Bridging the world of enzymes with electric fields

Integrando el mundo de las enzimas con los campos eléctricos

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Examines how electric fields influence enzyme catalysis and optimize biotechnological applications using advanced electrochemical methodologies. Describes electrochemical techniques such as protein film voltammetry to study enzymatic behavior under the influence of electric fields. Explore how electric fields improve the functionality of enzymes in industrial and medical applications, optimizing their stability and activity. This research significantly contributes to science and technology by introducing electric fields (EF) as a novel parameter in bioprocesses. It demonstrates how EFs influence enzyme catalysis, affecting the orientation and behavior of charged particles in biological systems, and disrupting biomolecular conformations to induce physiological changes. The study integrates advanced biotechnological techniques, showcasing the potential of EFs alongside traditional environmental factors like temperature, pH, and nutrient concentration. Furthermore, the research employs advanced electrochemical methodologies, such as nano-impact electrochemistry and protein film voltammetry, providing detailed insights into electron transfer and enzymatic mechanisms. Computational simulations further elucidate the effects of EFs on enzyme structure and function, particularly in redox reactions. These advancements enhance the industrial and medical applications of enzymes by optimizing processes and improving functionality under various conditions. By establishing EFs as an independent and reproducible variable, the study opens new avenues for future microbial and enzymatic research. Overall, this research offers groundbreaking knowledge in electrostimulation, setting the stage for innovative applications in biotechnology. Gain insights into enzymes from a thermodynamic perspective, linking electric field concepts and applications, and ultimately grasp their catalytic response to this stimulus. Electric fields significantly influence enzymatic catalysis through voltammetry and computational simulations, advancing biotechnological applications and providing a tool to modify catalysis.

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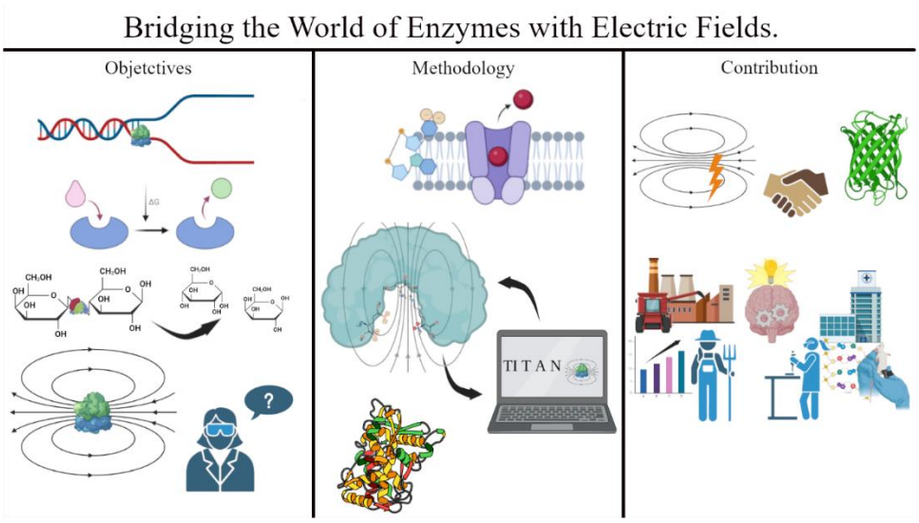
Abstract

