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ORIGINAL PAPER

Aptamer based vanillin sensor using an ion-sensitive field-effect transistor

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Abstract

An aptamer for vanillin was obtained and then used for the development of an aptasensor based on an ion-sensitive field-effect transistor (ISFET). This aptamer (a single-stranded DNA;ssDNA) was selected using the Capture-SELEX protocol, which suites well for selection of aptamers to small molecules. Among six aptamer candidates, the aptamer Van_74 with the highest affinity for vanillin was chosen (elution of 35% of the aptamer from a solid support in the presence of 2 mM of vanillin). Van_74 was characterized using nondenaturating PAGE of washouts from magnetic beads. It is shown that Van_74 binds to vanillin with an dissociation constant of >7.8 μM (determined by nondenaturating PAGE) and it was specific to vanillin in comparison with interferents: benzaldehyde, guaiacol, furaneol, ethyl guaiacol and ethyl vanillin. Also it was shown that change of buffer composition greatly affected the binding ability of Van_74. For biosensor fabrication aptamer was immobilised on the Ta_2O_5-sensitive surface of the ISFET via "click-chemistry". Detection scheme implied dehybridisation of the ssDNA probe from the aptamer and release in the solution during the addition of vanillin. As a result, the surface potential increase upon vanillin binding with the aptamer was detected by the transistor. The biosensor had a detection limit of 1.55×10^{-7} M and a dynamic range from 1.55×10^{-7} M to 1×10^{-6} M. Effective constant $K_{d,eff}$ for vanillin binding on biosensor surface was calculated to be $(9 \pm 3) \times 10^{-7}$ M. This allows selective detection of vanillin in the mixture of interferents and in samples of coffee extract.

Keywords Vanillin · Capture-SELEX · Aptamer, ion-selective field-effect transistor · Aptasensor, small target, low-weight target

Introduction

Vanillin (4-hydroxy-3-methoxybenzaldehyde) is typically used as a flavouring additive in beverages, baked goods, chocolates, and other sweet food. In addition, vanillin has antioxidant activity [1] and antimicrobial/antifungal effects in fresh fruit products and milk [2–4]. Therefore, vanillin concentrations should be carefully controlled during food production. Liquid chromatography [5, 6] and liquid chromatography coupled with solid-phase microextraction of vanillin and methyl vanillin [7], gas chromatography [8, 9] with UV spectrophotometry [10, 11], and mass spectrometry are often used for vanillin analysis in the food industry [12]. However, these methods usually involve time-consuming sample preparation, expensive procedures, and bulky equipment. As an alternative, electrochemical detection provides advantages such as high sensitivity, short analysis time, low cost, and simple handling. For selective electrochemical detection of vanillin in probes with a mixture of phenolic substances, additional electrode modification is thought to be required [13].

The ion-sensitive field-effect transistor (ISFET) is a type of metal–oxide–semiconductor field-effect transistor (MOSFET) in which the metal gate is replaced by a liquid [14]. Adsorption occurring on the liquid-sensitive surface interface modulates the current in the transistor channel, and the current change may serve as an analytical signal.

Aptamers are DNA or RNA oligonucleotides that fold in complex with the desired target with high affinity and specificity. Aptamers are chemically stable, reusable, and readily synthesised. DNA or RNA high-affinity aptamers may be isolated from random oligonucleotide libraries through an

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in vitro procedure called systematic evolution of ligands by exponential enrichment (SELEX) [15]. Currently, high-affinity aptamers can be obtained for various targets, including proteins, viruses, small organic molecules, and whole cells [16]. Despite the many types of aptamers that have been obtained, to the best of our knowledge, aptamers with affinity to vanillin have not yet been described in the literature.

Several sensors based on combination of aptamers and ISFETs are described in literature: the label-free and reagentless aptamer-based sensor for adenosine [17] and aptasensor for cocaine with Au nanoparticles as amplifying labels [18].

Here, a new electrochemical approach for vanillin detection based on ISFET and aptamer is presented. Capture-SELEX [19] was applied to obtain DNA aptamers against vanillin. After aptamer selection, a strategy to detect vanillin by immobilising the selected aptamer (Van74) with a short complementary probe on the Ta_2O_5-sensitive surface of silicon on insulator (SOI) ISFET was developed. Obtained results indicate that Van74 and the fabricated biosensor is a new potential method for on-site selective vanillin detection.

Experimental

Materials

All reagents were purchased from Sigma-Aldrich (Germany) unless otherwise indicated. Taq DNA polymerase (https://www.thermofisher.com/order/catalog/product/18038042), dNTP solution (https://www.thermofisher.com/order/catalog/product/R0241), TAE (https://www.thermofisher.com/order/catalog/product/B49) and TBE (https://www.thermofisher.com/order/catalog/product/B52) electrophoresis buffers, DNA extraction kits (https://www.thermofisher.com/order/catalog/product/K0702, https://www.thermofisher.com/order/catalog/product/K0503), and DNA cloning kits (https://www.thermofisher.com/order/catalog/product/K1232) were purchased from ThermoFisher Scientific (USA). Streptavidin-coated magnetic beads (https://www.neb.com/products/s1420-streptavidin-magnetic-beads) were supplied by New England Biolabs (Ipswich, MA, USA). SYBR Gold dye (https://www.thermofisher.com/order/catalog/product/S11494) was purchased from Invitrogen (supplied by ThermoFisher Scientific). *Escherichia coli* DHα strain (ThermoFisher Scientific, https://www.thermofisher.com/order/catalog/product/18258012) was used for plasmid transformation after DNA cloning.

