engineering proceedings

MDPI

Proceedings

Design of an Electrochemical Genosensor for the BDNF Gene Polymorphism Sequence Detection Using an Enzymatic Labelled DNA Probe

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Abstract: The BDNF gene is associated with high degrees of variability in antidepressant treatments. The Val66Met polymorphism is widely known as a source of this variability, warranting growing interest in genotyping patients that undergo antidepressant treatment to better suit their needs. This paper reports on an electrochemical genosensing platform, based on gold electrodes, capable of detecting this polymorphism, through the use of synthetic enzymatic labelled DNA-probes for 2 different *BDNF* alleles. The sensor showed promising results, and its applicability to real samples is currently being tested.

Keywords: Chronoamperometry; Depression; Electrochemical genosensor

Citation: To be added by editorial staff during production.

Academic Editor: Firstname Lastname

Published: date

1. Introduction

Major depressive disorder (MDD) is an incapacitating psychiatric illness, affecting around 280 million people worldwide [1]. Antidepressant drugs (AD) have remained the main pharmacological treatment for this condition since their discovery in the 1950s [2]. Despite the good tolerability and high efficacy of current medications, along with the progressive development and introduction of new ADs throughout the decades, treatment-resistant depression, side-effects that result in treatment abandonment and insufficient remission rates remain a cause of public health concern [3,4]. In fact, 50% of patients with MDD do not respond positively to first-line treatments or respond only minimally to monotherapy [5]. Evidence suggests that neuroplasticity-related genetic factors play a significant role in the pathophysiology of depression and AD response [6,7]. BDNF is an important neurotrophin involved in neuroplasticity processes and other brain-related disorders [8]. The BDNF gene is one of the most thoroughly studied genes regarding depression and AD response [9–11]. Furthermore, the rs6265 (Val66Met) non-synonymous single nucleotide polymorphism (SNP) in this gene has been established as a source of genetic variation [12]. Pharmacogenetic studies reveal great heterogenicity regarding this SNP and AD response, with some studies showing better remission rates in Met allele carriers [13], while others have shown better outcomes in

Val/Val genotypes [14]. As a result, there is a growing interest in patient genotyping to detect specific SNPs, such as Val66Met, to predict the most favorable treatment outcomes. Current SNP detection methodologies are vast, being usually based on the polymerase-chain reaction (PCR) technique [15]. Although this approach has been extensively optimized, this methodology also presents some disadvantages such as requiring expensive equipment, highly trained personnel, as well as being time consuming. As a result, cheaper and more flexible genotyping tools, like electrochemical genosensors, are attractive alternatives for the potentially faster and accurate detection of SNPs, namely the Val66Met SNP.

Electrochemical biosensors have been shown to present several advantages over conventional molecular biology techniques, such as low operation costs, fast experiment processing times and portability. More specifically, electrochemical genosensors are highly specific and sensitive platforms, that can be used to detect a quantitative signal derived from a hybridization event between an immobilized single-stranded DNA probe (ssDNA) on the electrode and its complementary DNA. Considering these devices as alternative and promising tools to standard methods, this work aims to report on the first disposable electrochemical genosensor device on a gold electrode for the sensitive and selective detection of the Val66Met SNP, by targeting two specific 74 bp fragments of the coding sequence: one for the Val allele genotype (target C) and the other for the Met allele genotype (target T). Additionally, electrochemical detection was carried out employing chronoamperometric detection techniques and the sensor was successfully applied for the detection of the Val66Met SNP.

2. Methods

The methodology consisted in the immobilization of a synthetic DNA-capture probe (39-mer), functionalized with a thiol group and complementary to the target T onto a disposable screen-printed gold electrode (SPGE). To enhance selectivity and overall hybridization efficiency, a sandwich hybridization format of the target C and T was designed using a fluorescein isothiocyanate (FITC) labelled signaling probe to which anti-fluorescein antibodies labelled with horseradish peroxidase (POD) enzymes were attached. The enzymatic amplification of the analytical signal was obtained by chronoamperometry using a POD/H_2O_2 system.

The optimization of the developed genosensor protocol involved, in short, four steps: a pretreatment step, a sensing phase, the sandwich format hybridization and the electrochemical detection, as seen in Figure 1.

