Theoretical aspects of several successive two-step redox mechanisms in protein-film cyclic staircase voltammetry

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ABSTRACT

Protein-film voltammetry (PFV) is a versatile tool designed to provide insight into the enzymes physiological functions by studying the redox properties of various oxido-reductases with suitable voltammetric technique. The determination of the thermodynamic and kinetic parameters relevant to protein's physiological properties is achieved via methodologies established from theoretical considerations of various mechanisms in PFV. So far, the majority of the mathematical models in PFV have been developed for redox proteins undergoing a single-step electron transfer reactions. However, there are many oxido-reductases containing quinone moieties or polyvalent ions of transition metals like Mo, Mn, W, Fe or Co as redox centers, whose redox chemistry can be described only via mathematical models considering successive two-step electron transformation. In this work we consider theoretically the protein-film redox mechanisms of the EE (Electrochemical–Electrochemical), ECE (Electrochemical–Chemical–Electrochemical), and EECat (Electrochemical–Electrochemical–Catalytic) systems under conditions of cyclic staircase voltammetry. We also propose methodologies to determine the kinetics of electron transfer steps by all considered mechanisms. The experimentalists working with PFV can get large benefits from the simulated voltammograms given in this work.

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1. Introduction

The voltammetric methods are used for more than 60 years to study the redox reactions of numerous small molecules and ions, and to provide access to relevant kinetic and thermodynamic information linked to various functions of considered compounds [1]. While the voltammetric experiments of small chemical systems are relatively easy to be performed, the transfer of electrons between the working electrode and large lipophilic molecules (such as lipophilic proteins, for example), however, is not an easy task, and it always suffers a number of drawbacks. The main cause for this is seen in the large inactive (insulating) part of the proteins that hinders significantly the direct electron exchange between the electrode and the redox active center of a given protein [2,3]. In the late 70s of the last century the group led by Hill was first that performed direct voltammetry of several water-soluble cytochromes at solid electrodes [4]. The effectiveness of the direct protein voltammetry was further increased by making adsorption of the studied proteins as a monolayer on the bare or modified surfaces of suitable electrodes. The worldwide accepted name of this new methodology given by Armstrong is a *protein film voltammetry* (PFV). Since the establishment of PFV some 15 years ago [5], this technique became a versatile toll to study the redox transformation of scores of enzymes, while providing closer insight into the physiological functions of various oxidoreductases [6–10]. The basic principles of PFV consider initial adsorption of a given protein in a form of a monolayer on the surface of suitable electrode (preferably graphite). The protein adsorption at the electrode surface is achieved by its self-assembling from the protein-containing electrolyte solutions. Hitherto, protein film voltammetry benefits from its sensitivity due to the extremely small sample requirements. For example, monolayer coverage of a protein of 50,000 Da requires just a few picomoles/cm^2 [5]. The PFV is a rather simple experimental methodology that provides access not only to the mechanism of redox transformation, but also to thermodynamic and kinetic parameters relevant to physiological features of many redox enzymes [2,3,5–10]. Moreover, this methodology is also extensively used as a versatile tool for simple detection of reactive oxygen species as hydrogen peroxide, hydroxyl and superoxide radicals [2,3,5,6]. The ability to investigate the redox protein reactivity with a given substrate over a wide range of conditions is enabled by studying the protein features with common voltammetric techniques such as cyclic or square-wave voltammetry [2–10]. In order to understand closely the mechanism of the enzyme–substrate interactions, and to have access to the thermodynamic and kinetic parameters