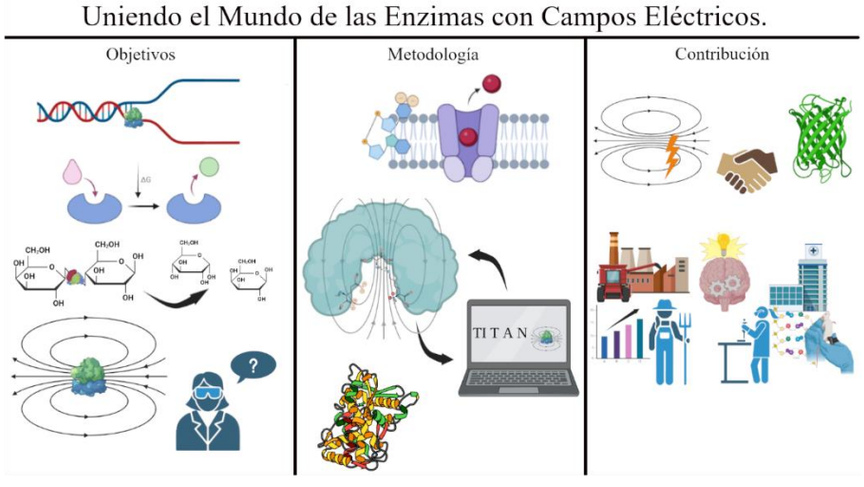
Enzymes are essential proteins involved in metabolism, gene expression, cell division, and immune responses. They play a significant role in industry due to their efficient catalysis of chemical reactions. The diversity of enzyme actions is attributed to their varying substrate specificities and reaction types. Recently, researchers have studied electric fields as a biophysical factor that can stimulate or inhibit biological or catalytic responses, although the mechanisms remain unclear. Understanding the role of enzyme amino acid structures and electric fields offers new insights into catalysis. It is crucial to establish a foundational understanding to comprehend these phenomena. By reviewing and relating fundamental concepts, we can broaden our interpretation and study of enzyme technology, leading to future research and potential applications.



Electrostatic, Catalysis, Biophysical

Abstract

Las enzimas son proteínas esenciales en procesos vitales como el metabolismo, la expresión génica, la división celular y las reacciones del sistema inmunológico. Son importantes en la industria por su eficiencia en catalizar reacciones químicas. La diversidad de acciones y aplicaciones de las enzimas se debe a sus diferentes especificidades de sustrato y reacción. En años recientes, investigadores han estudiado los campos eléctricos como factores biofísicos capaces de estimular o inhibir respuestas biológicas o catalíticas, aunque sus mecanismos no están completamente resueltos. Esto requiere comprender el papel de la estructura de los aminoácidos en las enzimas y los campos eléctricos, ofreciendo nuevos fundamentos sobre la versatilidad de la catálisis. Es esencial establecer un conocimiento básico que permita comprender estos fenómenos, revisando y relacionando conceptos fundamentales, para ampliar el panorama y lograr una mejor interpretación y estudio de la tecnología enzimática, con aplicaciones potenciales en futuras investigaciones.



Electrostática, Catálisis, Biofísica

Introduction

Enzymes play a crucial role in all stages of metabolism and cellular biochemical reactions. The planet's biodiversity has underscored the importance of certain enzymes, particularly those of microbial origin, which are extensively used in commercial-scale industrial applications (Nigram, 2013). Since the 20th century, there have been significant advances in studies related to the isolation, characterization, large-scale production, and bioindustrial applications of enzymes. Moreover, their manipulation has been made possible through techniques such as protein engineering, biochemical, metagenomic, and molecular techniques, enhancing the quality and performance of enzymes, thereby expanding their industrial applications (Chirumamilla *et al*., 2001; Nigram, 2013).

Consequently, the rapid development of the enzyme industry, as we know it today, has been particularly remarkable over the past four decades, thanks to significant progress in modern biotechnology (Kirk *et al.*, 2002). This ongoing advancement allows for the full exploitation of enzymes' potential in various industrial applications.

Enzymatic catalysis has expanded its application field to processes in the pharmaceutical, food, and beverage industries. However, to achieve optimal biocatalytic processes in the energy sector, such as the production of biofuels and natural gas conversion, further improvements in the stability and functionality of biocatalysts are required (Chapman *et al*., 2018).

The uniqueness of enzyme biological activity lies in the precision of their action, requiring minimal energy to catalyze a specific reaction (Ali *et al*., 2023). Although enzymatic design and manipulation are often approached from the perspectives of pH, substrate concentration, and temperature - crucial factors for catalysis - the exploration of other factors, such as electric fields (EF), has begun.

