Biomaterials 100 (2016) 143–151

Contents lists available at ScienceDirect

Biomaterials

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

Hybridization-based aptamer labeling using complementary oligonucleotide platform for PET and optical imaging

Jun Young Park [a, e], Tae Sup Lee [b], In Ho Song [b], Ye Lim Cho [a], Ju Ri Chae [a], Mijin Yun [a], Hyungu Kang [c], Jung Hwan Lee [d], Jong Hoon Lim [d], Won Gil Cho [e, **], Won Jun Kang [a, *]

[a] Department of Nuclear Medicine, Severance Hospital, Yonsei University College of Medicine, 50-1 Yonsei-ro, Seodaemun-gu, Seoul 03722, Republic of Korea
[b] Molecular Imaging Research Center, Research Institute of Radiological and Medical Sciences, Korea Institute of Radiological and Medical Sciences, 75 Nowon-ro, Nowon-gu, Seoul 01812, Republic of Korea
[c] R&D Strategic Planning, Bundang CHA Medical Center, 59 Yatap-ro, Bundang-gu, Seongnam-si, Gyeonggi-do 13496, Republic of Korea
[d] APGEN Inc, 220, Yeongsin-ro, Yeongdeungpo-gu, Seoul 07228, Republic of Korea
[e] Department of Anatomy, Yonsei University Wonju College of Medicine, 20 Ilsan-ro, Wonju 26426, Republic of Korea

ARTICLE INFO

Article history:
Received 1 January 2016
Received in revised form
12 May 2016
Accepted 17 May 2016
Available online 24 May 2016

Keywords:
Aptamer
Molecular imaging
Hybridization
Oligonucleotide
Radioisotope
PET

ABSTRACT

Aptamers are promising next-generation ligands used in molecular imaging and theragnosis. Aptamers are synthetic nucleic acids that can be held together with complementary sequences by base-pair hybridization. In this study, the complementary oligonucleotide (cODN) hybridization-based aptamer conjugation platform was developed to use aptamers as the molecular imaging agent. The cODN was pre-labeled with fluorescent dye or radioisotope and hybridized with a matched sequence containing aptamers in aqueous conditions. The cODN platform-hybridized aptamers exhibited good serum stability and specific binding affinity towards target cancer cells both *in vitro* and *in vivo*. These results suggest that the newly designed aptamer conjugation platform offers great potential for the versatile application of aptamers as molecular imaging agents.

1. Introduction

Molecular imaging uses target-specific probes in order to visualize a particular molecular target or biochemical process. Molecular imaging involves targeting ligands that can bind with high affinity to a specific cellular biomarker. Antibodies and peptides are established ligands to diverse biologic targets [1]. Aptamers are relatively new targeting ligands that offer a number of advantages over other targeting ligands in molecular imaging and theragnosis, and therefore have been proposed as promising next-generation ligands.

Oligonucleotides (ODNs) are single-stranded DNA or RNA molecules. Aptamers are specially designed oligonucleotides with a length of 20–100 bases. Aptamers are able to fold into three-dimensional (3D) structures that bind to specific target molecules with high affinity and specificity. Since the development of systemic evolution of ligands by exponential enrichment (SELEX), a number of aptamers have been developed to recognize proteins, phospholipids, sugars, other nucleic acids, and even specific cells [2]. Because of their ability to bind specifically to target molecules, aptamers are frequently compared to antibodies. However, aptamers offer several advantages over antibodies [3]. First, aptamers do not induce an immune reaction. Second, aptamers are chemically produced and their production is more reliable and less expensive. Third, aptamers are more stable in terms of pH and temperature. Lastly, aptamers can be produced for targets to which antibodies are difficult to generate.

