Direct and Mediated Electron Transfer in Enzyme Electrodes

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1 Introduction

The first experimental reports on the origin of bioeletrochemistry were related to the work of Luigi Galvani, in October 1786. In his work, Galvani demonstrated that a frog's leg twitched upon contact with a metallic arch at the extremities of the leg. Named "Animal Electricity", this work is evidence of the origin of bioelectrochemistry long before electrochemistry itself [1–4]. Over 200 years after this experiment, bioelectrochemistry has advanced so as to be able to manipulate biomolecules, connect them with solid electrodes, and ultimately build and apply bioelectrochemical devices [5–8]. These biodevices' performance depends on understanding all physical and chemical properties of both the redox biomolecules and the electrodes that support enzyme immobilization. Thus, this chapter will discuss the processes of enzyme immobilization and electron transfer, focusing on the enzyme glucose oxidase.

2 Redox Enzyme—Glucose Oxidase

Several biological processes depend on electron transfer processes that occur between molecules undergoing oxidation and reduction. This redox process generates the energy needed for the development of biological functions or the construction of electrochemical biodevices [9]. In the latter case, several redox enzymes are extensively used on solid surfaces, generating an optimal interface for electron exchanges to occur [10]. Because they belong to the oxidoreductase class, these enzymes act as

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F. N. Crespilho (ed.), *Advances in Bioelectrochemistry Volume 1*,
https://doi.org/10.1007/978-3-030-94988-4_2

Table 1 List of some redox enzymes and their active site

Enzyme	Active center	Ref.
Aldehyde dehydrogenase	Pyrroloquinoline quinone (PQQ)	[6]
Laccase oxidase	4 Cu (II) atoms	[11]
Alcohol dehydrogenase	Nicotinamide adenine dinucleotide (NAD)	[12]
Glucose dehydrogenase	Nicotinamide adenine dinucleotide (NAD)	[13]

excellent biocatalysts of specific substrates. Table 1 summarizes some redox enzymes and their active centers responsible for catalyzing their respective molecules.

One of the most classic enzymes used in bioelectrochemical devices is glucose oxidase (GOx), which often comes from *Aspergillus niger* fungi. GOx is a flavoprotein that catalyzes oxidation of β-D-glucose at its first hydroxyl group, using molecular oxygen as the electron acceptor, to produce D-glucono-delta-lactone and hydrogen peroxide. Sterically, GOx is dimeric, with its redox cofactor inside each monomer, called flavin adenine dinucleotid (FAD), as Fig. 1a, b show [14, 15].

Electrochemically, FAD undergoes oxidation according to the reaction in Fig. 2.

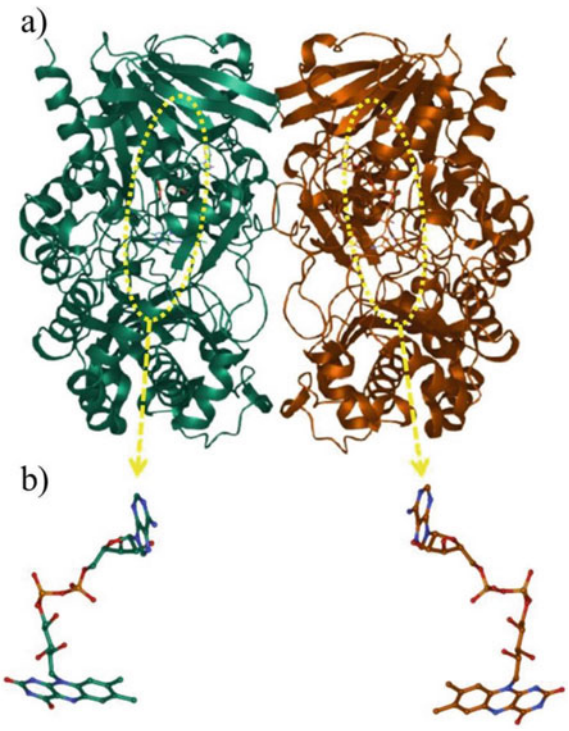

Fig. 1 **a** Computational representation performed on the protein data bank website for the GOx enzyme (1GPE) and for **b** FAD

