Amperometric Biosensors

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Amperometric biosensors utilizing oxidoreductases were classified into three generations: 1st generation biosensors employing oxidases based on the electrocatalytic monitoring of substrate consumption or product formation, 2nd generation biosensors employing oxidases or dehydrogenases based on the electrocatalytic recycling of suitable redox mediators, and 3rd generation biosensors employing oxidoreductases capable of direct electron transfer to bare or modified electrodes.

Keywords: biosensor ; current density ; direct electron transfer ; enzyme ; selectivity ; sensitivity ; nanomaterial ; self-assembled monolayers ; nanocomposite ; nanoparticle

1. Introduction

Chemical sensors consist of a chemical recognition system, a physicochemical transducer and a signal processing unit that transforms the concentration of the analyte into a quantifiable signal. A biosensor employs a biological recognition (biorecognition) element working on the principle of bioaffinity or biocatalysis. Bioaffinity sensors use receptor proteins, antibodies, or polynucleotides as biorecognition elements, whereas enzymes are used as bioelectrocatalysts. Several types of signal transducers can be distinguished, for example: mass-sensitive, optical, and electrochemical. Electrochemical biosensors can be based on the detection of a measurable current (amperometric), the accumulation of charge (potentiometric), changes in current as a result of a potentiometric effect at a gate electrode (field effect), changes in the conductive properties of a medium between electrodes (conductometric), or the changes of resistance and reactance close to an electrode (impedimetric).

Amperometric biosensors utilizing oxidoreductases were classified into three generations (**Figure 1**) as indicated above. Direct electron transfer (DET) forms the basis of 3rd generation biosensors [1]. DET is the ability of a solubilized or immobilized redox active protein to exchange electrons with an electrode upon contact; for example, oxidoreductases directly donating electrons gained from substrate oxidation to the electrode. Therefore, 3rd generation biosensors can be operated at lower potentials (close to the midpoint potential of the enzyme's prosthetic group) than those needed for the detection of enzymatic reaction products, e.g., hydrogen peroxide, in 1st generation biosensors, or even the redox potential needed to apply redox mediators to shuttle electrons between the enzyme cofactor and the electrode in 2nd generation biosensors. The specificity of 3rd generation biosensors is thus less affected by electroactive interferents like ascorbic acid and also by the absence of the redox active mediator.

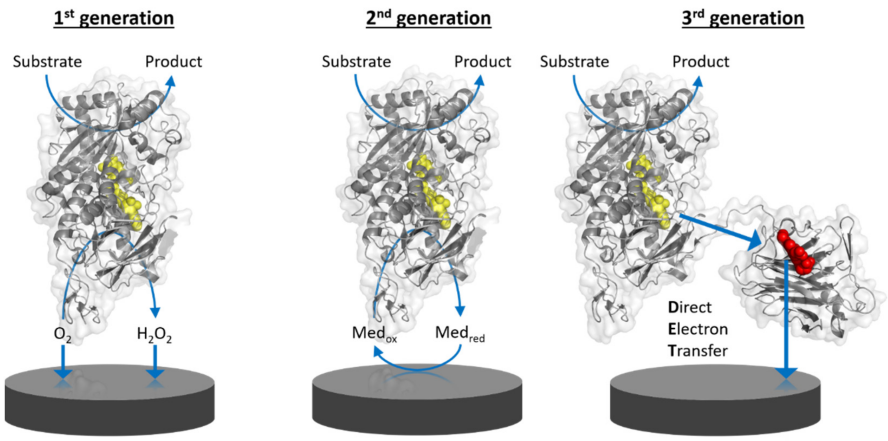

Figure 1. Schematic representation of the working principle of the three generations of enzyme-based amperometric biosensors. 1st generation: based on the electrocatalytic detection of enzymatic substrate conversion or product formation. 2nd generation: based on the electrocatalytic recycling of a redox mediator. 3rd generation: based on direct electron transfer from enzyme to electrode. Yellow molecules depict the FAD cofactor of flavoenzymes and the red molecule depicts the heme cofactor in the mobile cytochrome domain of a multi-cofactor enzyme.

2. Applications and Challenges of DET-Based Biosensors

2.1. Applications for DET-Based Biosensors

Potential applications of amperometric biosensors range from the monitoring of environmental markers like pesticides, medical analytes in blood or urine like glucose, lactate, and cholesterol, biomarkers for cancer or diabetes, to various industrially important substances as saccharides, alcohols, and phenols for process monitoring. Moreover, biosensors for the monitoring of cellulose-degrading enzymes and lignocellulosic biomass hydrolysis have been proposed [2][3]. Interestingly, enzymes for application in 3rd generation amperometric biosensors are mostly heme, flavo-, or quino-enzymes, but copper enzymes are also known to interact with electrodes through DET (**Figure 2**).