EF are considered biophysical factors capable of stimulating or inhibiting a biological response by exerting work to move a unit charge from one point to another in a given space. Due to the electrical properties and physicochemical nature of the material exposed to the field, it experiences forces of attraction and repulsion (Pataro *et al*., 2011). Consequently, these fields are essential for the reactivity and selectivity of enzymatic active sites, highlighting electrostatic interactions and their role in enzyme organization. Moreover, when applied appropriately, they can modify direct chemical interactions, reactivity, and catalysis by manipulating activation energies, which depend on molecule orientation (Fried & Boxer, 2017; Hanoian *et al*., 2015; Liu *et al*., 2014; Wu *et al*., 2020).

Enzymatic manipulation from the perspective of EF is based on protein composition, whose amino acid residues have a balance of positive and negative charges. The application of EF can affect these residues, causing reorientations and changes in catalysis or alterations in active sites. However, the application of EF presents challenges related to the lack of standardized measurement techniques for the phenomenon, which hinders the reproducibility of results, a consequence of the complex composition of biological systems (Colello & Alexander, 2003; Lewczuk *et al*., 2014).

Methodology

The Catalytic Power of Enzymes: A Thermodynamic and Structural Approach.

An enzyme is defined as a protein that acts as a biological catalyst, accelerating chemical reactions in living organisms. However, this definition can be ambiguous, making it relevant to link it with thermodynamic terms. In this context, a chemical reaction should be considered in terms of Gibbs free energy (ΔG), which represents the energy available to perform work in a system (Warshel *et al*., 2006; Lui, 2017; Aledo & Medina, 2019).

In biochemistry, chemical reactions vary according to the value of ΔG: if it is negative, the reaction is exergonic, releasing energy; if positive, it is endergonic, requiring energy. The uniqueness of enzymes lies in that they do not alter the ΔG of a reaction, but rather reduce the activation energy, that is, the energy barrier that substrates must overcome to reach the transition state and become products (equation 1) (Warshel *et al*., 2006; Lui, 2017; Aledo & Medina, 2019).

$$Enzyme - Substrate \leftrightarrow Produt \quad [1]$$

In this way, enzymes optimize reactions by reducing the activation energy, allowing them to occur at much higher speeds than a chemical or physical catalyst. In other words, enzymes do not alter the thermodynamic direction of a reaction, but they enable and accelerate the process by making the transition of states from substrates to products more accessible (Grahame *et al*., 2015).

However, the extraordinary power of enzymes is attributed to their chemical composition, that is, the sequence of amino acids, arrangement of atoms, chemical groups, as well as weak interactions like hydrogen bonds, ionic forces, and Van der Waals forces, among others (Roskoski, 2014).

All these components are distributed in such a way that they form a specific three-dimensional structure, capable of carrying out its catalytic function by efficiently interacting with the substrates. This task occurs in the active site, a specific region where catalysis takes place. The conformation of this site is crucial for the specificity of the substrate and the enzyme, as it determines the molecules that can bind and interact during catalysis (Roskoski, 2014).

The conformation of an enzyme can change in response to various stimuli such as changes in pH, temperature, presence of cofactors, and substrate concentration (Grahame *et al*., 2015). Recently, the use of EF has been explored as an additional variable capable of inducing changes in the enzyme structure, as well as the factors mentioned above. Understanding the underlying principles of this emerging variable is vital for advancing the study of enzymes and the effects derived from their exposure to this factor.

In this context, the relationship between EF and enzymes is not a novel topic. Evidence has been accumulating that links the catalytic ability of enzymes with electrostatic interactions (Stark, 1913; Warshel, 1981; Fried *et al*. 2014). This contrasts with Pauling's hypothesis (Pauling, 1946), which attributes such catalytic power to a reduction in the activation barrier, resulting from changes in conformational strain during the progression of the reaction. Furthermore, it has been observed that enzymes possess an intrinsic EF within their three-dimensional structure, and when interacting with an external field of different intensity, they modify their behavior, which is precisely what will be explored.

Results

Exploring the Interactions Between Electric Fields and Biology: From Fundamental Principles to Applications in Biological Systems.