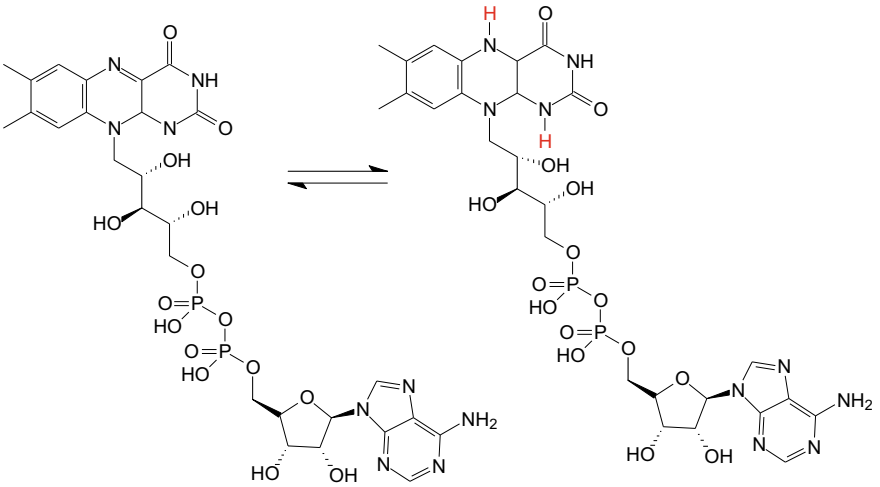

Fig. 2 Chemical structures of FAD and $FADH_2$

In the presence of two electrons and two hydrogen atoms, FAD, initially in the oxidized state, changes to the reduced state of $FADH_2$. This GOx redox cofactor reacts with glucose, according to the chemical reaction represented in Fig. 3 [15]. However, for an efficient electron transfer between the enzyme and its substrate on the solid surface of an electrode, the immobilization process becomes one of the biggest challenges for researchers, as we will discuss next.

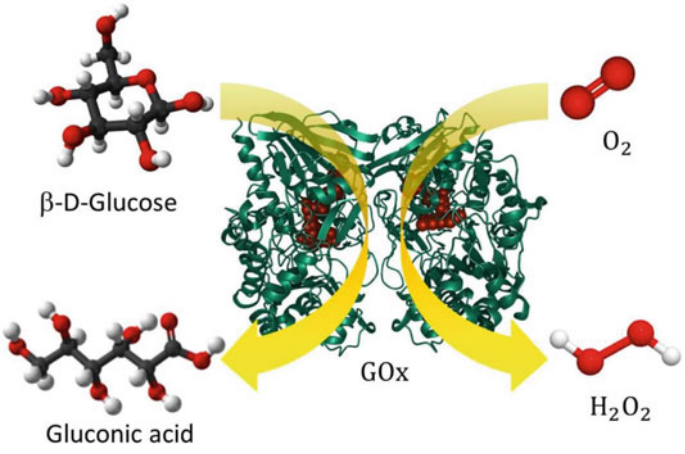

Fig. 3 **a** Schematic representation of the conversion of glucose into gluconic acid by the action of GOx-FAD

3 Enzymatic Immobilization Processes

The electron transfer process at the electrode/enzyme interface depends heavily on how the enzyme is immobilized on the electrode surface. The literature has classically reported five processes by which the enzyme can electronically connect with the electrode it is immobilized to. Each process has advantages and disadvantages for certain applications of this electrode biointerface. Figure 4 represents these immobilization processes: covalent bond, adsorption, cross-linking, electrostatic interaction, and affinity [16].

Immobilization through chemical bonding ensures strong fixation between the enzyme's functional groups and the electrode, thus promoting greater stability. However, the bioelectrode may generate a level of toxicity due to the chemical compounds used to fix the enzyme. In this respect, for in vivo applications, the presence of compounds that are bioincompatible with the environment can be an impediment to which the electrode will be applied [16].

Immobilization through physical adsorption can be considered the quickest and easiest of all methods. Another advantage is that chemical compounds are not needed to fix the enzyme, which makes the bioelectrode very applicable in in vivo implants. Nonetheless, in this technique the enzyme is weakly bound to the electrode surface, generating instability and inactivity of its active site [16].