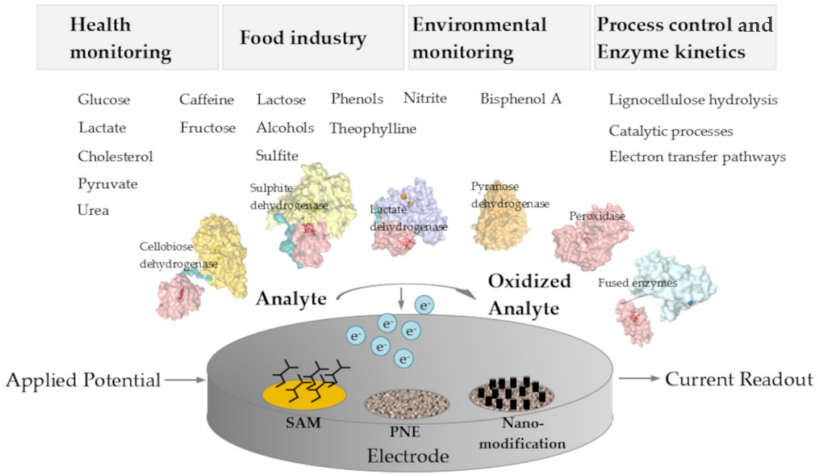

Figure 2. Schematic overview on application areas, analytes, enzymes, and the architecture of 3rd generation amperometric biosensors. SAM, self-assembled monolayer; PNE, porous nanostructured electrodes.

2.2. Challenges of DET-Based Biosensors

The prerequisite of direct electrochemistry with a reasonably high DET rate is a close proximity of the enzyme's prosthetic group to the electrode surface, since the electron transfer rate decreases exponentially with the distance, i.e., by a factor of ~10^4 when the distance is increased from 8 to 17 Å [4][5][6][7]. However, many articles claim DET, e.g. for glucose oxidase employed in glucose biosensors, but omit the presentation of appropriate data and reference experiments. DET is likely to occur when (1) the onset potential of the electrocatalytic reaction is close to the redox potential of the prosthetic group in the enzyme, (2) a significant catalytic current is measured upon substrate addition but does not occur when adding a similar substance that is not a substrate of the enzyme (e.g. L-glucose), and (3) the experimental design rules out the presence of free cofactors which can serve as redox mediators or other electroactive substances generating current. Notably, electrocatalytic currents obtained from the oxidation of H_2O_2 ($H_2O_2 \rightarrow 2H^+ + O_2 + 2e^-$, E^0 = 0.69 V vs. NHE [8]), or weakly bound coenzymes or prosthetic groups like NAD^+/NADH [9][10][11] or PQQ [12], may feign DET but actually act as redox mediators. The close distance between the cofactor and the electrode surface necessary for DET can be achieved either by employing single cofactor, multi-cofactor, or fusion enzymes with surface-exposed prosthetic groups or by means of electrode design and engineering employing chemical or nanomaterial modifications like carbon nanotubes, graphene, and their nanocomposites, which serve as electron relay between the buried redox center and the electrode [13] or the linking of artificial redox mediators to the enzyme itself [14][15][16].

3. Conclusions and Future Perspectives

Direct electrochemistry of oxidoreductases is utilized to generate biosensors for simple and low-priced on-line quantification of analytes. The analyte spectrum covers a broad range of relevant analytes like saccharides, phenolic compounds, and small molecules such as sulfite or alcohols, which are relevant for environmental monitoring, compounds of the food industry, and products of biotechnological processes. Many reports in the literature focus on glucose detection because of the high medical relevance for blood glucose determination, although much more enzymes with suitable electrochemical properties exist and can be applied to detect other substrates. Several of those are capable of DET and can be used for 3rd generation sensors when combined with the correct combination of electrode materials and various electrode modifications. The performance of DET-based biosensors has to be carefully reported and the necessary control experiments to rule out mediated electron transfer should be provided. Despite this point of caution, many studies on DET enzymes and 3rd generation biosensors are performed with great care and show a high level of insight and expertise. Some research groups particularly focus on the optimized electrode material design employing nanomaterials

and investigate optimized fabrication and protein immobilization strategies to achieve biosensors with higher sensitivity and stability. To date only one commercially available DET-based amperometric 3rd generation biosensor (LactoSens) exists compared to a great number of existing 1st and 2nd generation biosensors. Since not all oxidoreductases are capable of DET, the fusion of enzymes with electron-transferring cytochromes is a very promising strategy to increase the number of bioelectrocatalysts for 3rd generation biosensors and to increase the number of analytes.

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