

## Prodigiosin-Conjugated Aptamer for Attachment to the Surface of Brain Cancer Cells Mediated by Glutamate Receptor



Mohammadgha Ayatollahi<sup>a,b,\*\*</sup>, Gelayol Ayatollahi<sup>c</sup>, Mohsen Rashidi<sup>d</sup>,  
Seyedhossein Hekmatimoghaddam<sup>e</sup>, Mohammadhassan Mosshafi<sup>a</sup>, Ali Jebali<sup>e,\*</sup>, Maryam Iman<sup>f,\*</sup>,  
Hossein Shahdadi Sardo<sup>g</sup>

<sup>a</sup> *Pharmaceutics Research Center, Institute of Neuropharmacology, Kerman University of Medical Sciences, Kerman, Iran*

<sup>b</sup> *Department of Pharmacology and Toxicology, Schools of Pharmacy and Medicine, Yazd University of Medical Sciences, Yazd, Iran*

<sup>c</sup> *International Campus, Shahid Sadoughi University of Medical Sciences, Yazd, Iran*

<sup>d</sup> *Department of Physiology and Pharmacology, Faculty of Medicine, Mazandaran University of Medical Science, Sari, Iran*

<sup>e</sup> *Department of Laboratory Sciences, School of Paramedicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran*

<sup>f</sup> *Chemical Injuries Research Center, System Biology and Poisonings Institute, Baqiyatallah University of Medical Sciences, Tehran, Iran*

<sup>g</sup> *Targeted Drug Delivery Research Center, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran*

### ARTICLE INFO

#### Keywords:

Prodigiosin  
Aptamer  
Brain cancer cells  
Glutamate receptor

### ABSTRACT

The aim of this study was to introduce a prodigiosin-conjugated aptamer to attach to surface of brain cancer cells. Crystal structure of glutamate receptor and 106 nucleotide aptamers were separately interacted in NVT ensemble for duration of 50,000 ps at 310 K by Ascalaph designer software. Finally, delta intermolecular energy ( $\Delta$ INME) was separately obtained for each aptamer. For confirmation of simulation data, both brain cancer cells and normal brain cells were cultured, and separately incubated with the best selected aptamers. Finally, the adsorption percent of each aptamer was measured for both kinds of cells. Prodigiosin-conjugated aptamer 8, 10, 11, 23, and 69 could attach to all epitopes with high affinity and low  $\Delta$ INME. In vitro test showed that all aptamers could bind to brain cancer cells more than normal cells. Moreover, aptamer 10 had the highest adsorption to brain cancer cells and the least adsorption to normal brain cells.

### 1. Introduction

It is necessary to improve the method of delivering drugs to the target in brain cancer due to pharmacotherapy failure and inadequate blood drug levels that cause life threatening. Current methods of Pharmacotherapy do not lead to adequate efficacy and targeted drug delivery (TDD). It is accepted that TDD appears more effective, especially in cancers that local effect of drugs is necessary to remove confounded factors from normal cells. Aptamers (apts) as novel drug delivery tools (NDDT) can access this purpose by conjugating them with drug and on the other hand by binding to the targeted molecules and deliver the drug to their target with excellent efficacy. Aptamers (the word "aptamer" was derived from the Latin aptus meaning to fit and Greek meros meaning region) are single stranded DNA or RNA oligonucleotide with a 10 nucleotide length that can fold and bind to their various targets with high affinity and specificity [1–3]. It is reported

that aptamers have three-dimensional structure, and can interact with their targets similar to antibodies [1]. Oligonucleotide properties of aptamers cause to specific biological and chemical specifications for them and it has been shown that aptamer technology is a novel approach for different clinical applications, such as in vivo imaging, targeted therapy, and in vitro diagnosis [4]. It has been indicated that aptamers with their three dimensional structure have usually 20–60 nucleotide bases, and can fold due to intramolecular hydrogen forces [4–6]. Production of DNA aptamers is more inexpensive than RNA aptamers [7,8]. Aptamers can function as the sensors and also called chemical antibodies [2,3]. Tertiary structure of aptamers in addition to their skeletal structure indicates their characteristics [2,3]. Aptamers are produced by SELEX (Systematic Evaluation of Ligands by Exponential Enrichment) from combinatorial libraries [1]. Design of various aptamers is considered to deliver drug to the target and access to drug level in blood at steady state [9]. Review literatures showed

*Abbreviations:* TDD, Targeted drug delivery; apts, Aptamers; NDDT, Novel drug delivery tool; Selex, Systematic Evaluation of Ligands by Exponential Enrichment; G, Guanine; A, Adenine;  $\Delta$ INME, Delta intermolecular energy; ps, Picoseconds; CS, Computer simulation

\* Corresponding authors.