The single-stranded DNA library B1_bank and all the other nucleotides were synthesised by DNA Synthesis Ltd. (http://www.oligos.ru/, Russia). Sequences are listed in Supplementary Table S1. The B1_bank library was flanked by two primer binding sites of 18 and 19 nt, and a random fragment of 50 nt was separated to 10 and 40 nt by capture-sequencing of 12 nt. The capture oligonucleotide B1_cap containing a complementary capture sequence and labelled with biotin was used for reversible immobilisation of the DNA library on streptavidin-coated magnetic beads. Primers used for ssDNA pool amplification during aptamer selection were modified: the forward primer B1_for_Cy3 was labelled with the Cy3 fluorophore, and the reverse primer B1_rev_A contained a HEGL spacer and a poly(adenine) (20A) tail. Nonlabelled primers B1_for and B1_rev were used for DNA amplification at the final SELEX round before cloning the DNA pool.

Apparatus

Electrochemical measurements

ISFET I-V curves (showing dependence of the drain current I_{DS} on the reference electrode voltage V_{GS}) were recorded using an Agilent B1500A semiconductor device parameter analyzer (USA) and Cascade PM5 probe station (USA) with Agilent VEE Pro, as described previously [20].

Fluorescence measurement

The amount of Cy3-labelled DNA was monitored by Cy3 fluorescence. Measurements were carried out using a Tecan Infinite M200 microplate spectrofluorimeter (Tecan, Switzerland) using excitation at 540 nm and emission at 570 nm. Calibration curves were used to determine the amount of DNA in each probe.

Spectrophotometric measurements

Nonlabelled DNA was quantified using Tecan NanoQuant plates in a Tecan Infinite M200 microplate spectrofluorimeter.

Aptamer selection

The protocol for aptamer selection was similar to the Capture-SELEX procedure reported by Stoltenburg et al. [19]. 2 mM of vanillin (http://www.sigmaaldrich.com/catalog/product/sial/v1104?lang=en&region=RU) served as a target for selection.

DNA library immobilisation

B1_capture oligonucleotide was immobilised on streptavidin-coated magnetic beads. For the first SELEX round, 1.25 mL magnetic beads (5 mg, able to bind 2500 pmol of single-stranded 25 bp biotinylated oligonucleotide according to the supplier's information) was washed three times with 1 mL Wash/Binding Buffer (WBB: 0.5 M NaCl (http://www.sigmaaldrich.com/catalog/product/sial/s7653?lang=en&region=RU), 20 mM Tris-HCl [pH 7.5], 1 mM ethylenediaminetetraacetic acid

(http://www.sigmaaldrich.com/catalog/product/sial/eds?lang=en&region=RU)) and then resuspended in 1 mL B1_capture solution (5 pmol/μL). After 15 min of incubation, magnetic particles were again washed three times with WBB and three times with Selection buffer (SB: 100 mM NaCl, 20 mM Tris-HCl [pH 7.6] (http://www.sigmaaldrich.com/catalog/product/sigma/t5941?lang=en&region=RU, http://www.sigmaaldrich.com/catalog/product/sigma/t1503?lang=en&region=RU), 2 mM $MgCl_2$ (http://www.sigmaaldrich.com/catalog/product/sigma/m8266?lang=en&region=RU), 5 mM KCl (http://www.sigmaaldrich.com/catalog/product/sial/p9333?lang=en&region=RU), 1 mM $CaCl_2$ (http://www.sigmaaldrich.com/catalog/product/sigald/746495?lang=en&region=RU)). For subsequent SELEX rounds, only 80 μL of magnetic beads were used.

B1_bank (3 nmol) for the first SELEX round and the entire ssDNA pool for the following rounds were dissolved in SB, denatured at 90 °C for 8 min, incubated on ice for 10 min, and then incubated at room temperature for 2–3 min. The denatured ssDNA was added to B1_capture-coated magnetic particles and incubated overnight with interval mixing using an Eppendorf Thermomixer Comfort (Germany). The unbound ssDNA was washed away by rinsing the particles with SB seven times. Weakly bound sequences were eliminated by incubating the particles at 28 °C for 15 min, followed by seven washes with SB.

Incubation with target

Vanillin solution (2 mM in SB) was prepared fresh each week. For the first SELEX round, magnetic nanoparticles with hybridised B1_bank were resuspended in 200 μL of the target solution and incubated for 30 min. For the subsequent rounds, the target volume was 50 μL. Before each incubation with target, magnetic nanoparticles with hybridised ssDNA were incubated in the same volume of SB to monitor the background DNA elution. The solution above the nanoparticles was collected for further amplification of the eluted ssDNA. Starting from the second round, the amount of eluted DNA was controlled by Cy3 fluorescence.

Polymerase chain reaction (PCR) amplification of ssDNA

The eluted ssDNA pool was amplified in 10 (for the first round) or 5 (for all subsequent rounds) parallel PCR reactions in a volume of 100 or 50 μL, respectively. The reaction mixture contained 0.8 μM primers, 0.2 mM $MgCl_2$, 0.25 mM each dNTP, and 0.06 U/μL Taq DNA polymerase in 75 mM Tris-HCl (pH 8.8 at 25 °C), 20 mM $(NH_4)_2SO_4$, and 0.01% (*v*/v) Tween 20. Amplification was carried out in a Tercyk Thermocycler (http://www.dna-technology.ru/eng/, DNA-technologies, Russia) and included an initial denaturation step at 95 °C for 5 min; 12 cycles of 95 °C for 30 s, 66 °C for 30 s, and 72 °C for 30 s; and a final extension step at 72 °C for 2 min. After amplification, the dsDNA product was cleaned and concentrated using a PCR purification kit (Fermentas, Lithuania) according to the manufacturer's protocol.

ssDNA regeneration

DNA strands were separated by denaturing polyacrylamide gel electrophoresis (PAGE) followed by extraction and purification of the Cy3-labelled sense strand. DNA samples were denatured in formamide by heating to 95 °C for at least 10 min and loaded to 12% polyacrylamide gels containing 8 M urea. After 30-45 min of separation at 200 V in a Bio-Rad Mini Protein Tetra Cell System, the gel was stained with SYBR Gold, and the Cy-3 labelled band was excised from the gel. The ssDNA was extracted using the crush and soak protocol [21].