The pretreatment phase consisted in washing the SPGEs with ethanol and Milli-Q ultrapure water.

Secondly, a self-assembled monolayer (SAM) interface step was carried out, to ensure the orientation of the SNP DNA capture probe´s orientation. This SAM is comprised of the linear capture probes and MCH, in which 3 μL of DNA capture-T probe solution (0.5 μM) was immobilized onto the SPGE´s working electrode and stored in a humified and opaque Petri dish for 24h. Afterwards, the modified SPGE was rinsed twice with 200 μL of SSPE 2x buffer, eliminating the weakly attached DNA probes and 3 μL of MCH (0.5 mM) was added to the electrodes (sensing phase).

The sandwich format hybridization assay was achieved through a two-step hybridization (homogeneous and heterogeneous). The use of the sandwich hybridization format increases the selectivity of the assay. Firstly, the homogeneous hybridization occurs when the DNA-signalling probe (50 μM) binds to the DNA target in the buffer solution for 30 minutes. Then, the heterogeneous hybridization reaction is promoted, by adding the resulting solution of the homogeneous hybridization step to the modified electrode, binding the target/signalling probe solution to the immobilized DNA-capture probes for 60 min at 25ºC. After an hour, all electrodes were rinsed twice with 200 μL SSPE 2x to remove any non-specifically sequences (sandwich format hybridization). To uncover the electrochemical signal, POD enzyme in PBS/Casein buffer solution was applied to the

modified SPGEs surface for 15 minutes, before being rinsed. Lastly, the novel genosensor was connected to the potentiostat and 40 μL of commercial TMB/H_2O_2 substrate was added onto the electrode for 1 min. The enzymatically oxidized product was detected by chronoamperometry at -0.1 V, for 1 min (electrochemical detection). Three replicates were made for all measurements.

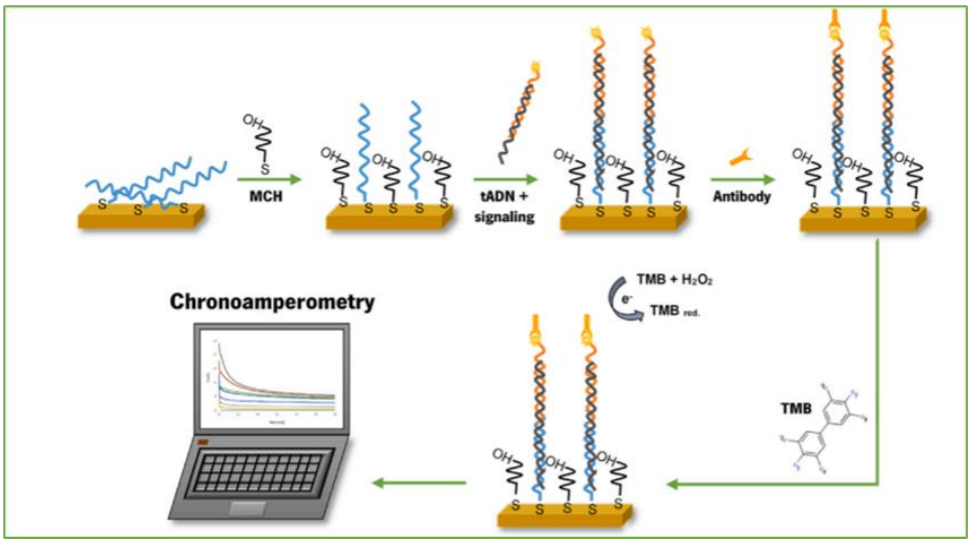

Figure 1. General procedure for the development of the electrochemical genosensor (adapted from [16] with permission).

3. Results and Discussion

For the construction of the SNP-specific electrochemical genosensing platform, a DNA oligonucleotide sequence specific to Val66Met SNP genetic variation is needed. In our work, two 74-mer synthetic oligonucleotide sequences (one for the Val allele variant, target-C, and another for the Met allele variant, target-T) were selected.

Two complementary sequences to the DNA-target probes C and T were utilized: a 35 bp DNA-signalling probe and a 39 bp thiolated DNA-capture probe, complementary for either the target-C or target-T probes.

For the development of the SPGE-based genosensor, capable of differentiating the Val66Met genotypes, all the experiments were carried out using both DNA-target probes (Target-C and Target-T), with the DNA-capture T probe as the complementary probe.

