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Affinity Biosensors For Phenylketonuria Diagnosis: A Review of Bioreceptors and Transducers Strategies

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Abstract

Phenylketonuria (PKU) is an inborn error of metabolism which arises from the mutations in phenylalanine hydroxylase (PAH) gene. PAH enzymes hydroxylate phenylalanine to tyrosine in the presence of the cofactor tetrahydrobiopterin (BH4), molecular oxygen and iron. Mutations in PAH gene led to lack of one of the essential enzymes, phenylalanine hydroxylase (PAH). Lack of this crucial enzyme brings about the accumulation of L-phenylalanine and their metabolites in the newborns' blood, urine and other body fluids, causing skin lesions, epilepsy, microcephaly, eczema, and scleroderma and, if untreated, also cause mental retardation. The amount of serum phenylalanine of healthy individual, is expected to be measured in the range of 50-110 µM, while phenylalanine in phenylketonuria patients is in the range of 0.6-3.8 mM in serum and 20-60 mM in urine. Metabolic diseases such as phenylketonuria are rare diseases but these types of illness reduce the quality of life at serious levels. If these diseases can be diagnosed early by the help of detection methods, mortality and morbidity can be prevented. For this reason, early diagnosis of metabolic diseases provides a better quality of life for the patients. Today, phenylketonuria could be determined using microbial inhibition, chromatographic and spectrophotometric methods. In Turkey, phenylketonuria test is performed by colorimetric method in screening centers. However, since these methods are time-consuming and expensive, complex instrumentation, preliminary preparation and special laboratory facilities are needed, the need in this area cannot be fully met. For this reason, there is an urgent need to develop simpler, faster and more economical assay methods and make them readily available at the clinic. For this reason, the development of new techniques and/or devices is a great need to be addressed urgently. In this context,

use of affinity biosensors which are known with their sensitivity, selectivity, low cost and rapid response, to the following of phenylketonuria disease will ensure that these disadvantages are overcome. This review aims at presenting the bioreceptors to selectively and sensitively diagnose phenylketonuria by using different transducers in affinity biosensors.

Keywords: Phenylketonuria, affinity biosensor, phenylalanine, bioreceptor, transducer

1. INTRODUCTION

Phenylketonuria, an inherited autosomal recessive disorder, is caused by a lack of the hepatic enzyme L-phenylalanine-4-hydroxylase, comprised of the metabolic pathway of phenylalanine (Phe). In a healthy individual, phenylalanine hydroxylase is responsible for converting L-phenylalanine to tyrosine in the presence of tetrahydrobiopterin (BH4), oxygen and iron (JERVIS et al., 1953), (Crujeiras et al., 2015). This hereditary disorder causes the accumulation of L-phenylalanine in body fluids (Blau et al., 2010). Current treatment modalities have been based on the reduction of L-phenylalanine, as the high levels of L-phenylalanine in body fluids lead to neurocognitive problems (Gok et al., 2016). Today, the main goal of newborn and child health studies is; not only reduce mortality rates but also prevent other problems caused by health problems. For this reason, it is very important to diagnose and treat the phenylketonuria which causes serious health problems.

This review discusses the recent advances in the affinity biosensors and detection strategies for phenylketonuria diagnosis. Additionally, key challenges and opportunities in further development and applications were outlined. Emphasis is mainly pointed out the affinity biosensors which modified with different molecular recognition element (bioaffinity elements) for phenylketonuria detection.

1.1. The Diagnosis of Phenylketonuria with Affinity Biosensors

Bioffinity biosensors, that show selective biorecognition of specific target species for triggering signals, represent a major class of modern biosensors (Cosnier, 2005; Wang, 2006; Merkoçi, 2013). Technological developments starting with the research conducted by Clark and Lyons have been contributed to the design of high-performance biosensors in different fields for biomedical purposes (Rasooly et al., 2006; Tothill, 2009; Soper et al., 2006; D'Orazio, 2011). This technology enables us to successfully detect target species with bioaffinity molecules via maximum association and minimum dissociations (Liu et al., 2012). The association and dissociations directly depends on the type of molecular recognitions (*i.e.*enzyme, antibody, DNA, RNA, aptamer) which is one of the most important components in biosensors.

1.1.1. Nucleic acid-based biosensors

As phenylketonuria is arisen from the mutations in PAH gene, successful recognition of the mutation is one of the possible ways to detect the disease. In order to achieve, there is a need for ultrasensitive detection technique which can rapidly and simply detect mutated DNA sequences by the help of low-cost technique. Whereas several analytical devices are found for DNA diagnostics, these techniques are expensive, time-consuming and needing well-equipped laboratories to operate. A variety of nucleic acid-based biosensors have been developed to meet the demand for simple portable devices for DNA diagnostics (Labuda et al., 2010). The working principle of nucleic acid-based biosensor could be summarized as the identification of biomarker with DNA or RNA based probe and the transformation of the biorecognition incident into signal after the hybridization of DNA probe and complementary sequence (Palchetti, 2014).