Cloning and sequencing

After the final SELEX round, ssDNA was amplified using nonlabelled primers (Table S1). dsDNA product was purified with a DNA purification kit and cloned using a CloneJet DNA cloning kit. *Escherichia coli* DHα strain rubidium competent cells were transformed with the ligation mixture. Plasmids were extracted with a GeneJet plasmid DNA extraction kit. The inserts were sequenced using ABI PRISM BigDye Terminator v. 3.1 reagent kits and sequencing primers from the CloneJet kit on an automatic sequencer (3730 DNA Analyzer, Applied Biosystems, Foster City, CA, USA). Sequences were processed using BioEdit software and analysed online with Clustal Omega (http://www.ebi.ac.uk/Tools/msa/clustalo/).

Screening of aptamers binding ability

Six aptamer candidates containing Cy3 labels at the 5′-end were chemically synthesised by DNA-Synthesis Ltd. Briefly, 300 pmol of each aptamer candidate was hybridised with 200 μg of magnetic beads covered with the capture probe. After DNA hybridisation, magnetic beads were divided into six equal probes. Three probes were incubated with 2 mM vanillin solution in SB, and the three other probes were incubated with SB as a control. After 45 min of incubation with gentle shaking, magnetic beads were separated using a magnetic rack, and the eluted DNA amount was measured by Cy3 fluorescence.

Aptamer characterisation by PAGE

For 10 experimental points 100 μl of streptavidin-coated magnetic beads of the initial concentration (c = 4 mg/ml, the beads bind 500 pmol of single-stranded 25 bp biotinylated

oligionucleotide per mg) were taken. Beads were washed 3 times in WBB and then incubated in 50 μl of WBB with addition of 4 μl of B1_cap (100 pM/ μl) for 2 h. After incubation beads were washed 3 times in WBB and 3 times in SB. 8 μl of aptamer (100 pM/μl) in 42 μl of SB was denatured at 90 °C for 8 min, incubated on ice for 10 min, and then incubated at room temperature for 2–3 min. The denatured aptamer was added to B1_capture-coated magnetic particles and incubated overnight with interval mixing (1200 rpm for 30 s, 9 min 30 s without mixing) using an Eppendorf Thermomixer Comfort (Germany). The unbound ssDNA was washed away by rinsing the particles with SB seven times. Weakly bound sequences were eliminated by incubating the particles at 28 °C for 15 min, followed by seven washes with SB.

Magnetic beads were aliquoted in 10 eppendorf tubes for 5 μl in each; 40 μl of SB (or another buffer) and 5 μl of target with desired concentration were added to these samples. Incubation with target was carried out at 21 °C for 1 h with constant mixing 850 rpm. Aliquots of 5 μl from washout were used in PCR (20 μl of PCR premix). 5 μl of PCR product without purification were mixed with 2–3 μl of TriTrack DNA Loading Dye (https://www.thermofisher.com/order/catalog/product/R1161) and loaded to nondenaturing PAGE. After 1 h of separation at 120 V in a Bio-Rad Mini Protein Tetra Cell System, the gel was stained with SYBR Gold and scanned by Microtek Bio-1000F scanner (http://www.microtek.com/products.php?KindID=5&ID=379).

The GelQuant.NET software provided by biochemlabsolutions.com was used to analyze the intensity of DNA strips in the gel.

Biosensor fabrication

Chip fabrication

n-type ISFET with a Ta_2O_5-sensitive surface was fabricated using standard 1.2 μm fully depleted SOI complementary metal-oxide-semiconductor technology on 4′-SOI SIMOX (separation by implantation of oxygen) wafers (p-type) with a 0.18-μm-thick active layer, 0.38-μm-thick insulating layer, and 12–22 Ω·cm resistivity at the SMC Technological Centre (Russia). Fifty nanometres of tantalum were deposited by physical vapour deposition onto a 10 nm SiO_2 gate dielectric after the formation of a polysilicon gate. Then, a standard metallisation step was carried out after thermal oxidation of tantalum film at 850 °C in oxygen for 10 min. The channel length of the transistor was 2 μm, channel width - 96 μm (Fig. S1).

Aptamer immobilisation and biosensor activation

After screening for aptamer binding ability, selected candidates were synthesized using a 5′-end alkyne label by DNA Synthesis Ltd. Chips with ISFET were immersed in 3% 3-aminopropyltriethoxysilane (http://www.sigmaaldrich.com/catalog/product/aldrich/440140?lang=en&region=RU) in ethanol, and the surface was then activated to Cu-catalysed click-chemistry by reaction with azidobutyric-*N*-hydroxy-succinimide (https://www.lumiprobe.com/p/azide-nhs-ester, Lumiprobe, Russia) [22]. The alkyne-modified aptamer was "clicked" onto the transistor-sensitive surface by the azide-alkyne cycloaddition reaction catalysed by copper-containing reagent (Cu(II)-TBTA (https://www.lumiprobe.com/p/click-chemistry-catalyst, Lumiprobe, Russia)). After aptamer immobilisation, the aptamer was hybridised with a short complementary probe (5′-Bio-GTC-Spacer18-CATCGAGACTCC-3′) for 2 h.

