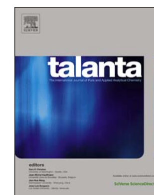
Talanta 162 (2017) 232–240

Contents lists available at ScienceDirect

Talanta

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



Selection of DNA aptamers against penicillin G using Capture-SELEX for the development of an impedimetric sensor

Nathalie Paniel, Georges Istamboulié, Athar Triki, Clément Lozano, Lise Barthelmebs, Thierry Noguer*

Laboratoire BAE, Université de Perpignan Via Domitia, 52 Avenue Paul Alduy, 66860 Perpignan Cedex 9, France

ARTICLE INFO

Keywords:
Aptamers
Capture-SELEX
Penicillin
Impedimetric sensor
Milk analysis

ABSTRACT

This paper describes for the first time the selection of aptamers selective to penicillin. Aptamers were selected using a specific process called Capture-SELEX (Systematic Evolution of Ligands by Exponential Enrichment). This technique is based on the selection of DNA aptamers using penicillin G in solution while the ssDNA library is fixed on a support. One aptamer showing a good affinity to penicillin was finally selected and tested in electrochemical sensor configuration, using electrochemical impedance spectroscopy as detection technique. The developed aptasensor allowed the detection of penicillin in a wide concentration range, comprised between 0.4 and 1000 μg L^{-1} Such performance was compatible with milk analysis, as the maximum residue limit tolerated in this matrix is 4 μg L^{-1}. The selectivity of the developed sensor was also studied, showing that the sensor was also able to bind other beta-lactam antibiotics, although with a weaker affinity. Finally the sensor was used for detection of penicillin G in milk. It was shown that a simple sample treatment with isopropanol followed by filtration was sufficient to eliminate matrix effects, allowing the determination of penicillin in milk at concentrations compatible with legislation requirements.

1. Introduction

β-lactam antibiotics are a wide class of antibiotics, represented by all the antibiotic agent that contain a β-lactam ring in their molecular structures. This includes the penicillin derivatives (penams), cephalosporins (cephems), monobactams and carbapenems. In veterinary practice, benzylpenicillin or penicillin G (G for gold standard) is the most frequently used β-lactam antibiotic for prevention and treatment of bacterial infection like mastitis for dairy cattle [1,2]. 60–90% of parenterally administered penicillin is eliminated in the urine within 6 h. The biliary route is also a major excretory pathway. For dairy cattles, penicillins are additionally eliminated in milk although often in trace amounts in the normal udder, and may persist for up to 90 h. Milk and yogurt are the major representatives of the dairy products that are the basics of food in industrialized countries. Before commercialization, cow milk is thermally treated to eliminate the possible pathogenic bacteria. The pasteurization (60 °C – 30 min or 72 °C – 15 s) and sterilization (120 °C – 20 min) only reduce penicillins residues approximately 10–20% and penicillin G can persist at a small rate that might be responsible of allergic reaction in sensitized individuals [1,3]. Penicillin residues may also be responsible for the induction of antibiotics resistant bacteria and in other level can negatively affect the growth of starter cultures for fermented dairy products [1,3–5]. To avoid health hazards due to the presence of penicillin G and penicillin residues in milk a maximum residue limit (MRL) have been established at 4 ppb for penicillin G [6]. Beside baby food must not contain penicillin antibiotic at all. Various methods have been developed for controlling the presence of β-lactam in food and feed samples and can be classified in two groups, confirmatory and screening methods. Confirmatory methods are represented by liquid chromatography (HPLC), gas chromatography (GC), high performance liquid chromatography (HPLC) and mass spectrometry (MS) [7–10]. Unfortunately, these chromatographic methods are time consuming, expensive and require complex sample preparation. Screening methods includes the microbial approaches based on bacterial growth inhibition [11,12] and immunochemical techniques like enzyme-linked immunosorbent assays (ELISA) [12–14]. The major default of these methods is the fail to identify the individual residues in the case of the microbial approach and the requirement of specific antibodies for the immunochemical techniques. Moreover, these methods are expensive and time-consuming when a lot of samples must be analyzed. Therefore, it is of great importance to develop a rapid and reliable method for quantifying penicillin G in food. Recently, biosensors have emerged as promising alternatives to conventional analytical systems for various

* Corresponding author.
E-mail address: noguer@univ-perp.fr (T. Noguer).

http://dx.doi.org/10.1016/j.talanta.2016.09.058
Received 25 July 2016; Received in revised form 23 September 2016; Accepted 24 September 2016
Available online 28 September 2016
0039-9140/ © 2016 Elsevier B.V. All rights reserved.

applications in environmental monitoring, food analysis and clinical diagnostic. The main advantages of these devices are related to their simplicity, versatility, portability, low cost and potential for accurate and real-time detection. Moreover, biosensors can be used in very dirty environments with minimal sample preparation [15–24]. Actually, some electrochemical and optical immunosensors are developed to detect penicillin in food. For example, Li et al. (2015) have developed an immunosensor for electrochemical detection of penicillin G in milk at trace level. Anti-penicillin G was immobilized in a bi-layer lipid membrane support modified with gold nanoparticles. This biosensor was able to detect 2.7×10^{-4} ng/L of penicillin G. The capacities were under the MRL of penicillin G in milk. However, most of the developed biosensors request some complex steps before realized the measurement and used antibodies for recognition elements. Among potential alternative recognition elements, aptamers have raised as very promising tools for diagnostics. Aptamers are oligonucleotides (DNA or RNA) that can bind with high affinity and specificity to various targets, including a large number of molecules containing randomly created sequences, such as drugs, proteins or other organic or inorganic molecules [25–35]. These single chains with short number of oligonucleotides 20-100 bases) are able to fold into well defined three-dimensional structures, which exhibit high affinity and specificity for target molecules. Owing to their many advantages such as cost effectiveness, flexibility, easy of modification, high stability, regeneration capacity, and compatibility with large-scale production [36–41] aptamers are a first choice candidate for the determination of penicillin in dairy products. Aptamer sequences against a target are commonly selected using the *in vitro* SELEX procedure (Systematic Evolution of Ligands by Exponential Enrichment), which was first reported in 1990 [42,43]. The SELEX method is an iterative process allowing the identification of unique RNA/DNA molecules from large populations of random sequence oligomers (DNA or RNA libraries) that bind to the target molecule with high affinity and specificity. Once the SELEX process is complete, the aptamer sequence can be established, and unlimited amounts of the aptamer can be subsequently prepared using chemical synthesis.

