Journal of Pharmaceutical and Biomedical Analysis 107 (2015) 244–250

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

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

Aptamer-conjugated magnetic nanoparticles for extraction of adenosine from urine followed by electrospray ion mobility spectrometry

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ARTICLE INFO

Article history:
Received 11 November 2014
Received in revised form
28 December 2014
Accepted 7 January 2015
Available online 15 January 2015

Keywords:
Adenosine
Aptamer
Magnetic nanoparticles
Ion mobility spectrometry
Lung cancer

ABSTRACT

Magnetic nanoparticles (MNPs) conjugated with aptamer was developed for the selective extraction of adenosine in urine samples followed by electrospray ionization–ion mobility spectrometry (ESI–IMS). The ion mobility spectrum of adenosine showed two peaks at low concentrations and two more peaks related to dimer of adenosine at high concentrations. However, the ion mobility spectrum of eluent at low concentration showed only the peaks related to dimer of adenosine. In other words, aptamer captured two adenosine molecules between the top G-quartet and the two short stems, where they bonded to each other. The mass spectrum of the eluent also validated the presence of dimer (m/z 535.95). The effect of extraction parameters on extraction efficiency including sorbent amount, elution conditions (solvent type and volume) and adsorption conditions were investigated. Under the optimized conditions, the linear dynamic range was found to be 0.05–5.00 $\mu g\,mL^{-1}$ with detection limit of 0.02 $\mu g\,mL^{-1}$. The extraction efficiency was 94% and the relative standard deviation was 4% for three replicate measurements of adenosine at 0.25 $\mu g\,mL^{-1}$ in urine samples. As a practical application, the method was applied for the determination of adenosine in urine samples of patients with lung cancer, and the obtained results were in good agreement with those obtained by HPLC–UV method. Therefore, the proposed method is an alternative clinical analysis.

1. Introduction

Sample pretreatment is the most important step of removing matrix and clean-up prior to the instrumental analysis. During the last two decades, different methods based on solid-phase extraction (SPE) have been reported for the extraction of analytes with trace amounts of biological fluids. The main limitation associated with ordinary stationary phase SPE columns is low selectivity. Therefore, the selective SPE methods such as molecular imprinted polymers (MIPs) and immunoaffinity have been developed [1]. Immunoaffinity is a powerful technique based on high affinity between antigen–antibody interactions. The use of antibodies in the analysis is associated with specificity, however there are some limitations mainly attributed to the nature of these protein receptors. Recently, antibodies are replaced by aptamers on solid support with more advantages [2].

Aptamers are single-stranded DNA or RNA (ssDNA or ssRNA) molecules that can bind to pre-selected targets including proteins, small organic compounds and nucleic acids with high affinity and specificity. Aptamers specifically bind to the target molecule due to their unique three-dimensional folded structures [3]. Several techniques have been suggested to use aptamers as affinity ligands on the stationary phase including on-line aptamer affinity chromatography [4], off-line preconcentration (extraction, purification) using aptamers on solid phase support (e.g., SPE column, magnetic beads and nanoparticles (NPs)) [5–7] and microfluidic formats [8]. The use of aptamer conjugated with magnetic modified adsorbents, called magnetic solid-phase extraction (MSPE), has been a topic of great interest to researchers [9]. By using nanosized adsorbents, the extraction capacity and efficiency increases, because of the significantly higher surface area-to-volume ratio. Moreover, Fe_3O_4 NPs properties are feasible for sample extraction without any sample centrifugation or filtration step. Another advantage of MNPs is the capability of surface modification with various functional groups leading to selective sample extraction [9,10]. In this work, we used aldehyde modified Fe_3O_4 NPs conjugated with aptamer as MSPE for the extraction of adenosine in human urine.

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http://dx.doi.org/10.1016/j.jpba.2015.01.021
0731-7085/

Adenosine is an endogenous purine nucleoside, which has an important role in many physiological processes. A number of studies have shown solid tumors experience severe necrosis due to the rapid growth of cancer cells and the fast rate of cell metabolism. As a result of tissue hypoxia, adenosine concentration in the extracellular fluid of solid tumors increases. Also, this enhancement of adenosine concentration lead to disorder in anti-tumor immune response function and consequently tumor cells stay alive. Therefore, adenosine can act as a tumor-marker and its concentration in the urine can be determined to monitor the progress of the diseases [11]. Huizenga et al. indicated that the structure of the complex between ssDNA-aptamer and adenosine in solution consists of two small Watson–Crick helices and two G-quartets. Four guanosines assembled via hydrogen bonds and two G-quartets stack on each other to form a stable structure in the presence of metal cations. Aptamer catches two adenosine molecules between the top G-quartet and the two short stems form a pocket [12].

Several analytical methods have been reported for analysis of adenosine using high-performance liquid chromatography (HPLC) coupled with different detection systems such as UV absorbance [13], fluorescence [14], mass spectrometry (MS) [15] and tandem mass spectrometry (MS–MS) [16]. Also, different approaches based on aptasensors have been developed using fluorescence [17], chemiluminescence [18], electrochemiluminescence [19] and electrochemical [20,21] methods.

Ion mobility spectrometry with electrospray ionization source (ESI–IMS) is an analytical technique for qualitative and quantitative analysis of many compounds based on mobility of gas phase ions in an electric field [22]. The IMS has been utilized in biomolecule analysis and the invaluable reviews have been reported [23,24]. Despite prompt response and high sensitivity, IMS has a limitation for the analysis of complex samples, because of its low selectivity [5]. Aptamer, as an alternative to antibody has this potential to be considered as a specific sorbent to compensate the weakness of IMS. Our group has used an aptamer-based sorbent for extraction of tetracycline in biological fluids [5]. In this work, the performance of the combination of aptamer conjugated with MNPs followed by ESI–IMS is evaluated for the analysis of adenosine in urine samples of patients with lung cancer.