Biosensor measurements

All measurements were carried out in a well-like structure which was made of organic ink. Organic ink consisting of microcrystalline wax SP19 (40%) (http://spwax.com/pages/products/Microcrystalline.html) and vaseline (60%) (http://www.sigmaaldrich.com/catalog/product/sial/16415?lang=en&region=RU) was deposited onto the surface of the chip using a Nordson EFD Ultimus V pneumatic dispenser (Germany; 5.7 bar at 22 °C) and Janome JR2303 robot positioning system (Japan) in the form of filaments (5.5 mm in width and 4.5 in length, 0.25 mm in wall thickness, and 1.625 mm in height). Measurements were obtained with tungsten electrodes connected to contact pads.

Measurements of the biosensor began with the determination of operating mode. For that the modified ISFET with well-like structure containing of 25–30 μl of SB was used. Then time-dependent changes in the current I_{DS} ($V_G = \text{const}$) were recorded and during record 1–1.5 μl of target of different concentrations (solution in SB or another buffers) was added.

As a biosensor, the ISFET was used in subthreshold mode. Operating mode was determined from I-V_G curves ($V_{DS} = 0.1$ V); the threshold voltage V_t and subthreshold slope S were used. Time-dependent changes in the current I_{DS} ($V_G = \text{const}$) were recorded and surface potential change $\Delta\varphi_i \approx \frac{S}{S_0} n\varphi_t \frac{I_{i+1}-I_i}{I_i}$ was used as a signal.

For each experiment 2–3 baseline measurements (in buffer solution) for ISFET stabilization were performed before sample addition. For data processing surface potential calculations ($I_{DS} \rightarrow \Delta\varphi$) were made. Signal from sample was calculated as absolute value of difference between baseline and sample after ~300 s of sample addition.

Coffee extract was obtained from 6 g of coffee beans which were placed under N_2 steam and bubbled into SB during 3 min.

Results and discussion

Aptamer selection and identification

The Capture-SELEX protocol described by Stoltenburg et al. [19] was used for aptamer selection. This method implies reversible fixation of the ssDNA library on the support instead of immobilisation of the target molecule, thus providing a huge advantage for selection of aptamers to small molecule targets by eliminating steric hindrances for aptamer-target complex formation. Compared with the original Capture-SELEX protocol, smaller volumes of target solution were used for all selection rounds to reduce labour and reagent consumption.

The original ssDNA library, named B1_bank, was used for aptamer selection. The library was flanked by two primer binding sites of 18 and 19 nt, and a random fragment of 50 nt which was separated to 10 and 40 nt by capture-sequencing of 12 nt. The capture sequence facilitated fixation of the library on the capture oligo. It was designed to have a high binding efficiency during library hybridisation within the minimum length.

The ssDNA library was hybridised on a 12 nt capture probe attached to a magnetic support via the biotin-streptavidin interaction. During incubation with the target, oligonucleotides capable of folding into a complex with the target molecule switched from the capture probe to the complex, thus passing into the solution. Before each incubation with the target, negative selection against the selection buffer without the target step was introduced to estimate the background DNA elution. After incubation with the target, the solution with eluted ssDNA was amplified by PCR with elongated reverse primers, and DNA strands were separated on denaturing PAGE. The Cy3-labelled strand was then removed from the gel, extracted, and purified. The entire ssDNA pool then was evolved to the next SELEX round.

The selection was stopped after eight cycles. Enrichment of ssDNA pool during SELEX is presented in Supplementary (Fig. S2). After the last round, the eluted ssDNA pool was amplified with nonlabelled primers for the subsequent cloning and sequencing. A portion of the nonlabelled DNA pool was again amplified with Cy3-labelled forward primer and elongated with the polyA tail reverse primer to obtain a fluorescently labelled ssDNA pool for assessment of the pool binding ability towards the target. The pool was hybridised on the support and divided into two equal parts. One part was incubated with 2 mM vanillin solution, and the other part was incubated with selection buffer. The amount of the eluted DNA was quantified by Cy3 fluorescence. Notably, 20% more ssDNA was eluted in the presence of the target, indicating that the pool contained sequences with binding ability for vanillin.

The nonlabelled DNA was cloned into the pJet 2.1 vector using blunt-end ligation. Plasmids were extracted from 96 *E. coli* colonies and used directly for sequencing. After sequence analysis, 83 sequences were aligned using the Clustal Omega algorithm. All the sequences were unique. AGG and GGA motifs were abundant among them. The phylogenetic tree showed that all the sequences can be divided into seven clusters counting 12, 12, 14, 14, 11, 16 and 4 ssDNA sequences (Supplementary, Fig. S3). The selected sequences were aligned collectively (Fig. 1) and pairwise to get maximum similarity (Fig. S4). GxAG motif was found at 20–24 position before the capture sequence. AGG at 46–48 position and GGA at 52–54 were found in the candidate sequences (Fig. 1) and were abundant in the entire sequences DNA pool. GGxGxAxA at 57–62 position, G at 72 position, GG at 78–79 are also common for the aptamer candidates. Van_01 and Van_74 showed the highest homology and had the same GCGGC at 50–54 position (Fig. S4).