The present work describes the selection of aptamers sequences for penicillin by the use of a particular SELEX process called Capture-SELEX. This protocol was first developed by Stoltenburg and collaborators [44] on the basis of the FluMag-SELEX procedure described by the same authors in 2005 [45]. It is based on the selection of DNA aptamers using penicillin in solution while the ssDNA library is fixed on a support [44]. At the end of the Capture-SELEX, aptamer sequences were analyzed and some sequences of interest were retained. A validation of recognition capacity of the candidates against the penicillin was carried out using affinity chromatography and electrochemical impedance spectroscopy, in the aim of developing an electrochemical aptasensor dedicated to milk analysis.

2. Material and methods

2.1. Capture SELEX

2.1.1. Chemicals

Trizma hydrochloride (Tris-HCl), ethylenediaminetetraacetic acid (EDTA), calcium chloride ($CaCl_2$), magnesium chloride ($MgCl_2$), potassium chloride (KCl), sodium chloride (NaCl), ethanol (98% and absolute), manganese chloride ($MnCl_2$), penicillin, polyacrylamide, urea, formamide, glucose, sodium acetate ($C_2H_3NaO_2$) were purchased from Sigma (France).

2.1.2. Capture-SELEX library, capture oligonucleotides and primers

The used library (LB Capt 2N) contained two random nucleotides sequences (N10 and N40) that were separated by a docking sequence of 12 nucleotides (Table 1) [44]. The two random regions were flanked in 3' and 5' by invariant primer annealing sites of 18 nucleotides each (Table 1) [46]. The capture oligonucleotide (CO) (Table 1) contained a complementary sequence to the docking sequence; it was modified in 5' by a hexaethylene glycol (HEGL) spacer, three additional nucleotides (CTG) and a biotin allowing its immobilization. The primers used for oligonucleotides amplification during aptamers selection were both modified in 5' position: the forward primer (FP FAM) was attached to a 5-carboxyfluorescein (FAM) fluorochrome, while the reverse primer (RP HEGL A20) contained a HEGL spacer and a poly(adenine) (20 A) tail (Table 1). For the cloning step, a PCR amplification of the purified and concentrated DNA resulting from the last selection round was performed using non-tagged FP and RP primers (Table 1). Prior to sequencing, a validation of the good insertion of the DNA sequence into the pGEM-T Easy vector (Promega) was carried out by PCR using T7 and SP6 primers (Table 1), whose annealing sites were present on both sides of the DNA insertion area. A positive control (PC) was added consisting in an invariant DNA sequence of 62 nucleotides, that was flanked in 5' by T7 and FP primers and in 3' by the complementary sequences of RP and SP6 primers (Table 1). All the oligonucleotides were synthesized and chemically-modified by Microsynth (Switzerland).

2.1.3. Immobilization of the docking sequence A1 on streptavidin-coated magnetic beads

The protocol used was based on the one described by Stoltenburg et al. (2012) [44]. Streptavidin-coated magnetic beads (M-270 from Invitrogen), diameter 2.8 µm, were used to immobilize the CO modified with a biotin group. 3.3 mL of magnetic beads were transferred from the stock solution into a fresh tube and placed onto a magnetic support. The supernatant was discarded and the beads were washed three times with 500 µL of binding and washing (BW) buffer (10 mM Tris-HCl pH 7.5, 1 mM EDTA and 2 M NaCl). After the last washing, the supernatant was discarded and the beads were resuspended in an equal volume of BW buffer and CO solution to obtain a final concentration of

Table 1
Sequences and modifications of the oligonucleotides used for capture-SELEX, cloning, and sequencing steps.

	Sequences 5′–3′ and modifications	n
LB Capt 2N	GGGAGGACGAAGCGGAAC-**N10**-TGAGGCTCGATC-**N40**-CAGAAGACACGCCCGACA	98
FP FAM	**FAM**-GGGAGGACGAAGCGGAAC	18
RP HEGL A20	**Poly-dA20-HEGL**-TGTCGGGCGTGTCTTCTG	18
CO	**Biot-CTG-HEGL**-GATCGAGCCTCA	12
FP	GGGAGGACGAAGCGGAAC	18
RP	TGTCGGGCGTGTCTTCTG	18
T7	TAATACGACTCACTATAG	18
SP6	ATTTAGGTGACACTATAG	18
PC	TAATACGACTCACTATAGGGGAGGACGAAGCGGAACTATTTCCGCCTGAGGCTCGATCATGGAGTATATCACTATGATTTAGTGAGTTA-CATGTGGGCCAGAAGACACGCCCGACACTATAGTGTCACCTAAAT	134

N10 and N40: random regions, FAM: fluorochrome, Poly-dA20: 20 adenine, HEGL=Hexaethylene glycol=spacer=$(CH_2\text{-}CH_2\text{-}O)_6$, Biot: Biotin.

Download English Version:

https://daneshyari.com/en/article/5141165

Download Persian Version:

https://daneshyari.com/article/5141165

Daneshyari.com