An EF is defined as a force field generated by electric charges. The response in the EF varies depending on the nature of the material (its polarization capacity) and the inherent or acquired surface charges (Kandelousi, 2018). Additionally, it can be conceived as a vector field where forces of attraction and repulsion are exerted (Pataro *et al*., 2011). This fundamental concept dates back to Benjamin Franklin's discovery, who observed that like charges repel each other while opposite charges attract (Home, 1972). Coulomb's Law (Equation 2) (Ida, 2000) is a quantitative relationship between two-point charges and the space surrounding them, describing the force they generate, which is also fundamental in the study of electrostatic phenomena at different levels. Thus, the EF is grounded in fundamental principles of electrostatics, enabling the understanding and prediction of electrical interactions between charges.

$$F = K \frac{q_1 q_2}{r^2} \quad [2]$$

Where
q_1 *y* q_2 = magnitude of charges in coulomb (C).
r^2= distance separating the charges in meters (m).
F= force of attraction or repulsion in Newtons (N).

The force of attraction or repulsion between molecules is intrinsically linked to the distance between the charged particles, being inversely proportional to the distance separating them (Ida, 2000; Zhou & Pang, 2018). This phenomenon leads to two types of behavior: conductors and insulators.

Biological systems, characterized by their versatility, can exhibit both properties, classifying as semiconductors, as they contain charges capable of moving systems (Colello & Alexander, 2003).

In biological materials, the distribution of charges tends to be homogeneous; however, in exceptional cases, they may concentrate in specific regions within a molecule, creating a charge separation or polarization (Colello & Alexander, 2003).

The application of an EF to a molecule entails significant changes in its charge distribution, which can result in the breaking or formation of bonds that affect or favor the generation of products. A detailed understanding of this process could be achieved by characterizing the fundamental electronic state of the species involved in the reaction (Vaissier-Welborn, 2024).

In this regard, it connects with the electrostatic property of biological systems, such as enzymes. This characteristic refers to the interaction between charge densities and their distributions, which can be permanent or induced, encompassing permanent or induced dipoles, as well as solvation effects, hydrogen bonds, and hydrophobic forces, analyzed from an electrostatic perspective. In this context, distributions tend to distort to adapt to a new environment, achieving once again an electrostatic preorganization. Thus, the effect of the enzymatic environment on the reaction's evolution is considered as an electrostatic potential associated with an EF. This EF will dictate the temporal evolution of the system's charge distributions, suggesting to induce an interaction energy in a substrate proportional to its dipole, in accordance with the physics convention that indicates the orientation of dipoles towards positive charges (Vaissier-Welborn, 2024).

As an example, in aqueous environments, like biological ones, ions (Na^+, Ca_2^+, K^+, and Cl^-) interact with the EF. We also find this in proteins, composed of amino acids with ionizable groups, polar, and side chains. In a typical biological environment with pH 7.4, eight amino acids act as hydrophobic and nonpolar, adopting dipole characteristics. Under similar pH conditions, seven amino acids are hydrophilic and polar, capable of acquiring a charge or behaving ionically. Two amino acids have a negative charge and three, a positive one. In summary, the net charge and polarity of a protein depend on its distribution, which is intrinsically related to its sequence and, in turn, to the pH (Ida, 2000; Zhou & Pang, 2018). Other biological molecules, such as nucleic acids, vitamins, lipids, and cofactors, which possess a net charge, are also influenced by electric fields (Colello & Alexander, 2003; Ren *et al*., 2012; Zhou & Pang, 2018; Vaissier-Welborn, 2024).

Under physiological conditions, proteins, enzymes, and peptides generally adopt their native conformation, thereby favoring their biological functions. However, exogenous factors can disturb this balance, triggering a transition to denatured states. Research based on molecular dynamics simulations has revealed that agents like EF can induce conformational changes in enzymes, even affecting their denaturation and stability (Budi *et al*., 2005; English *et al*., 2009). Biologically, it is not possible to find an EF in its traditional definition and representation, but rather in a relatively more abstract form where electrochemical phenomena manifest as transmembrane potentials derived from ionic exchange. It is well-known that any system with mobile charges, when exposed to an EF, generates an electric potential. This potential is the result of electron flow in metallic conductors or ions in electrolytes. The point where this exchange occurs is known as the electrode (Colello & Alexander, 2003) (see Figure 1).