Chosen aptamer candidates with the Cy3-label at the 5′-end were synthesised for further investigation. For each sequence, the binding ability to vanillin was analysed as described for the ssDNA pool (Fig. 2). One of the candidate - Van_60 - was poorly hybridised on the support. Among the other candidates, Van_74 and Van_01 showed the best binding ability to vanillin. Additionally, 35% and 22% more DNA was eluted in the presence of the target. Van_74 was selected for further characterization and biosensor development.

Aptamer characterization

Aptamer Van_74 was selected for further investigation as aptamer with the best binding ability to vanillin among testing candidates. Initially, an investigation of interaction of the aptamer with different concentrations of vanillin was carried out. For this purpose, magnetic beads coated with Van_74 were incubated in vanillin solutions with concentrations 2.0×10^{-3} - $\times 4.9 \times 10^{-7}$ M of vanillin. PCR amplification of washout showed that the signal (band intensity) from vanillin at concentration lower than 7.8×10^{-6} M had no difference from the background (Fig. 3a, b). Washing of magnetic beads incubated with SB was used as background.

The specificity of Van_74 was studied by investigating interaction with possible interferents: benzaldehyde, guaiacol, furaneol, ethyl guaiacol and ethyl vanillin. For this, magnetic beads coated with Van_74 were incubated in interferents's solutions at the concentration of 280 μM. Analysis of nondenaturating-PAGE revealed that Van_74 is specific to

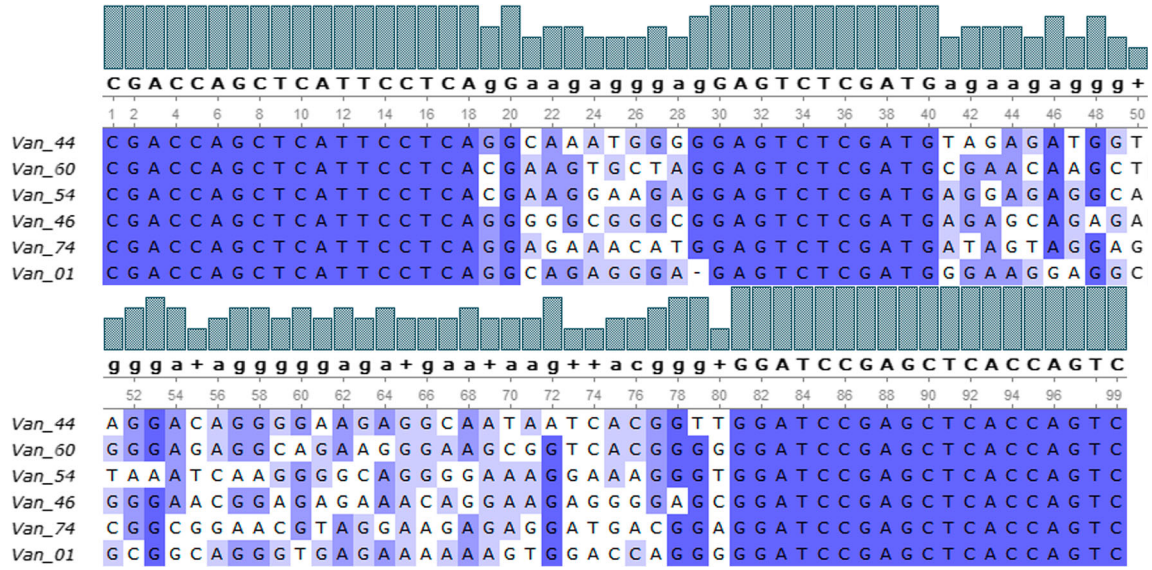

Fig. 1 Alignment of ssDNA sequences of aptamer candidates: Van_01, Van_44, Van_46, Van_54, Van_60 and Van_74. Alignment was performed using Ugene software (http://ugene.net/ru/) with the use of MUSCLE algorithm

vanillin: band intensity is ~70% higher in comparison with other compounds (Fig. 3c, d). All structural groups (−OCH_3, −CHO, − OH and benzene ring) are probably important for formation of aptamer-vanillin complex. Comparison of vanillin and ethyl vanillin showed that additional –CH_2-group in ethyl vanillin impacted on binding ability of Van_74. However, in the case of ethyl vanillin the binding efficiency was higher by ~12% compared to other interferents. This suggested that there was still small interaction between ethyl vanillin and Van_74. It is confirmed by the signal from the mixture of all compounds, which is higher than the signal from only vanillin. At the same time, the signal from the mixture that does not contain both vanillins is comparable with the background.

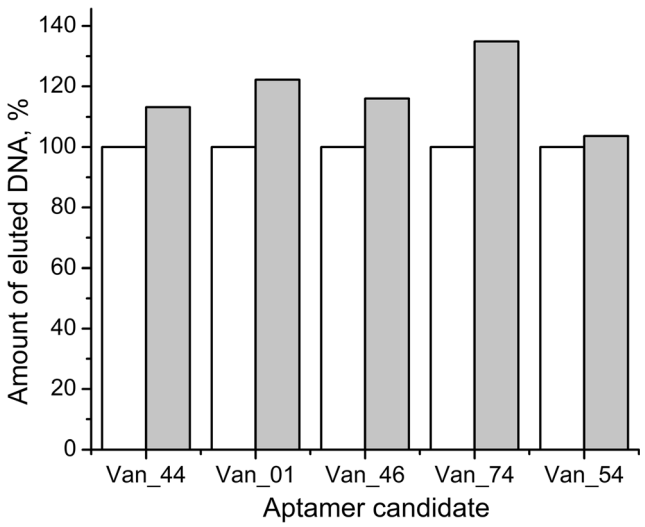

Fig. 2 Comparison of DNA elution from the support with 2 mM of vanillin quantified by Cy3 fluorescence