2. Experimental

2.1. Reagents

Adenosine (99%) was obtained from Sigma–Aldrich (China). Methanol, acetic acid (100%), (3-aminopropyl) triethoxysilane (APTES, 98%), glutaraldehyde (GA, 25% solution in water), ammonium hydroxide (25%, w/w), Tris–HCl were purchased from Merck (Darmstadt, Germany). Acetonitrile was provided by Sigma–Aldrich (Seelze, Germany) and ethanol (99.6–99.9%) was obtained from Bidestan Co. (Qazvin, Iran). Ferric chloride hexahydrate ($FeCl_3{\cdot}6H_2O$), ferrous chloride tetrahydrate ($FeCl_2{\cdot}4H_2O$), magnesium chloride and sodium chloride were analytical grade and purchased from Riedel-dehaën (Seelze, Germany). The 5′-amino-modified DNA oligonucleotides (5′-AGAGAACCTGGGGGAGTATTGCGGAGGAAGGT-3′ for the aptamer and 5′-TGAAGCAGACTGAGGTACGTGTGAGGAGGGAG-3′ for the scrambled oligonucleotide) with a C6 spacer arm were synthesized in Oligo Macrogen (Seoul, South Korea). The sequence of the aptamer of adenosine used in this work has been reported by Yan et al. [18]. Standard stock solution of adenosine (1000 μg mL^{-1}) was prepared in MeOH–H_2O (50:50, v/v). Working solutions of adenosine were prepared daily by diluting stock solution with methanol and 0.1 M acetic acid (99:1, v/v). Deionized water was prepared by OES (Overseas Equipment & Services) water purification system (OK, USA).

2.2. Apparatus

Fourier transform infrared (FT-IR) spectra were recorded with a Jasco FT-IR, model 6300 (Tokyo, Japan). Magnetite properties of the prepared nanoparticles were determined using a vibrating sample magnetometer (VSM) (Kashan, Iran). Graphs of field emission-scanning electron microscope (FE-SEM) were obtained by Hitachi S4160 (Tokyo, Japan) and X-ray diffraction (XRD) patterns were recorded with Philips XRD (Model XPERT). Liquid chromatography–mass spectrum (LC–MS) was recorded with Shimadzu LC/MS, model 2010 EV (Kyoto, Japan).

2.3. Electrospray ionization–ion mobility spectrometry

The electrospray ionization–ion mobility spectrometer (ESI–IMS) was designed and constructed in our group at Isfahan University of Technology [24]. In summary, it consists of the IMS cell, the electrospray needle, two high voltage power supplies, a pulse generator, an analog to digital converter and a computer that briefly would be explained. The IMS cell length was divided into two sections: desolvation region (6 cm) and drift region (13 cm). These two parts separated by a Bradbury–Nielsen ion gate, made of two series of parallel wires biased to a potential for blocking ion passage to the drift tube. A six-port injection valve (Rheodyne, Reno, NV, USA) with a 20-μL loop, was used for the sample introduction. The electrospray needle (P/N 7768-01, Hamilton, Reno, NV, USA) with a Teflon tube, was fixed at one end of the cell. The grid potential was removed for a short period of time by a pulse generator, worked at frequency of 25 Hz. Nitrogen gas, passed through a 13X molecular sieve (Fluka, Buchs, Switzerland), was used as the drift and desolvation gases. A high-speed A/D module (12-bit dynamic range) was used for recording spectra, and each IMS spectrum was the average of 100 individual scans. The ESI–IMS operating conditions were set at the previously reported values [25].

2.4. Aptamer immobilization

Amino-modified MNPs were synthesized by coprecipitation iron salts, according to a previously reported procedure [26]. In order to covalent immobilization of aptamer on the surface of amino-modified MNPs, APTES-modified MNPs were activated with glutaraldehyde. Then these groups can react with amino-modified aptamers. Therefore, 1.5300 g of APTES-modified MNPs was added to 5% glutaraldehyde aqueous solution and stirred overnight at room temperature. The materials were subsequently washed three times with deionized water to remove excess glutaraldehyde. After drying, 8 mg functionalized MNPs were weighed and then 40 μL of 1000 μg mL^{-1} solution of the amino-modified aptamer was added and final volume was adjusted to 500 μL by deionized water. Then the mixture was sonicated for 5 min. The reaction was allowed to proceed by shaking overnight. Finally, magnetic nanoparticles coated with aptamer were washed three times with Tris–HCl buffer (pH 7.3) and then the solution of aptamer-MNPs were resuspended in 500 μL of Tris–HCl buffer and stored at 4 °C.

2.5. Extraction procedure

Prior to extraction, aptamer was renatured. In this regard, 8 mg of aptamer-MNPs were resuspended in 500 μL of (5 mM Tris–HCl, 20 mM NaCl and 10 mM $MgCl_2$, pH 6.6) was heated at 82 °C for 2.5 min and then let to reach the room temperature [4]. Then, 200 μL of extracted sample was added to the mixture and stirred for 5 min. The mixture was allowed to reach equilibrium for 20 min at 5 °C, for complete capturing of the target by the aptamer. Magnetic adsorbents were magnetically separated by a strong magnet (with