\*\* Corresponding author at: Pharmaceutics Research Center, Institute of Neuropharmacology, Kerman University of Medical Sciences, Kerman, Iran.

E-mail addresses: [Ayatollahi@gmail.com](mailto:Ayatollahi@gmail.com) (M. Ayatollahi), [alijebal2011@gmail.com](mailto:alijebal2011@gmail.com) (A. Jebali), [Iman2008@gmail.com](mailto:Iman2008@gmail.com) (M. Iman).

<https://doi.org/10.1016/j.colcom.2018.03.006>

Received 5 February 2018; Received in revised form 18 March 2018; Accepted 25 March 2018

2215-0382/© 2018 Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

that natural pigments are produced in insects, plants, animals and bacteria that have different properties and also environmental compatibility. Natural pigments are nontoxic, non-carcinogenic and are also safe. Some of them are flavenoids and anthraquinones, which produced in plants and animals [10]. One of the natural pigments is prodigiosin that is produced in a bacteria called *Serratia marcescens*. Prodigiosin is in vitro cytotoxic, anti-cancer and anti-malarial (cytotoxicity can be measured with MTT assay) [10]. Prodigiosin is toxic for primary cancer cells and useful in treatment of the patients with B cell chronic lymphoblastic leukemia [11], especially prodigiosin 25-C as a derivative of prodigiosin that has also immunosuppressive activity [12]. Aptamers can also be used in cardiovascular diseases [13], central nervous system diseases [14] and the other diseases. Aptamers are considered as gold standard drug delivery tools for their targets.

Crystal structure of glutamate receptor, GluA1, was obtained from protein data bank (DOI: <https://doi.org/10.2210/pdb3saj/pdb>). Then, the protein was segmented to 5 epitopes in Hyperchem software (Fig. 1). Next, the geometry of each epitope was separately optimized by gradient-conjugate algorithm in order to decrease potential energy. Finally, each epitope was saved as pdb file. Hyperchem 8.0.10 was sourced from MakoLab Company, USA. For building of aptamer library, 106 nucleotide aptamers were prepared with 2 kinds of nucleotides named guanine(G) and adenine(A) in Hyperchem software. In the final step, the 5' end of all aptamers was attached to Prodigiosin as a bacterial cytotoxic pigment. The geometry of each aptamer-conjugated Prodigiosin (Fig. 2) was separately optimized the same as epitopes by gradient-conjugate algorithm. Finally, all of them were saved as pdb file, and were separately interacted with each epitope by dynamic CS in NVT ensemble for duration of 50,000 ps at 310 K by Ascalaph designer 1.8.94 which provided from Agile Molecule Company, Sweden. At the end of simulation, delta intermolecular energy ( $\Delta$ INME) was separately obtained for each aptamer.

For confirmation of simulation data, the best selected aptamers were evaluated in vitro. Two types of cell lines abovementioned including brain cancer cells (U-87 MG) and normal brain cells (SK-N-MC) were cultured in DMDM enriched with FBS in concentration of 10%. Next, the cell suspension (10,000 cells/mL) was separately prepared, and incubated with the best selected aptamers (100 picomolar) at 37°C for 24 h. At the end, cells were centrifuged at 5000 rpm, and the optical density of supernatant was read at 260 nm. Finally, the adsorption percent of each aptamer was measured for both kinds of cells.

$\Delta$ INME for various epitopes of glutamate receptor after interaction with 106 prodigiosin-conjugated aptamers was shown in Supporting

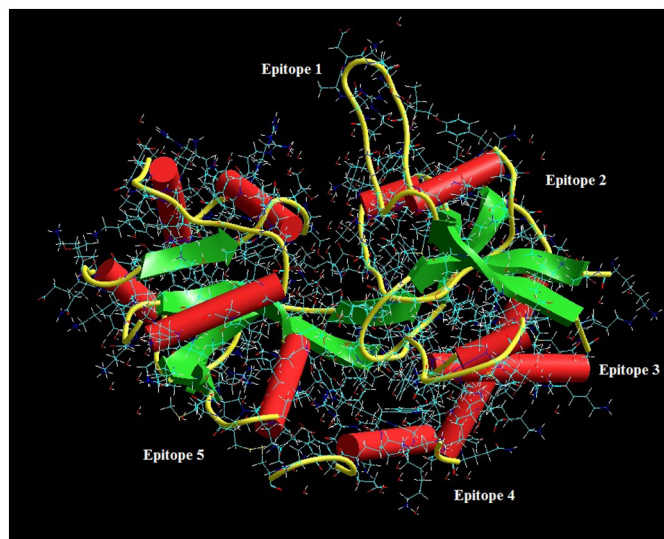


Fig. 1. Five epitopes of glutamate receptor, illustrated by Hyperchem software.