Since the obtained aptamer was intended to be used in fabrication of a biosensor based on ISFET the choice of working buffer was essential. The most important limiting factor affecting the sensitivity of ISFET comes from the increased distance of charges from the interface, the so-called Debye length. Beyond this distance surface potential change cannot be properly detected [23]. The Debye length is a function of electrolyte concentration [24]. Thus, experiments with different buffer concentrations were carried out to evaluate the impact of the buffer on the possibility of the aptamer interaction with vanillin (vanillin concentration - 1.5 mM). It was shown that change of buffer composition greatly affected the binding ability of Van_74 (Fig. 4). Aptamer worked best in the SB which was used during SELEX. Reduction of NaCl and Tris/HCl content by half (compared to SB) greatly impaired binding ability of aptamer. The use of buffers with even lower concentrations also worsened the binding between Van_74 and vanillin. Thus, these components of buffer and slight change in pH played a crucial role in formation of aptamer-target complex.

For further investigation of a biosensor SB was used as working solution.

Biosensor characterisation

The biosensor was fabricated using an ISFET with tantalum oxide gate dielectric and aptamer Van74, which was immobilised on the sensitive surface.

It is known that ISFET responds to the change of surface potential near the sensitive surface [23]. Adsorption of

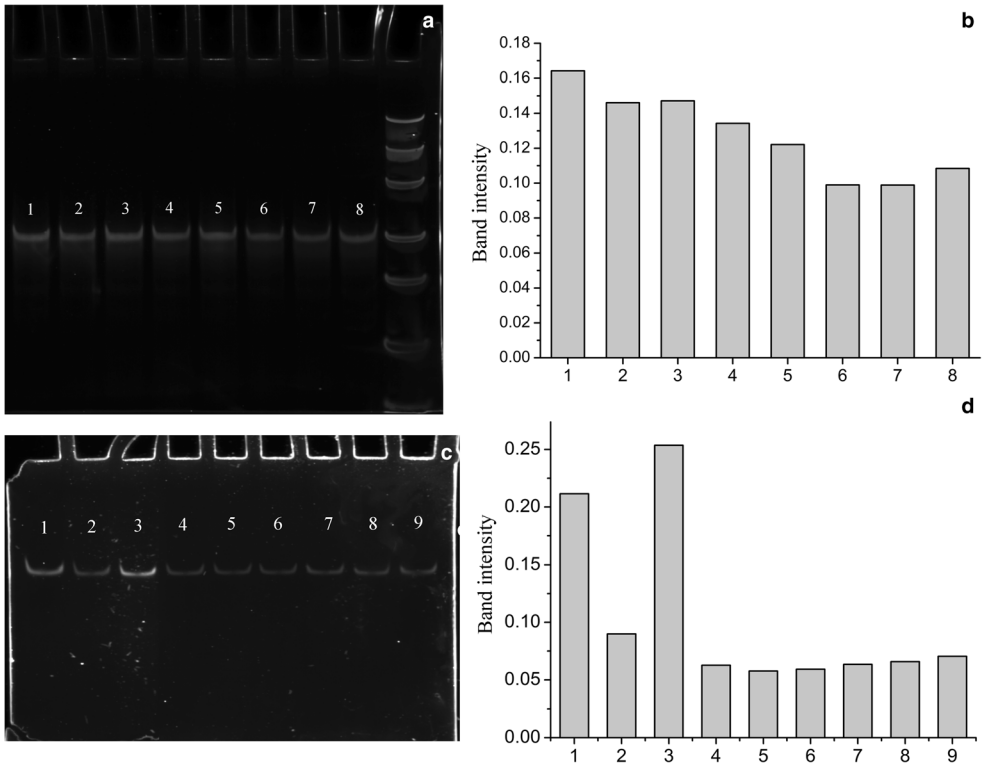

Fig. 3 Nondenaturating PAGE of PCR amplified (20 cycles) washout probes from magnetic beads (**a**) and image analysis of phoresis with GelQuant.NET (B) for different concentrations of vanillin in SB: **1**–2.0×10^{-3} M, **2**–5.0×10^{-4} M, **3**–1.3×10^{-4} M, **4**–3.1×10^{-5} M, **5**–7.8×10^{-6} M, **6**–1.9×10^{-6} M, **7**–4.9×10^{-7} M, **8** - SB. Additional experiment with 8 cycles of PCR is in Supplementary (Fig. S5). Nondenaturating PAGE of PCR amplified (12 cycles) washout probes from magnetic beads (**c**) and image analysis of phoresis with GelQuant.NET (D) for different targets at the concentration of 280 μM in SB: **1** – vanillin, **2** – ethyl vanillin, **3** - mix 6 (benzaldehyde, guaiacol, furaneol, ethyl guaiacol, ethyl vanillin, vanillin; each at 280 μM), **4** – mix 4 (benzaldehyde, guaiacol, furaneol, ethyl guaiacol; each at 280 μM), **5** – guaiacol, **6** - ethyl guaiacol, **7** - benzaldehyde, **8** - furaneol, **9** - SB

relatively low-weight organic molecules like vanillin doesn't provide high enough input to the surface potential charge distribution in comparison with local pH changes or biomolecule adsorption. This is particularly true in case of negatively charged deoxyribose-phosphate backbone of immobilized DNA aptamers on the sensitive surface. Thus, using ISFET

