concentrations of PG (0–2.2 μL 10x) were added to the wells containing CS-IS-modified SNPs-Streptavidin complex in the Tris–HCl buffer (100 μL, pH 7.4) and the fluorescence spectra were measured after 5 min.

2.7. Optimization of the incubation time of PG

1.5 μL 10x PG was mixed with CS-IS-modified SNPs-Streptavidin complex in the Tris–HCl buffer (100 μL, pH 7.4). The mixtures were incubated at room temperature from 0 to 7 min. Next, fluorescence

Table 1
Oligonucleotide sequences used in this study.

Oligonucleotide	Sequence (from 5′ to 3′)
Aptamer (Apt)	CCCTCCTTTCCTTCGACGTAGATCTGCTGCGTTGTTCCGA [2,4]
Complementary strand (CS)	TCGGAACAACGCAGCAGATCTACGTCGAAGGAAAGGAGGG
Immobilized strand (IS)	CCTTTCCTTCGACGTAGATCTGCTGCGTTGTTCCGA-Biotin