Aghaei et al. (2017) conducted a research about the detection of single nucleotide mutation on chromosome 12q23.2 which is arisen from G to A transition at position 546 on intron 10 of the PAH gene for the screening of the phenylketonuria. A summary of working principle of DNA biosensor based on hematoxylin tagged screen-printed gold electrode could be seen in Figure 1 (Aghaei et al., 2017). In literature, raman spectroscopy (Brauchle et al., 2014), fluorescent (Sun et al., 2015), surface plasmon resonance (SPR) (Ding et al., 2015), calorimetric (Jung et al., 2015), X-ray crystallography (Bao et al., 2015), quartz crystal microbalance (QCM) (Kargl et al., 2015), and mass spectrometry (Marrazza et al., 1999) have been studied to detect the single nucleotide DNA mutations. But Aghaei et al. were chosen electrochemical transducer due to others' disadvantages like high cost, the need to use simple processes for synthesis, and, longer analysis time. Screen printed gold electrodes were used and working electrode were decorated with thiol modified oligonucleotide probe 5'HS-(CH2)6-SH-TGTGGACCCAACGTGAAAAGT-3') to detect complementary target DNA. After the hybridization, hematoxylin was accumulated on surfaces to monitor reduction signal with differential pulse voltammetry. The working ranges for the designed screen-printed gold electrode were reported to be 20 pM–150 nM, and the limit of detection (LOD) was 8.5 pM. Additionally, the recovery values in simulated real samples were found 98.12 % (Aghaei et al., 2017).

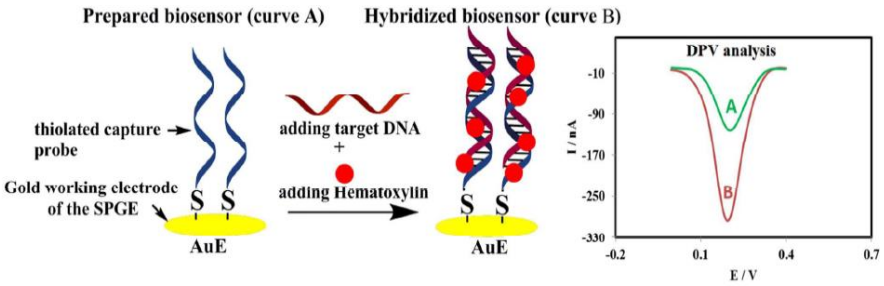

Figure 1. The development of a DNA biosensor for the diagnosis of phenylketonuria (Aghaei et al., 2017)

1.1.2. Enzyme-based biosensors

Enzyme-based biosensors have been extensively studied in qualitative and quantitative analysis of a variety of analytes in biomedical applications and some of them have been commercialized like self-monitoring of blood glucose levels (Heller et al., 2008; Ispas et al., 2012). Moreover, enzyme-based biosensors can be utilized in food freshness, analyzing of glucose content in drinks, detection of cholesterol in butter, food components of sugars, infective organisms test (Kurbanoglu et al., 2020). As it has been known that phenylketonuria is caused by the lack of phenylalanine hydroxylase enzyme which catalyzes the L-phenylalanine to convert tyrosine in the presence of molecular oxygen and tetrahydrobiopterin, the usage of phenylalanine hydroxylase as bioaffinity molecule is sensitive way to measure phenylalanine concentration, thus phenylketonuria. Therefore, several enzyme-based biosensors have been developed (Villalonga et al., 2007; Weiss et al., 2007; Naghib et al., 2012; Naghib et al., 2014).

Villalonga et al. (2007) were studied to detect the change in amperometric response after the enzymatic reaction of L-phenylalanine by the help of functionalized surfaces with phenylalanine dehydrogenase (Figure 2). Gold electrodes were chosen and modified with perthiolated-cyclodextrin to immobilize adamantane modified phenylalanine dehydrogenase. Sodium 1-adamantanecarboxylate were used to immobilize enzyme to cyclodextrin with the host-guest associations. The linear range of developed affinity biosensor were found 20 μM-3 mM and the sensitivity was observed as 21.1 nA/mM. The quite low value for limit of detection was found 15 μM (Villalonga et al., 2007).