Box

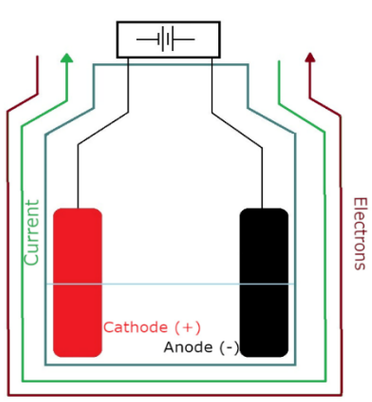


Figure 1

General Schematic of an Electrobio-reactor

Source: Own elaboration

In Figure 1, the direction of ion movement in relation to the current and the EF is observed. It is crucial to highlight that the current flow is oriented in a positive direction, a significant clarification since metals carry a negative charge, whereas electrolytic solutions contain both positive and negative charges. When an accumulation of electrons occurs in the metallic conductor at the left electrode, a negative charge is generated on the surface, favoring the attraction of positive ions (cations), which is why it is called the cathode. Conversely, at the anode, the attraction of anions is due to the lack of electrons, creating a positive charge.

This process implies that anions donate an electron to the anode, while cations head towards the cathode, leading to an exchange of charges. However, this exchange is not so simple because many ions do not easily give up their electrons, attributable to their electronegativity (Colello & Alexander, 2003).

Moreover, an ion barrier known as the 'electrical double layer' or insulating barrier is formed.

This barrier originates from a coating of water that covers the electrode in an aqueous environment, due to the polar nature of water and the excess charge on the electrode surface, requiring more energy to complete the exchange of molecules (Colello & Alexander, 2003; Gonella *et al*., 2021).

This situation, termed overpotential of energy, is essential for overcoming the electrochemical reaction at the electrode-aqueous medium interface (Eberhard, 1986). At this point, two types of reactions can be distinguished to accomplish the exchange: the first occurs when the electrode acts as an inert electron, i.e., chemically inactive, and the second occurs if the electrode acts as a donor/acceptor of ions, i.e., chemically active (Colello & Alexander, 2003).

This is where redox reactions become significant, as they facilitate charge transfer through the reduction and oxidation of ions at the cathode and anode, respectively. Additionally, water decomposition mechanisms can occur at any electrode, a phenomenon known as electrolysis (Colello & Alexander, 2003).

For instance, in a covalent solution like water, which contains few ions, a current can be generated by adding a diluted acid or a salt like NaCl, which provides enough ions to conduct the current. However, when using H_2SO_4, which contains H^+ ions and $(SO_4)^-$, the hydrogen ions are attracted to the cathode, where they collect electrons, forming a gas molecule. At the anode, the negative sulfate ions are attracted, undergoing neutralization and reacting with water to produce more H_2SO_4 and release O_2 (Chang *et al*., 2020).

It is crucial to emphasize that the presence of electrolysis during the application of EFs is not always desirable, as it can generate toxic byproducts for a biological system (Jasper *et al*., 2017). One way to prevent this phenomenon is to create a barrier that inhibits the diffusion of byproducts, allowing the flow of ionic current (Colello & Alexander, 2003). Understanding the nature of the electrodes to be used, as well as the composition of the fluids involved, is one of the alternatives for achieving this.

Another critical aspect is the manner in which the EFs are applied, as variations in magnitude and direction can have a substantial effect on the rate of enzymatic reactions. The application of EFs above 0.1 V can alter the energies of molecular orbitals, modifying the activation barriers of reactions and the kinetic parameters associated with catalysis. Working with lower field intensities allows the molecules to remain intact but oriented and polarized according to their dipole moment and polarization (Shaik *et al*., 2018).

Applications of Electric Fields in Enzymes

In the field of enzymes, research since the 1970s has laid the groundwork for a more quantitative than qualitative application of EF (Stark, 1913; Warshel, 1981; Warshel *et al*., 2006; Roskoski, 2014; Grahame *et al*., 2015). Phenomena triggered by exposure to these fields affect the spectroscopy of molecules (Stark effect), promote electron transfer and redox reactions. They also induce polarized spin conductivity, alterations in molecular geometry, isomerization of molecules, and even spin-crossover transitions in Fe (II) complexes, acting as molecular switches (Léonard *et al*., 2021).