Many of the experimental parameters, namely DNA-capture and DNA-target probes concentration, MCH concentration and incubation time, homogeneous and heterogeneous hybridization steps, anti-FITC-POD antibody concentration and incubation time, involved in the development of the genosensor were optimized.

The experimental variables were optimized according to the largest ratio obtained between the chronoamperometric signals measured at -0.1 V (vs the Ag pseudo-reference electrode) for 0 (blank, B) and 0.2 nM of synthetic target DNA (signal, S): signal-to-blank or S/B ratio.

Table 1 summarizes all the optimized parameters, as well as tested ranges and selected values, in the development of the genosensor.

Table 1. Optimization of the developed electrochemical genosensor.

Parameters	Tested Range	Selected Value
DNA-capture probe concentration (μM)	0.25-1.0	0.5
MCH incubation time (min)	0-60	30
MCH concentration (mM)	0.25-1.0	0.5
Homogeneous hybridization incubation time (min)	15-60	30
Heterogeneous hybridization incubation time (min)	30-120	60
Homogeneous hybridization incubation temperature (ºC)	25; 98	25
Antibody concentration (U/mL)	0.5-2	1
Antibody incubation time (min)	15-60	15

After the optimization of the sensor, different concentrations of both DNA-target probes were tested, in order to establish a linear relationship between target concentration and current intensity. In fact, a calibration curve with a linear range of 0.10 $nmolL^{-1}$ – 2.0 $nmolL^{-1-}$ was obtained.

4. Conclusion

In this paper, the development of a novel, disposable electrochemical genosensor, capable of detecting the Val66Met BDNF gene polymorphism with high selectivity is described. The genosensor was constructed through the use of a SAM interface in a sandwich format on a gold electrode. Moreover, 2 enzymatic labelled DNA probes of the rs6265 SNP, one for each allele, were successfully detected through chronoamperometric techniques, employing a POD/H_2O_2 system, with distinct electrochemical currents achieved between the complementary probe (DNA-target probe T) and non-complementary probe (DNA-target probe C). Additionally, a calibration curve as obtained, linear in the range 0.10 $nmolL^{-1}$ – 2.0 $nmolL^{-1}$.

Some parameters, such as the detection limit are currently being optimized. Additionally, the genosensor is also being tested on real samples, paired with PCR techniques, to warrant its real-world use.

Author Contributions: Conceptualization, M.S. and M.F.B.; methodology, R.C., S.M., S.C., R.M., C.D-M., A.C, M.S. and M.F.B.; validation R.C., S.M., M.S and M.F.B.; formal analysis, R.C., S.M., M.S. and M.F.B.; investigation, R.C., S.M., M.S. and M.F.B.; resources, R.C., S.M., S.C., R.M., C.D-M., A.C, M.S. and M.F.B.; data curation, R.C., S.M., M.S. and M.F.B.; writing—original draft preparation, R.C., S.M., M.S. and M.F.B.; writing—review & Editing, R.C., S.M., S.C., R.M., C.D-M., A.C, M.S. and M.F.B.; visualization, .C., S.M., S.C., R.M., C.D-M., A.C, M.S. and M.F.B.; supervision, C.D-M., A.C., M.S. and M.F.B.; project administration, M.S. and M.F.B.; funding acquisition, C.D.-M. and A.C.; . All authors have read and agreed to the published version of the manuscript.

Funding: This work was funded by the Bilateral Cooperation FCT/CAPES 2018/2019 Processo 4.4.1.00 CAPES (CAPES-FCT2017484310 P 200.137.174.210), the Ibero-American Program on Science and Technology (CYTED— GENOPSYSEN, P223RT0141) by the European Cooperation in Science and Technology (COST, ENOTTA-CA21147), and was also financially supported by Portuguese national funds through projects UIDB/50006/2020, UIDP/50006/2020, LA/P/0008/2020 from the Fundação para a Ciência e a Tecnologia (FCT)/Ministério da Ciência, Tecnologia e Ensino Superior (MCTES). Maria Fatima Barroso thanks FCT for the FCT investigator grant (2020.03107. CEECIND).

Conflicts of Interest: The authors declare no conflict of interest.

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