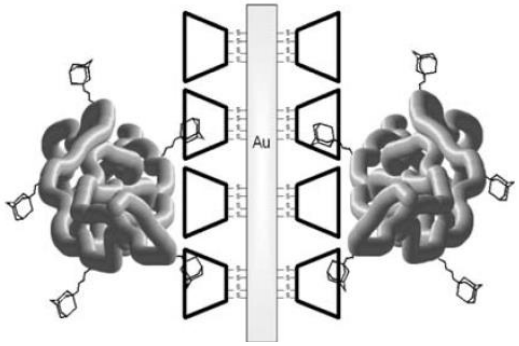

Figure 2. Supramolecular-mediated immobilization of adamantane-modified phenylalanine dehydrogenase on CD-coated Au electrodes (Villalonga et al., 2007).

Then, Weiss et al. reported a research about the development of enzyme-based biosensor for the measurement of phenylalanine in urine to diagnose phenylketonuria. During the experiment, unique electrodes comprised of a carbon paste electrode with nicotinamide a nicotinamide adenine dinucleotide (NAD^+), phenylalanine dehydrogenase, uricase, and 3,4-dihydroxybenzaldehyde (3,4-DHB) were used to detect phenylalanine. As a result of the studies, the detection limit of the modified sensor was found to be 0.5 mM. The selectivity studies were also performed and the biosensor response was not affected in the presence of ascorbate, urate, tyrosine and alanine (Weiss et al., 2007).

Naghib et al. developed enzyme-based biosensor by immobilizing phenylalanine dehydrogenase on dextran-based polymer. First, polymer-blend film was coated with dextran, polyvinylpyrrolidone and carboxymethylcellulose in the presence of lysosomal protein and glutaraldehyde with spin coating to immobilize enzyme, phenylalanine dehydrogenase. Then, enzyme immobilized electrode surface was coated with the semi-permeable cellulose acetate membrane to increase stability of electrode response (Figure 3). The performance of electrodes was tested for amperometric detection of phenylalanine and the results shown that the working ranges for the electrode were in range of 0.5 mM–6 mM. The sensitivity of enzyme electrode was 12.014 mA/M cm^2 and the LOD was 0.5 mM (Naghib et al., 2012).

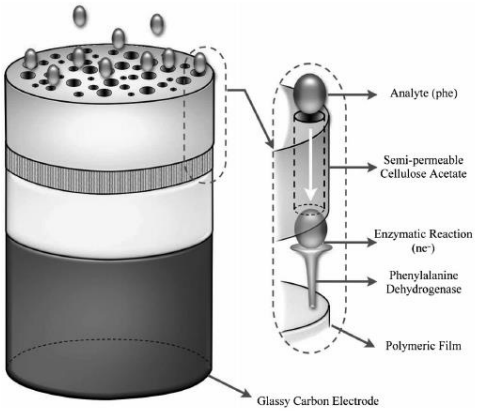

Figure 3. Schematic representation of electrode (Naghib et al., 2012).

Naghib et al. were again conducted research to developed affinity biosensor based on the direct detection of phenylalanine with phenylalanine dehydrogenase immobilized on graphene oxide nanosheets (GO)-chitosan modified gold electrode (Figure 4). The performance of electrodes was enlightened with differential pulse voltammetry. The linear range of enzyme electrode was found between 0.5 to 2.5 mM. The LOD value was found as 416 nM. The selectivity of enzyme electrode was also studied in the presence of glucose, estriol, dopamine, glycin, L-cysteine, ascorbic acid and ethanol and negligible increment in signal was seen in voltammogram (Naghib et al., 2014).

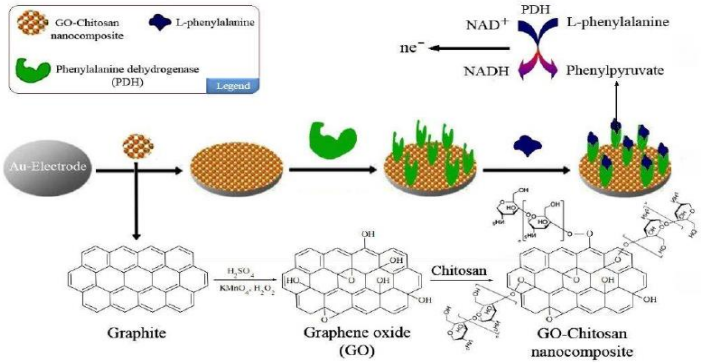

Figure 4. Schematic representation of proposed biosensor (Naghib et al., 2014).

In 2018, electrochemical paper-based enzymatic platform coupled to screen printed graphene modified electrode was developed to monitor the level of phenylalanine (Figure 5). The performance of biosensor was measured with differential pulse amperometry. The linear range of enzyme-based biosensor was reported as 5-600 µM and the LOD value was 0.2 µM. The selectivity test conducted with bilirubin, hemoglobin, cysteine, tryptophan, tyrosine, leucine, methionine and ascorbic acid. Only ascorbic acid caused an increment about 10.2% in background signal. However, added value of ascorbic acid during selectivity test was too high compared to physiological level. Therefore, the method was found quite sensitive to phenylalanine (Moreira et al., 2018).