In 1997, Armstrong and colleagues developed a voltammetric technique called Protein Film Voltammetry (PFV), primarily aimed at studying the behavior of complex metalloproteins. This technique involves the adsorption of the protein onto an electrode to which an electric potential is applied, and the flow of electrons between the electrode and the active site is monitored, measured as current consumption (essentially similar to cyclic voltammetry). Thanks to this technique, important thermodynamic and kinetic information has been obtained on various proteins (cytochrome P450, catalases, cytochrome c nitrite reductase, hemoglobin, etc.), enabling a greater understanding of their mechanisms of action (Armstrong *et al*., 1997; Davis *et al*., 2021).

In recent decades, researchers have actively explored the use of EF in various applications, spanning fields as diverse as health and biology, such as in cancer treatment and cell sorting, as well as in engineering and technological applications, where the aim is to improve heat transfer, study colloidal hydrodynamics, and address stability issues (Kandelousi, 2018).

Thanks to technological advances and the development of tools that allow for more complex and specific techniques, the field of electrochemistry has become actively involved in the study of protein models (Davis *et al.*, 2021). Among them, nano-impact electrochemistry (NIE) stands out, through which more in-depth study of mechanisms such as electron transfer has been achieved (Han *et al*., 2016; Davis *et al.*, 2021). Since electron transfer is a crucial process in biochemical reactions, special emphasis has been placed on its study. Perhaps one of the most relevant in the biological field is the relationship between conductance capacity and secondary structure (Zhang *et al*., 2015; Yu *et al*., 2019; Davis *et al*., 2021).

In addition to the electroporation of cell membranes, the electrostimulation of cellular metabolism through electric or electromagnetic fields has become a valuable tool in non-invasive processes to stimulate living organisms. These processes include organism proliferation, enzymatic reactions, biopolymer synthesis, morphological changes, membrane transport phenomena (Berg, 1993; Berg, 1995), and bone tissue regeneration (Tsong, 1990).

It is important to note that these effects are conditioned by factors such as exposure time, intensity of the applied current, distance between the electrodes, among others (Pataro *et al*., 2011). Most studies have focused on chemical catalysis and computational simulations, largely overlooking enzymatic aspects. Among the pioneer researchers, Fried *et al.* (2014), have explored the Stark vibrational effect, which involves the spectral shift of atoms and molecules in the presence of an EF.

This approach allows measuring the EF experienced by the substrate when it binds to the active site, thus supporting the importance of electrostatic contribution in enzymatic catalysis (Boxer *et al.*, 1987). Furthermore, using Stark spectroscopic vibration, Fried *et al.* (2014) identified that the active site of the enzyme ketosteroid isomerase (KSI) presents an EF that stabilizes the C=O bond dipole, orienting it towards the transition state, correlating with an improvement in the catalytic rate.

Computational studies of the enzyme cytochrome P450, present in the bacterium *Jeotgalicoccus sp*. ATCC 8456, have used a software called "elecTric fIeld generaTion And maNipulation (TITAN)". This software aids in generating uniform and non-uniform EF and quantifying local EF present in proteins and other biomolecules. This approach has allowed exploring the effects and evolution of EF in biological systems. In particular, the study focused on the hydrogen transfer reaction of the P450 enzyme, and its modifications induced by the application of an externally oriented EF. The main impact was observed on the Fe-O bond (Stuyver *et al.*, 2020). In a simulation by Lai *et al.* (2010), it was demonstrated that an external EF has the capability to control the activation of the enzyme's catalytic activity, its O_2 consumption, its resting state geometry, and the spin state arrangement, thus highlighting the relevance of electric fields in enzymatic processes (Nam et al., 2024).