Aptamers have been used in various clinical applications including *in vitro* assays, molecular imaging, biomarker discovery, and therapeutics. Therapeutic aptamers hold great potential but their use in systemic therapy has thus far been limited due to their rapid systemic clearance. However, aptamers have also been studied in molecular imaging and have shown great promise as

* Corresponding author.
** Corresponding author.
E-mail addresses: wch01@yonsei.ac.kr (W.G. Cho), mdkwj@yuhs.ac (W.J. Kang).

http://dx.doi.org/10.1016/j.biomaterials.2016.05.035
0142-9612/

imaging agents. Direct conjugation of optical probes to aptamers is widely used in cellular-level and small-animal-level studies [4]. Aptamers can be linked to magnetic resonance imaging (MRI), computed tomography (CT), and ultrasound imaging probes [5–7]. However, nuclear imaging, including positron emission tomography (PET) and single photon emission computed tomography, offers the highest potential for clinical translation. The technetium-99m (Tc-99m) labeled aptamer targeting the extracellular matrix protein tenascin-C (TTA1) indicated excellent potential for nuclear imaging probes [8]. Another study illustrated the use of the Tc-99m labeled MUC-1 aptamer for cancer imaging by targeting the MUC-1 protein, which is an established biomarker for several cancers [9]. Two recent studies showed fluoride-18 *in vivo* cancer PET imaging using aptamers for tenascin-C and protein tyrosine kinase 7 [10,11]. However, the radiolabeling of each aptamer requires multiple steps and labeling conditions may vary according to the heterogeneity of the aptamers.

Hybridization is the formation of double-stranded nucleic acids between two complementary DNA or RNA strands [12]. Oligonucleotides can be designed to recognize the cellular DNA or RNA through Watson-Crick base pairing [13]. Research on the application of aptamers as an imaging agent remains in its early stage. The main purpose of this study was to develop the complementary oligonucleotide (cODN) as the platform for the conjugation of imaging probes to the aptamers via hybridization properties. To demonstrate the applicability of the cODN platform, the targeting ability of the hybridized aptamer was evaluated *in vitro* and *in vivo*.

2. Material and methods

2.1. Reagents

All reagents were commercially available and used as received. 11-Azido-3,6,9-trioxa-1-undecanol mesylate was purchased from FutureChem Co. (Seoul, South Korea). Hexynyl-functionalized complementary oligonucleotide and AS1411 aptamer were purchased from ST Pharm Co. (Seoul, South Korea). 5′-Cy5 labeled complementary oligonucleotide (5′-Cy5-cODN) and cytosine-rich oligonucleotide (CRO) were obtained from Aptamer Sciences Inc. (Seoul, South Korea). ^{18}O-enriched water (>98%) was purchased from Rotem (Beer Sheva, Israel). QMA (Sep-Pak Light Accell Plus®) and C18 (Sep-Pak Plus®) cartridges were purchased from Waters (Milford, MA, USA).

2.2. Preparation of ^{18}F-labeled cODN

No-carrier-added ^{18}F-fluoride ion was produced via the [^{18}O(p,n)^{18}F] nuclear reaction on a PETtrace 16.5 MeV cyclotron (GE Healthcare, USA). ^{18}F-fluoride ion was dried azeotropically with subsequent addition of acetonitrile. The precursor mesylate (7 mg, 23.5 μmol) was added to the ^{18}F-KF–K222 complex and the mixture was stirred at 100 °C for 10 min. After cooling, isolation of ^{18}F-fluoro-PEG-azide (^{18}F-FPA) was performed using high-performance liquid chromatography (HPLC). The product was trapped on C18 cartridges and eluted with ethanol. ^{18}F-FPA was then analyzed by HPLC. A mixture of 5′-hexynyl modified oligonucleotide (200 μg, 41.4 nmol), 1 M *N,N*-diisopropylethylamine in acetonitrile (10 μL) and 100 mM copper (I) iodide in acetonitrile (20 μL) was reacted with ^{18}F-FPA (750–1100 MBq) at 70 °C for 20 min. The resulting mixture was purified by HPLC. ^{18}F-5′-cODN was trapped on C18 cartridge and recovered with ethanol elution. Analysis of ^{18}F-5′-cODN was confirmed using HPLC. The HPLC conditions were described in Supporting Information.