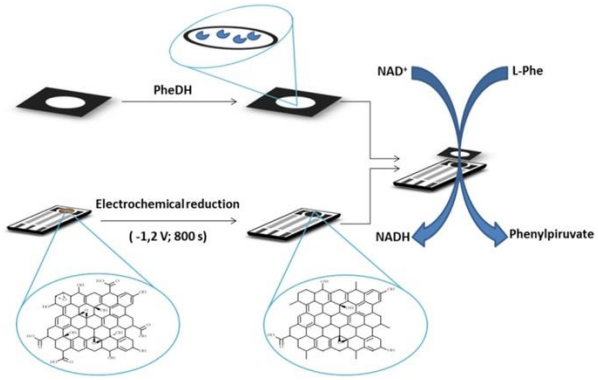

Figure 5. Schematic representation of paper-based enzymatic platform coupled to screen printed graphene modified electrode and its working principle (Moreira et al., 2018).

Recently, a new enzyme-based biosensor was developed by Arslan et al. (2020). The working principle of biosensor based on the amperometric detection of phenylalanine with phenylalanine dehydrogenase and toluidine blue O decorated platinum electrode. The linear range of biosensor was found 0.01-0.25 µM and the LOD value was estimated as 1 nM. The selectivity of proposed biosensor was shown in the presence of ascorbic acid, uric acid, ethanol, methanol, paracetamol, cystine, cysteine and glucose and no significant differences in signal after the addition of these molecules was observed (Arslan et al., 2020).

1.1.3. Aptamer biosensors

Then, aptamers are unnatural nucleic acid ligands demonstrating particular binding affinity for amino acids, drugs, proteins and other molecules which were developed in 1990s for in-vitro selection and amplification for the isolation for RNA sequences that could particularly bind to target molecules (Tuerk et al., 1990; Ellington et al., 1990). These functional RNA oligonucleotides were then named as aptamers (Jayasena, 1999). Subsequently about 20 years', DNA and RNA aptamers have been categorized as adhering firmly to a wide range of targets (for instance; proteins, peptides, amino acids, drugs, metal ions) thanks to the constructing of fast, automated, selection technologies (Song et al., 2008). In literature, only

one research which was used aptamer as bioaffinity molecule for the detection of phenylketonuria has been found. Hasanzadeh et al. were developed apta-assay based on the usage of aptamer immobilized on gold nanoparticle modified gold electrode (Apt/AuNSs/Au) (Figure 6). The performance of aptasensor was tested with differential pulse voltammetry and voltammetric results showed wide linear ranges around 0.72 µM-6mM. The LOD values were 0.23 µM. Additionally, the selectivity of the Apt/AuNSs/Au electrode was also studied in the presence of Tyrosine, Glycine, Serine, l-Valine, Tryptophane, Arginine, Proline, Aspartic acid, Methionine, and Leucine and no significant effect caused by interfering compounds was observed in voltammograms (Hasanzadeh et al., 2018).

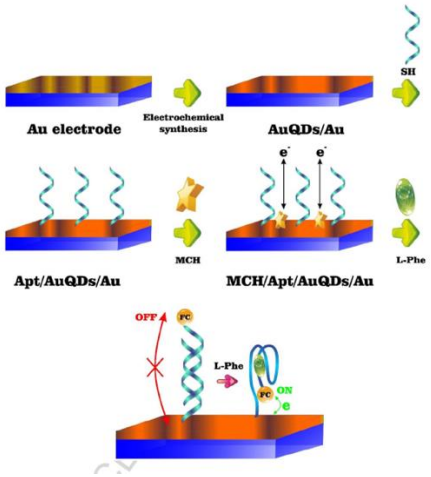

Figure 6. Schematic representation of the fabrication and working principle of Apt-AuNSs-Au electrode (Hasanzadeh et al., 2018).

2. DISCUSSION AND CONCLUSION

Many affinity biosensor-based assays for the detection of different diseases have been reported in literature up to now. This trend has been shown us the importance of affinity biosensor technology in biomedical applications.

The literature reviewed in this paper demonstrates that affinity biosensors are able of describing and measuring phenylketonuria (PKU) disease. With an importantly low detection limit (8.5 pM), wide dynamic range (20 pM to 150 nM), and good results in real sample analysis, this biosensing system can promisingly ease the analysis of DNA sequences in clinical and biomedical applications, especially for detection and early screening of the PKU.

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