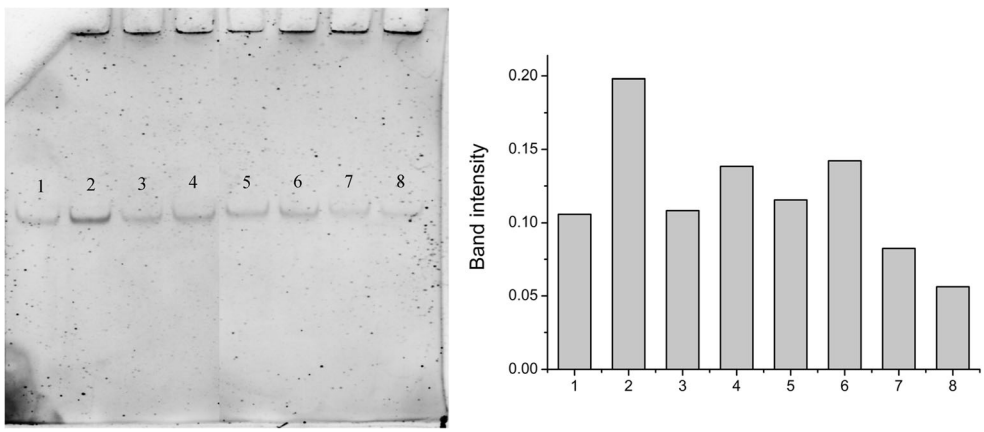

Fig. 4 Nondenaturating PAGE of PCR amplified (12 cycles) washout probes from magnetic beads (**a**) and image analysis of phoresis with GelQuant.NET (**b**) for different buffer solutions: **1** – SB: 100 mM NaCl, 20 mM Tris-HCl [pH 7.6], 5 mM KCl, 2 mM $MgCl_2$, 1 mM $CaCl_2$, total molarity – 128 mM; **2** – buffer 1 with vanillin 1.5 mM; **3**–40 mM NaCl, 10 mM Tris-HCl [pH 7.6], 5 mM KCl, 2 mM $MgCl_2$, 1 mM $CaCl_2$, total molarity – 58 mM; **4** – buffer 3 with vanillin 1.5 mM; **5**–5 mM NaCl, 2 mM Tris-HCl [pH 7.3], 1 mM KCl, 1 mM $MgCl_2$, 0.5 mM $CaCl_2$, total molarity – 9.5 mM; **6** – buffer 5 with vanillin 1.5 mM; **7**–1 mM NaCl, 2 mM Tris-HCl [pH 7.1], 0.5 mM $MgCl_2$, 0.25 mM $CaCl_2$, total molarity – 3.75 mM; **8** – buffer 7 with vanillin 1.5 mM

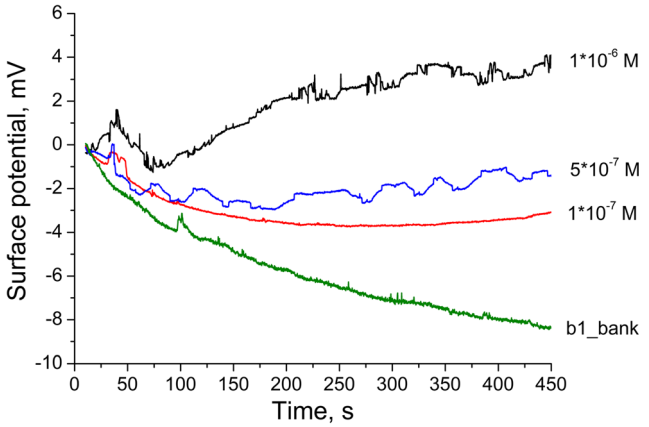

Fig. 5 Real time signal of the modified ISFET (in $\Delta\varphi$) after vanillin addition at different concentrations. Data aren't normalized

for detection of interaction between vanillin and aptamer is a complicated task.

So for further investigation capture-SELEX approach was used. In capture-SELEX ssDNA pool was immobilized on magnetic beads via hybridization with biotin modified probe. In the selection scheme, interaction of vanillin with aptamers resulted in dehybridisation from the probe. In the biosensor operation a reverse scheme was applied: the probe was hybridised with Van_74 which was immobilised on the sensitive surface of the ISFET. Such scheme avoided the limitation of low sensitivity of ISFET for direct detection of interaction between vanillin and the immobilised aptamer. In this case, vanillin replaced the hybridized probe on the sensitive surface resulting in decreasing negative charge near the surface that was detected by the ISFET (Fig. 5). As shown in Fig. 5, addition of different concentrations of vanillin to the biosensor increased the surface potential. In contrast, addition of vanillin (1×10^{-6} M) to initial B1_bank library, immobilised via alkyl primer, did not generate any notable increase in surface potential as the DNA probe remained hybridised (Fig. 6a).

The calibration curve for vanillin determination showed that vanillin can be quantified over a concentration range from 1.55×10^{-7} M to 1×10^{-6} M. The limit of detection (LOD) for the biosensor was estimated to be 1.55×10^{-7} M. Binding efficiency of aptamer in the biosensor was characterised by $K_{d,eff}$ and was calculated to be $(9 \pm 3) \times 10^{-7}$ M (Fig. S6).

In comparison with another electrochemical sensors presented in Table 1, the obtained biosensor showed equal performance. However, our approach had greater potential for miniaturisation, with all benefits provided by CMOS technology, and may have applications in the development of a multisensory ISFET array on a chip.