2.3. Preparation of hybridized aptamer

The sequence of the complementary oligonucleotide platform was 3′-GTCGGTGTGGTGGTC-5′. For the hybridization, a fully matched sequence (5′-CAGCCACACCACCAG-3′) was added to the 3′ terminus of AS1411 and CRO. The AS1411 sequence was 5′-GGTGGTGGTGGTTGTGGTGGT GGTGGTTTCAGCCACACCACCAG-3′ and the CRO sequence was 5′-CCTCCTCCTCCTTCTC CTCCTCCT CCTTTCAGCCACACCACCAG-3′. The hybridization of aptamers using the Cy5-or ^{18}F-labeled cODN platform was performed in annealing buffer (10 mM Tris pH 7.5, 1 mM EDTA, 50 mM NaCl, 10 mM $MgCl_2$). The mixture was incubated at 95 °C for 5 min, then slowly cooled to room temperature. Hybridization efficiency of 5′-Cy5-cODN hybridized aptamers (Cy5-hyAS1411 or Cy5-hyCRO) was analyzed by electrophoresis and that of ^{18}F-5′-cODN hybridized aptamers (^{18}F-hyAS1411 or ^{18}F-hyCRO) was determined using HPLC.

2.4. Lipophilicity

The ^{18}F-5′-cODN hybridized aptamers were added to the solution containing phosphate-buffered saline (PBS, 1 mL) and octanol (1 mL). After vigorous vortexing for 5 min, the two layers were separated by centrifugation (3000 rpm, 5 min). Aliquots (100 μL) of octanol and PBS buffer were taken from each layer and radioactivity was measured using a Wizard2® 2480 Automatic gamma counter (PerkinElmer, Canada). The log *P* values were calculated as a ratio of counts in the octanol fraction to counts in the PBS fraction.

2.5. Electrophoresis

Electrophoresis was performed on run at 135 V for 20 min using the Mupid-One agarose gel electrophoresis system (Advance, Tokyo, Japan). Autoradiography and fluorescence images were acquired on a Typhoon FLA 7000 image analyzer (GE Healthcare) and analyzed using Multi Gauge software v3.0 (Fujifilm, Tokyo, Japan).

2.6. In vitro stability

The *in vitro* stability of ^{18}F-5′-cODN and ^{18}F-hyAS1411 was analyzed by measuring the radiochemical purity using HPLC. An aliquot (10–20 μL) of ^{18}F-5′-cODN or ^{18}F-hyAS1411 was diluted with PBS (pH 7.4) and incubated at 25 °C and 37 °C for 4 h. For the serum stability test, Cy5-or ^{18}F-cODN hybridized aptamers in annealing buffer were mixed with mice serum at the final concentration of 50% and incubated at 37 °C for 2 h. At selected time points (10, 30, 60 and 120 min), 5 μL of aliquots were taken and then analyzed by electrophoresis on the MetaPhor® 3% agarose gel.

2.7. Cell culture

C6 cells (No. CCL-107) and CHO cells (No. CCL-61) were obtained from American Type Culture Collection (Manassas, VA, USA). All cell culture media, supplements, and serum products were purchased from Invitrogen (Carlsbad, CA, USA). C6 cells and CHO cells were cultured in Dulbecco's Modified Eagle Medium and RPMI-1640 Medium, respectively, supplemented with 10% fetal bovine serum and 0.1 mg/mL gentamycin, and maintained in a humidified atmosphere of 5% CO_2 at 37 °C.

2.8. Animal model

Athymic female nude mice were purchased from Orient Bio Inc. (Gyeonggido, South Korea). For the tumor model, 1×10^6 C6 cells were implanted subcutaneously in the right shoulders of 7-week-old mice. Tumors were allowed to grow to a volume of