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Programmable scanning and Y-modulated Detection Methods for Catalytic Effect Estimation in Amylase Crystals in LVEM Protocols

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ABSTRACT

This work investigates the enhancement of α-amylase catalytic activity under the influence of electric fields, focusing on structural and conformational modifications induced by pulsed electromagnetic fields. We propose employing Y-modulated detection methods to analyze defective but catalytically active crystalline structures of α-amylase and their interaction with classic substrates such as amylose. This approach offers new perspectives on understanding enzyme behavior under external stimuli and potential improvements in biotechnological processes. Practical applications include novel methodologies for producing porous starches via α-amylase-mediated hydrolysis enhanced by electric fields.

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It is known that the catalytic activity of alpha-amylase increases in the presence of an electric field, which is based on structural and conformational changes, particularly pronounced under pulsed electromagnetic fields with different modulation leading to reversible changes (and their relaxation dynamics) in biopolymer structure [1-3]. The electromagnetic nature of this effect is confirmed by its modulation by metal ions present in salts of alpha-amylase, resulting in phenomena related to the domain of "spin chemistry" and spintronics (for example, when using iron or cobalt) [4].

The practical application of this effect discovered over the past decade has not been long in coming, as there are already methods for preparing porous starch using alpha-amylase-catalyzed hydrolysis under the influence of such an electric field [5,6]. The effect of pre-gelatinization on alpha-amylase-stimulated starch hydrolysis under electrical field action has also been studied, along with its contribution to glucoamylase-catalyzed starch hydrolysis [7,8]. In general, this fits into methods of influencing enzyme catalysis by means of an electric field, well-known since the early 1990s [9,10]. Specifically, in the case of amylases, one can consider electrostatic effects at active sites of these enzymes [11,12].

Rapid implementation of electrophysical techniques regulating amylase activity (analogously to translational medicine transitioning directly from laboratory research to practice) as well as pathways enhancing various enzyme activities through exposure to electric fields (including pulsing ones) on industrial scales seems sufficient evidence, in our view, for the use of electron beam scanning as another method for identifying active sites of these enzymes or their "hot spots" (if we talk about granular and disperse forms of biocatalysts), including through intensity and profile modulation of the beam. Electrophysics underlying several bioelectroconversion methods in glycomics and glycobiology, just like dominating biosensor methods involving electron transfer and bio-electrochemical channeling (such as the use of amylases and ferrocene as an electron carrier), undoubtedly gives us both a chance to route charge transfer flows across the surface of enzymatic or biocatalytic samples using programmable electron microscope beams and visualize the electrophysical result of this impact on the sample (in particular, using methods similar to EBIC/EMF or conventional mapping of charge distribution across dark and light areas on ungrounded samples using Y-modulation schemes) [13-17].

In connection with this, we propose to apply Y-modulated detection for analyzing imperfect surfaces (and catalytically active crystals) of amylase and its effects on classical and predominant substrate—amylose [18-21].

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