The specificity of the ISFET biosensor was further studied by investigating the sensor response to the addition of benzaldehyde, guaiacol, ethyl guaiacol, ethyl vanillin and furaneol at the concentration of 1 μM. Benzaldehyde, guaiacol, ethyl guaiacol and ethyl vanillin were chosen as available structural analogues, and furaneol was taken as non-ring representative of major group of dominant substances contributing to the flavour of roasted coffee beans [34]. As shown in Fig. 6a, the analytical response of the biosensor to these substances differed from the response to vanillin and was comparable with biosensor behaviour under nonspecific adsorption on the sensitive surface without probe dehybridisation. Notably, under conditions used in this study (300 s after target addition) ISFET biosensor may selectively detect 1×10^{-6} M vanillin in the present of mixture of these compounds at 1×10^{-6} M concentration each (Fig. 6b) This suggested good selectivity of Van74 for vanillin in the presence of benzaldehyde, guaiacol, ethyl guaiacol, ethyl vanillin and furaneol as it was shown previously by PAGE study.

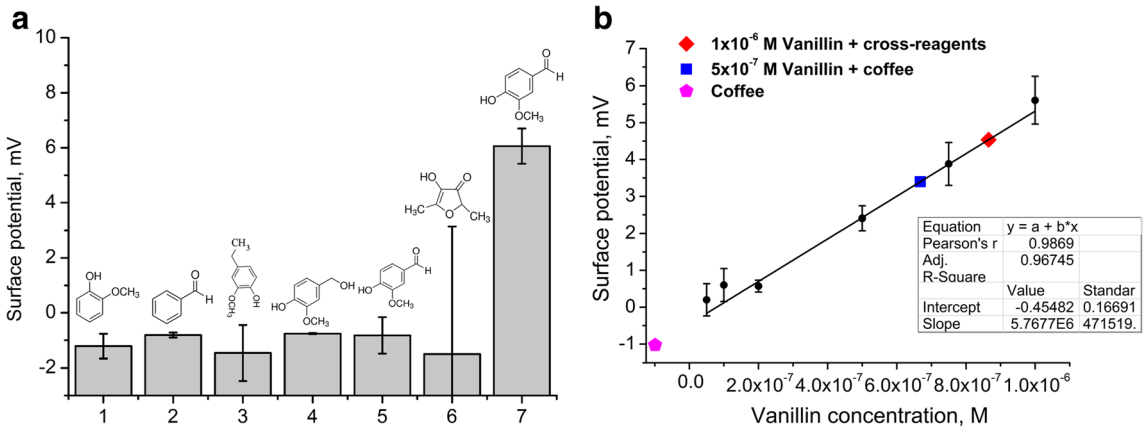

Fig. 6 Signal of the modified ISFET with Van_74 or initial B1_bank to addition of 1×10^{-6} M targets (**a**): **1** – guaiacol (Van_74), **2** – benzaldehyde (Van_74), **3** - ethyl guaiacol (Van_74), **4** – ethyl vanillin (Van_74), **5** – vanillin (B1_bank), **6** – furaneol (Van_74), **7** – vanillin (Van_74). Calibration curve for the sensor with estimation of real and artificial samples (**b**)

Table 1 Electrochemical sensors for vanillin detection presented in the literature

Sensor type	LOD, μM	Range, μM	Ref.
MWCNT-modified electrode	7	–	[25]
Screen-printed graphite electrode	0.4	5–400	[26]
Arginine-functionalised graphene nanocomposite	1	2–10	[27]
Ag nanoplates on graphene	0.33	2–100	[28]
Nitrogen-doped graphene/carbon nanotubes	0.0033	0.01–10	[29]
Gold nanoparticles stabilised in poly(allylamine hydrochloride)	0.055	0.90–15.0	[30]
Graphene nanoflake-modified glassy carbon electrode	0.0124	0.01–53	[31]
Palladium nanoparticles on porous aromatic frameworks	0.000002	0.00001–0.00082	[32]
Cobalt sulfide nanorods	0.07	0.5–56	[33]
ISFET with DNA aptamer	0.155	0.155–1.0	This work

Analyte addition technique was used to measure vanillin concentration in real sample of coffee extract. Addition of extract to the modified ISFET resulted in negative transistor response due to matrix effect of coffee. Vanillin was added to real coffee extract at the concentration of 5×10^{-7} M to estimate the recovery ratio. The matrix signal decrease from extract was added to the signal of extract with additional vanillin, and 6.9×10^{-7} M of vanillin was detected (Fig. 6b).

Despite the promising results demonstrated in this study, the use of ISFET and aptamer has some limitations. First, it is still a difficult task to fabricate ISFET with sensitive surface that allows to detect small values of signal on baseline drift over time. Secondly, limitations come from aptamer selection scheme. The binding ability of the aptamer at different molarities of the solution showed that there is no guarantee the aptamer will work in a buffer solution other than the solution in which it was selected. Thus, when initially selecting an aptamer, it is necessary to take into account not only the potential interferents, but also the conditions under which the further analysis will be performed. Despite these limitations, the use of the combination of ISFET and aptamer showed successful results with correct approach, as demonstrated in this paper.

Conclusion

In summary, for the first time, an aptamer for vanillin was successfully selected by applying the Capture-SELEX protocol. This aptamer was further used to develop an ISFET biosensor with a dehybridisation scheme. The LOD of the biosensor was estimated as 1.55×10^{-7} M, with a concentration range from 1.55×10^{-7} M to 1×10^{-6} M. Notably, the sensitivity and selectivity of the biosensor were comparable to other existing detection methods of vanillin. This novel approach may provide a powerful screening tool for on-site vanillin detection.

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Data availability The data that support the findings of this study are available from https://ndownloader.figshare.com/files/9605290. https://doi.org/10.6084/m9.figshare.5545492.

Compliance with ethical standards The author(s) declare that they have no competing interests.

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