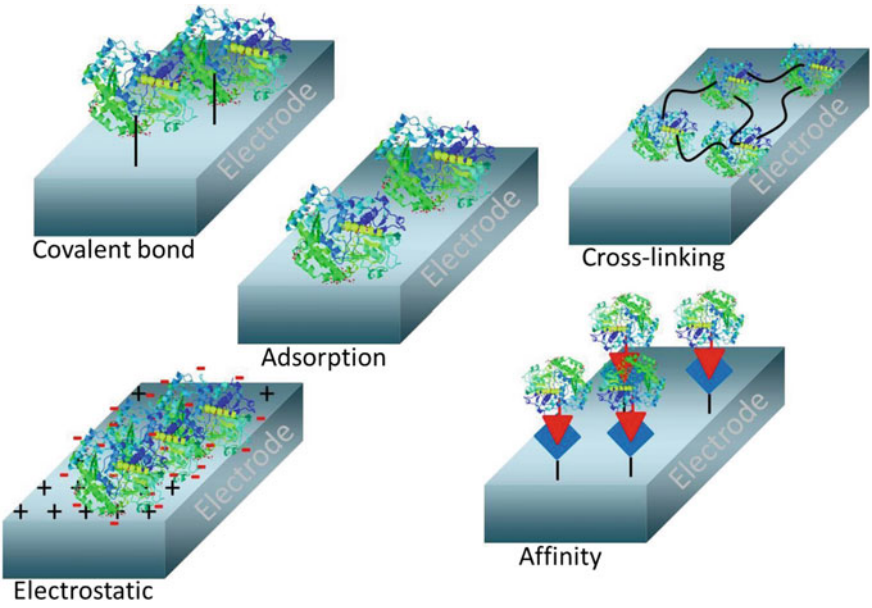

Fig. 4 **a** Schematic representation of the immobilization process in the electrode/enzyme interface

Both cross-linking and the biochemical affinity method require specific receptor substances and biomolecules, making the process a little more expensive and time-consuming. However, these processes guarantee electrode stability. In the case of affinity, other parameters must be considered, such as optimal pH of the medium and biocompatibility of the groups used to guarantee enzyme fixation on the electrode. Once immobilization on the electrode surface is completed, another aspect that must be investigated is the enzyme's structure and active site, as will be discussed below [16].

4 Direct Electron Transfer and Its Dependence on the Distance Between Enzyme and Electrode

A computer simulation performed on the PDB website with the structure of GOx (1GPE) showed that GOx exhibits its prosthetic group FAD in a non-centralized way inside each monomer, as Fig. 5 illustrates. The distances found from these simulations were 14.05, 19.36, 19.55, and 26.35 Å.

Some works have reported that the minimum optimal distance for the electron to tunnel from the enzyme to the electrode surface is 14 Å or less. In this sense, Fig. 7 shows four schematic representations (a–d) in which the enzyme succeeds or fails to exchange electrons with the electrode surface. As most immobilization processes do not guarantee the deposition of the enzyme through the face with the shortest distance, there is usually no direct electron communication at the electrode/enzyme interface. This is due to the volume of the enzyme's tertiary structure, which generates distances above 14 Å. This inhibits direct electron transfer, represented in Fig. 6a–c [17–19].

Martins and co-authors [19] presented an inherently effective strategy for direct electron transfer to occur using GOx and a graphene oxide-modified flexible carbon

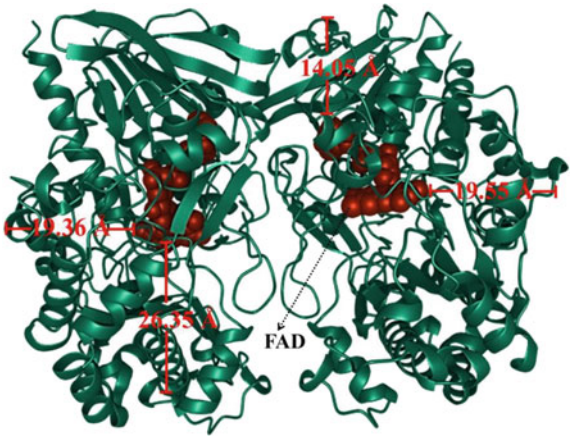

Fig. 5 **a** Schematic representation performed on the PDB website for GOx (1GPE)