While some researchers have applied EF as a means of enzymatic stimulation, the mechanism is not yet fully elucidated. It is primarily attributed to structural changes, but it has also been studied that transmembrane enzyme, such as ATPase, can harness the free energy from the EF to modify their catalysis, although this depends more on the characteristics of the EF than on the enzyme (Westerhoff *et al.*, 1986). This leads to the so-called 'electrogenic enzymes,' of which ATP synthase is probably the most representative.

In this enzymatic complex, the exchange of Na^+ and K^+ ions has been well defined for several decades (Hernández *et al.*, 1983); however, based on this premise, the electrochemical implications in protein and cellular systems were studied more deeply, leading to the description of another factor known as 'electrochemical homeostasis' or 'ionic homeostasis,' which in turn led to the description of mechanisms of ionic exchange regulated by electromotive forces, where transmembrane proteins are of primary importance (Mehta *et al.*, 2008).

It should be noted that the opposite effect can occur, namely, the denaturation of enzymes, resulting from the association or dissociation of functional protein residues, inducing charge separation (induced dipole) (Poojary *et al.*, 2017; Zhou &Pang, 2018). Although the process of enzymatic inactivation is better characterized compared to stimulation, it is presumed that this phenomenon may be due to the additional formation of active sites or their modification, reducing the activation energy of the reaction. This may be related to the induction of a favorable orientation of the substrate and the enzyme. Therefore, it is crucial to continue research in this area to understand and establish the real contribution of EF (Zhou &Pang, 2018).

While enzymes have been classified according to their biochemical function and all share essential thermodynamic parameters (optimal pH and temperature, activation energy, pKa, redox state, etc.); oxidoreductases, especially those involved in respiratory processes, stand out among others due to the electrochemical potential, which adds as an additional factor (Elliot *et al.*, 2002; Léonard *et al.*, 2021).

Due to this electrochemical nature, the active sites of these enzymes can be presented in three defined states as oxidized (O), intermediate (I), or reduced (R), where each state then has different properties and therefore can lead to different metabolic pathways (Elliot *et al.*, 2002); where the response will depend on the existence of additional electron transport centers (e.g., heme groups or Fe-S) (Craig & Marszalek, 2002).

In 1984, Serpesu & Tsong (1984) used a solution of blood cells to study the effect of imposing an EF on the activity of (Na,K)ATPase; the results obtained from this work showed that protein modifications resulted from changes in the flow of Na^+, K^+, and Rb^+ ions as well as the addition of compounds that showed to inhibit or favor ionic exchange under the study conditions.

Conclusions

EF are a phenomenon commonly associated exclusively with physical aspects and, due to their traditional definition and representation, are seldom linked to biological processes; however, it has been established that EF, and therefore electric potential, are parameters implicitly involved in enzymatic processes.

Nevertheless, the manipulation of enzymes through EFs still presents several challenges due to the rigorous conditions necessary for their study; this is why many works within this field employ bacterial models in specific processes where the results are associated with enzymatic processes. However, this has not been a limitation for studying in depth the effect and relationship that EFs have with enzymes. While concrete observations of the implications at the structural and/or conformational level have not yet been achieved, contemporary tools and methods allow for a more precise approach. Combined with decades of research, it is possible to conclusively assert that electric fields are a physicochemical factor of utmost importance for enzymatic processes and a useful tool in optimizing these processes, which are extremely attractive for various industries.

Declarations

Conflict of interest

The authors declare no interest conflict. They have no known competing financial interests or personal relationships that could have appeared to influence in this chapter.

Author contribution

Alonso- Vargas, Monserrat: Conceptualization, methodology, investigation, writing—original draft preparation.

García- Esquivel, Yarely: Methodology, investigation, writing—review and editing, supervision.

Cadena- Ramírez, Arturo: Conceptualization, resources, data curation, writing—review and editing, visualization, supervision, project administration, funding acquisition.

Availability of data and materials

No extra data are available.

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Abbreviations

EF	Electric Fields
TITAN	Electric Field Generation and Manipulation
ΔG	Gibbs Free Energy
I	Intermediate
KSI	Ketosteroid Isomerase
NIE	Nano-impact Electrochemistry
O	Oxidized
PFV	Protein Film Voltammetry
R	Reduced

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Differences

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