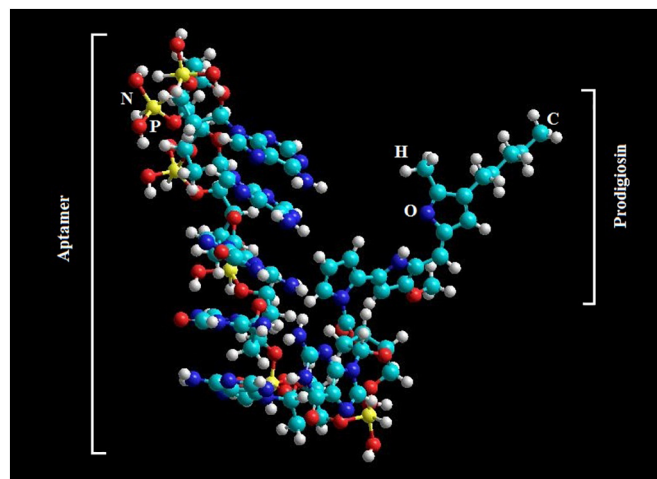


Fig. 2. Schematic diagram of conjugated aptamer.

Table 1

The best aptamer which could attach to all epitopes of glutamate receptor with least  $\Delta$ INME.

Epitope number	Aptamer number	Intermolecular energy ( $\Delta$ INME)
Epitope1	10	-5.146
Epitope2	23	-3.974
Epitope3	11	-12.781
Epitope4	8	-8.418
Epitope5	69	-8.024

information. As demonstrated, each aptamer-conjugated prodigiosin can interact with specific epitope. Table 1 shows the best aptamer which could attach to all epitopes of glutamate receptor with least  $\Delta$ INME. As seen, aptamer-conjugated prodigiosin 8, 10, 11, 23, and 69 can attach to all epitopes with high affinity and low  $\Delta$ INME. The sequence of aptamer 8, 10, 11, 23, and 69 was 3'-GGGGGGGAGGGGGG GGGGGG-5', 3'-GGGGGGGGGAGGGGGGGGGG-5', 3'-GGGGGGGGGGA GGGGGGGGG-5', 3'-GGAAGGGGGGGGGGGGGGG-5', and 3'-GGGGG GGGGGAAAAGGGGG-5', respectively.

Fig. 3 shows the adsorption percent of each aptamer for both types of cells. As seen, all aptamers could bind to brain cancer cells more than normal cells. Moreover, aptamer 10 had the highest adsorption to brain cancer cells and the least adsorption to normal brain cells.

Review literatures showed that delivering of drug to the target especially in cancers, which are life threatening is very important and delivery tools are necessary to access this object [8,15]. Review literatures have also directed the aptamers as novel tools which appear useful in Prostate and breast cancers [8]. Molecular dynamics simulation is another beneficial tool to find new drug and aptamer, which previously confirmed by other researchers [3,7]. Design of a physical, chemical and biological TDDTs causes the blood drug levels at steady state. TDDTs are mechanical pumps, polymers, micro particulate and patches, which dermally used [6,9,16]. Aptamers have been shown that to be beneficial tools in TDD for prostate cancer and breast cancer with high affinity, specificity and selectivity. Aptamers can bind to various targets such as small molecules, proteins, viruses and cells [17]. They can bind to specific target molecules and even can bind to ribosomes and other cellular proteins and then split in the presence of target molecules. Aptamers have clinical and industrial uses. They also termed chemical antibodies and preferred to them. A report directed successfully to use 10 nucleotides aptamers for applications [18] but to access more accurate results, the decision was made to arrange 20 nucleotide aptamers (totally 106 aptamers) by CS in above-mentioned research. For this purpose the protein was searched in computer termed glutamate receptor and was segmented to 5 segments that each one named

Download English Version:

<https://daneshyari.com/en/article/6977046>

Download Persian Version:

<https://daneshyari.com/article/6977046>

[Daneshyari.com](https://daneshyari.com)