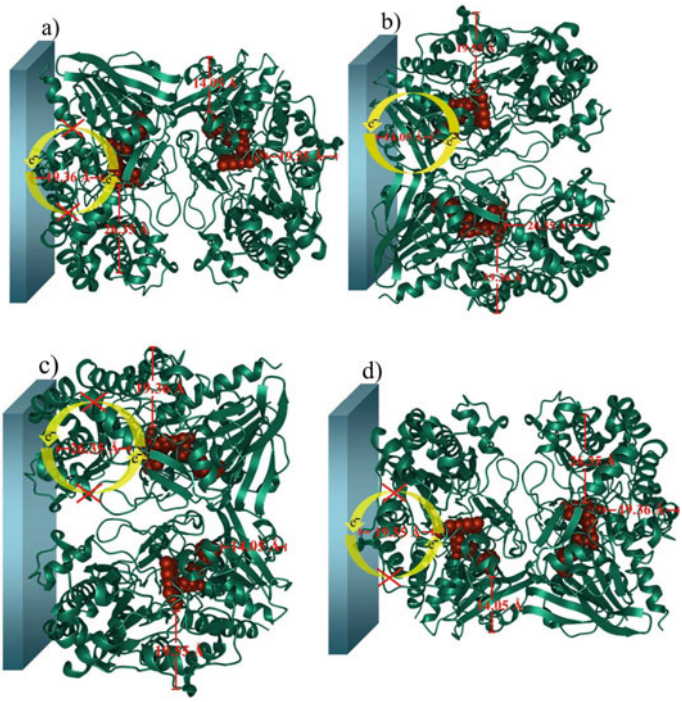

Fig. 6 Schematic representations **a–d** of the immobilization process through GOx's faces. Direct electron transfer only occurs when the enzyme adsorbs on the side that generates the shortest distance between FAD and the electrode surface

fiber (FCF) electrode. In their work, they showed that the presence of graphene oxide in the fiber structure shortens the distance between FAD and the electrode surface, ensuring fast and efficient electron exchange.

Marcus theory is another approach that can be considered in the study of distance dependence on the rate of electron transfer at the electrode/enzyme interface [20–22]. This theory holds that, for an enzyme adsorbed on a solid surface (homogeneous transfer), the oxidation k_{oxi} and reduction k_{red} rate constants require certain energy to re-accommodate all atoms under high overpotential conditions. This is known as reorganization energy (λ). Equation 1 is derived from Marcus theory and describes the rate of electron transfer using the Fermi–Dirac distribution, since electron transfer can occur to or from any Fermi level in the electrode:

$$k_{\text{red/oxi}} = \frac{k_{\max}}{\sqrt{4\pi\lambda}/RT} \int_{-\infty}^{\infty} \frac{\exp(-1/4\lambda RT[\lambda \pm F(E - E^0) - RTx])}{1 + \exp(+)} dx \qquad (1)$$

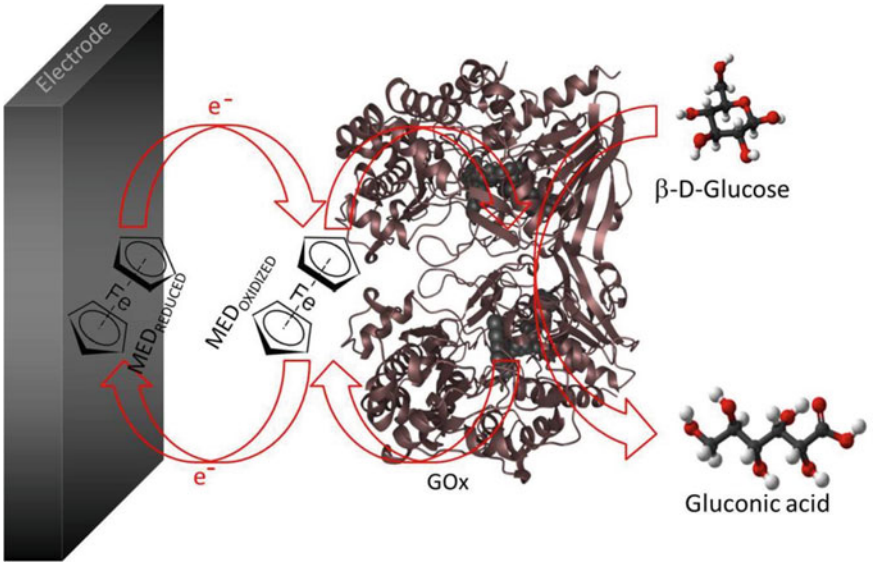

Fig. 7 Schematic representations of the electron transfer process between GOx enzyme and electrode surface mediated by ferrocene

