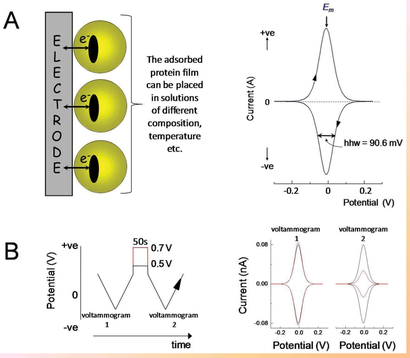
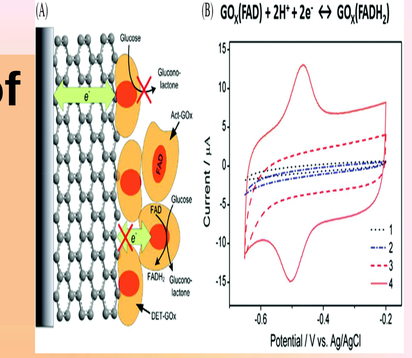


New Voltammetric Method to Determine Michaelis-Menten Kinetic Constant of Enzyme-Substrate Reactions in Protein-Film Voltammetry

Sofija Petkovska, Milkica Janeva, Viktorija Maksimova, Rubin Gulaboski

Faculty of Medical Sciences, Goce Delcev University, Stip, Macedonia

e-mail: sofija.petkovska@ugd.edu.mk

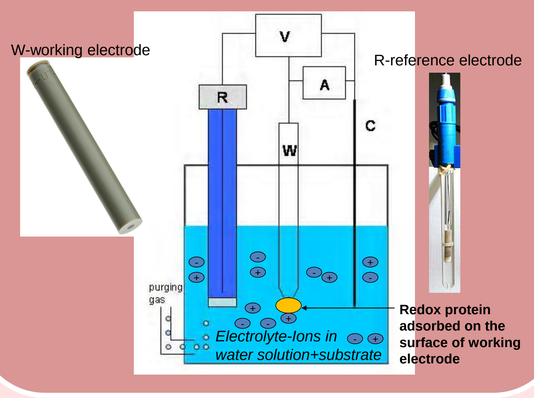


Introduction

Being recognized as an efficient tool for mechanistic enzymology, the protein-film voltammetry is simple approach to get thermodynamic and kinetic information for the redox chemistry of many enzymes. This technique requires a small amount of redox active enzymes, whose molecules are organized in monomolecular film on the working electrode surface and behave independently of each other. In this work we present a simple and time-independent method developed under conditions of cyclic voltammetry for the determination of kinetics of the chemical step of an electrocatalytic-regenerative (EC') mechanism in protein-film scenario. Theoretical results of a surface EC' mechanism reveal that the limiting cyclo-voltammetric catalytic current depends solely on the rate of the chemical regenerative reaction. In the region of large overpotentials, the limiting current of the steady-state cyclic voltammograms is independent on all kinetics and thermodynamic parameters related to the electrode reaction of adsorbed redox enzyme. The approach proposed relies on the dependence of the maximal catalytic current of experimental cyclic steady-state voltammograms as a function of the catalyzing agent concentration.

Methods & Materials

We performed theoretical calculations in cyclic voltammetry, considering a redox reaction of a given redox active protein firmly adsorbed in a form of monolayer to the working electrode surface in absence and in presence of specific substrate to that enzyme. We studied all the parameters affecting the voltammetric features of the redox active protein. All calculations have been performed with MATHCAD software.



Results

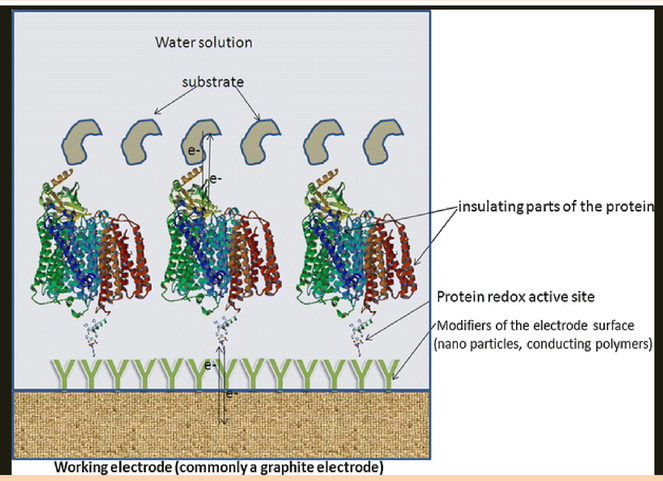


Figure 1. Protein-film voltammetry is a technique that requires small amount of prenzyme Attached to the surface of the working electrode. By applying potential, the redox features of the attached enzyme, as weel asThe interactions between a given enzyme and substrate can be studied with cyclic voltammetry

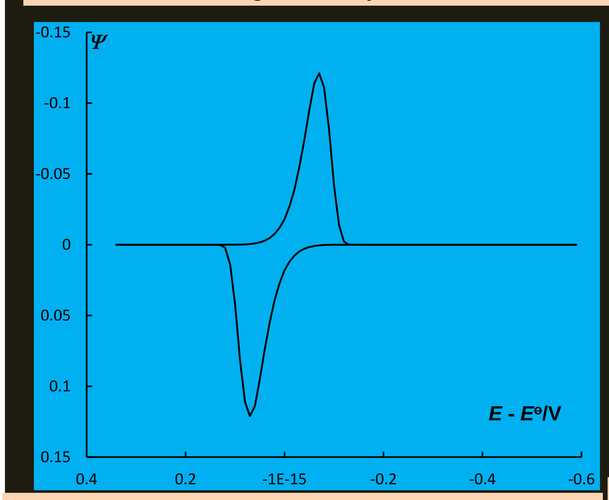


Figure 2. Simulated cyclic voltammogram of a given redox-active protein recorder in Protein-film voltammetry