where λ is the reorganization energy in eV (the energy required to reorient all atoms from the equilibrium state to the product state) and k_{max} is the maximum electron-transfer rate constant at high overpotential. Based on this theoretical simulation using the Marcus theory equation, the linear behavior obtained decreases linearly with increasing distance between the active enzymatic site and the electrode surface, as demonstrated by Marcus and co-authors [19].

5 Mediated Electron Transfer

Redox mediators are substances of low molar mass that can easily receive and donate electrons and thus facilitate the transfer of electrons between enzymes and the electrode surface. They are needed to mediate electrode/enzyme electron exchanges due to the distance between the redox site and the electrode. Some redox enzymes have a very bulky tertiary structure where the active site is located. This makes it impossible to tunnel the electron from the enzyme to the electrode. In this sense, numerous studies [18, 23–25] have reported the use of redox mediators in bioelectrodes. Depending on whether the application is in vivo or in vitro [7, 8], using mediator species in the electron exchange process may limit this application due to the toxicity that some mediators can generate. However, for biodevice applications that do not fall under these limitations, the great advantage of using a redox mediator is the ease of oxidizing and reducing the active site of an enzyme immobilized on an electrode

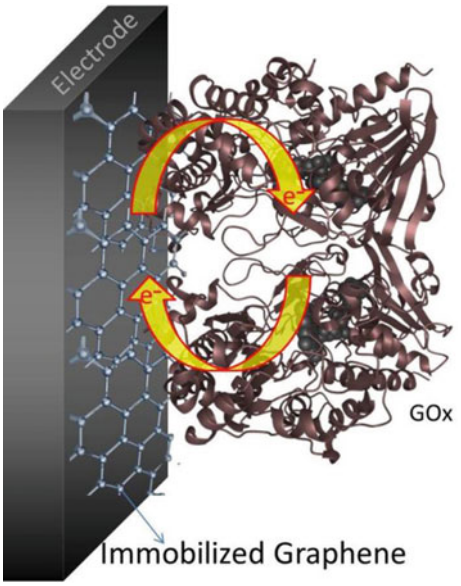

Fig. 8 Schematic representation of the electron transfer process between GOx enzyme and electrode surface mediated by graphene

surface. Figure 7 presents a scheme of how the redox mediator ferrocene acts in the electron exchange reaction between GOx and the electrode surface. Ferrocene both mediates electron exchange and enables FAD-GOx to react with more glucose molecules, generating gluconic acid as an end product.

Another advance in the field of mediated transfer at the electrode/enzyme interface is the use of nanomaterials (e.g., metallic nanoparticles [26], nanotubes [27], or graphene [19]). However, in this case, the communication between enzyme and electrode can be considered as facilitated rather than mediated. This consideration is because a nanomaterial generally does not have an oxidized and reduced state, as it occurs within the definition of a redox mediator species. Facilitation is due to the nanomaterial reducing the distance between the redox site and the electrode surface, as Fig. 8 illustrates.

Advances in the synthesis, development, manipulation, and functionalization of numerous advanced materials have also furthered the understanding of the properties of the electrode/enzyme interface applicable in bioelectrochemistry.

6 Conclusion

In this chapter, we discussed the physical and chemical properties at the electrode/enzyme interface, emphasizing GOx immobilized on a solid electrode surface. Some factors can limit charge transfer between an enzyme and an electrode. The distance between the enzymatic redox site and the electrode surface may be the

main factor for mediated or direct transfer of electrons in a bioelectrode to occur. However, the presence of nanomaterials and/or redox mediating species at the electrode/enzyme interface can reduce this distance, facilitating electron communication between enzyme and electrode as well as the catalysis of specific substrates in the presence of these enzymes.

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