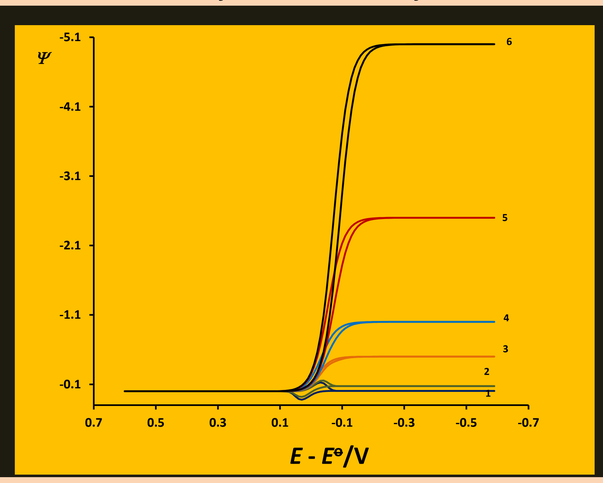


Figure 3. Cyclic voltammograms of a given redox-active protein recorder in Protein-film voltammetry in presence of Several substrate concentrations. Elevations of the limiting currents at negative potentials is specific for this mechanism

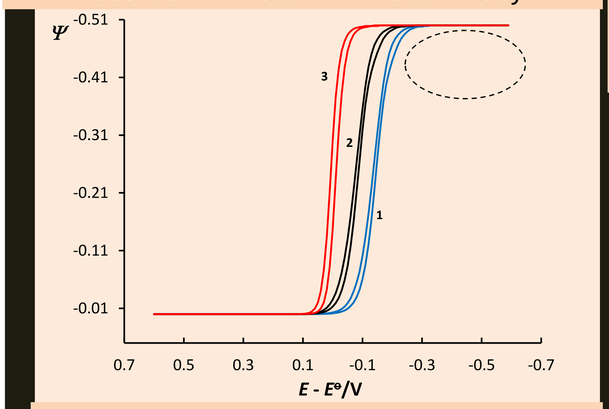


Figure 4. Cyclic voltammograms of a given redox-active protein recorder for several different kinetics of electrode reaction.
The limiting currents at negative potentials is Insensitive to the electrode kinetic parameter.

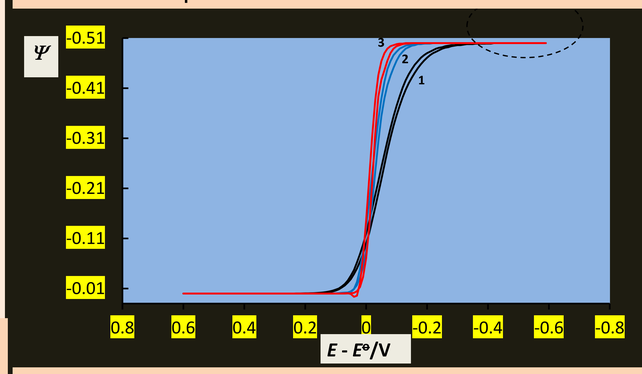


Figure 5. Cyclic voltammograms of a given redox-active protein recorder for several different symmetry barriers of electrode reaction.
The limiting currents at negative potentials is Insensitive to the electrode transfer coefficient.

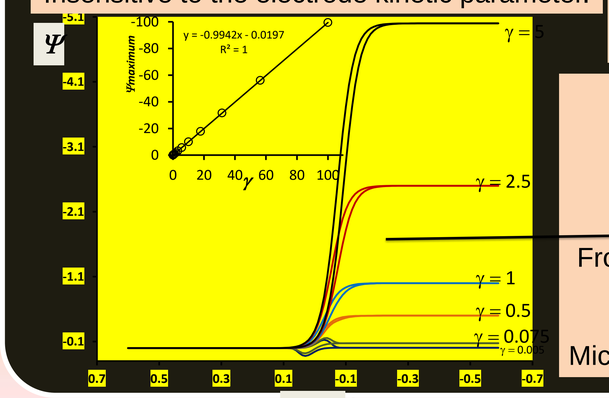


Figure 6. The limiting currents of the Cyclic voltammograms of a given redox-active protein Are linear function only of the substrate molar concentration.
From the slope of this dependence in real experiments (given in the inset of figure 5), one can determine directly the Michaelis-Menten kinetic constant of enzymatic reaction

Conclusions

We developed a simple and time-independent theoretical method under conditions of protein-film voltammetry to get insight into the kinetics of Enzyme-substrate reactions. The method relies on the linear dependence of the limiting currents of a given protein in cyclic voltammetry on the substrate concentration only. At large overpotentials, the limiting currents are completely independent on kinetics and thermodynamics of the electrode reaction. By plotting the magnitudes of the limiting cyclo-voltammetric currents as a function of substrate molar concentration, one should obtain a linear line, from which slope simple evaluation of the Michaelis-Menten Constant is possible.

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Supplementary Material:

https://link.springer.com/article/10.1007/s10008-008-0665-5

Acknowledgements

This work has been supported by the Goce Delcev University Stip, Macedonia, and the Alexander von Humboldt Foundation, Germany