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relevant to those interactions, one must get a help of mathematical models developed for relevant enzymatic systems. So far, the majority of the theoretical models in PFV are developed for a single-step electron transfer systems under conditions of linear cyclic voltammetry-LCV [3,5–10]. Alongside, our group started developing theoretical models relevant to PFV under conditions of square-wave voltammetry (SWV) [6,11–20]. In the last few years we started modeling complex redox reactions of enzymes that can be interconverted from one oxidation step to other via two or more consecutive electron transfer steps [12,14,17]. Such enzymes are common in the biological systems, where one finds many classes of proteins containing electroactive centers that undergo redox transformations in several successive electron steps [21–24]. Of these, the most important are flavoproteins, then proteins with quinone moiety [21–23], and the proteins containing polyvalent ions of transition metals like Mo, Mn, W, Fe or Co as redox centers [24]. In this paper we present a theoretical study of three redox mechanisms that are relevant for redox proteins undergoing multiple successive electrochemical transformations under conditions of cyclic staircase voltammetry. The considered systems are assigned as EE (Electrochemical–Electrochemical), ECE (Electrochemical–Chemical–Electrochemical) and EECat (Electrochemical–Electrochemical–Catalytic) redox mechanisms. This study will help the experimentalists in elucidating the complex redox mechanisms of significant redox proteins, and it will give qualitative criteria for distinguishing particular mechanism from the similar ones. The current work is seen as a complementary to our previous works published elsewhere [12,14,17].

2. Mathematical models

The considered systems in this work are assigned as surface EE, ECE, and EECat redox mechanisms and these can be described by following reaction schemes:

I. EE mechanism
Electrochemical step #1: $A(ads) + ne- \rightleftarrows B(ads)$
Electrochemical step #2: $B(ads) + ne- \rightleftarrows C(ads)$
II. ECE mechanism
Electrochemical step #1: $A(ads) + ne- \rightleftarrows B(ads)$
Chemical step: $B(ads) + Y \xrightarrow{k_f} C(ads)$
Electrochemical step #2: $C(ads) + ne- \rightleftarrows D(ads)$
III. EECat mechanism
Electrochemical step #1: $A(ads) + ne- \rightleftarrows B(ads)$
Electrochemical step #2: $B(ads) + ne- \rightleftarrows C(ads)$
Catalytic step: $C(ads) + Sub \xrightarrow{k_{cat}} B(ads)$

We assume that all redox active participants in the electrode mechanisms I–III are strongly adsorbed (ads) at the electrode surface, thus we neglected the mass transfer via diffusion in our modeling procedure. The symbol Y stays for an electrochemically inactive reactant, whose concentration is much higher than the initial concentration of all adsorbed electroactive species in the reaction mechanism II. Therefore, the chemical step in the reaction mechanism II is assumed to be of pseudo-first order. The physical parameter k_f (s^{-1}) is the pseudo-first order rate constant of the chemical step in the mechanism II. This parameter is related to the bulk concentration of Y as follows $k_f = k'_f c(Y)$, where k'_f is the real chemical rate constant having units mol^{-1} cm^3 s^{-1}. By *Sub* we assign an electrochemically inactive *substrate* (or catalytic reagent) in reaction mechanism III. The bulk concentration of the substrate Sub present in electrochemical cell is supposed to be much higher than the initial concentration of all adsorbed electroactive species. Consequently, the chemical step in the reaction mechanism (III) is also considered to be of pseudo-first order. By k_{cat} (s^{-1}) we define a pseudo first order catalytic rate constant that is related to the bulk concentration of the substrate via equation $k_{cat} = k'_{cat} c(Sub)$.

In the last expression, k'_{cat} is the real chemical (i.e. catalytic or Michaelis–Menten) rate constant (mol^{-1} cm^3 s^{-1}), while $c(Sub)$ is the molar concentration of the substrate Sub present in excess in the bulk solution in electrochemical cell. The electrode mechanisms I–III can be mathematically defined by the following set of equations:

A. For EE mechanism (redox mechanism I):

$$\frac{d\Gamma(A)}{dt} = \frac{I_1}{nFS} \tag{1}$$

$$\frac{d\Gamma(B)}{dt} = -\frac{I_1}{nFS} + \frac{I_2}{nFS} \tag{2}$$

$$\frac{d\Gamma(C)}{dt} = -\frac{I_2}{nFS} \tag{3}$$

B. For ECE mechanism (redox mechanism II):

$$\frac{d\Gamma(A)}{dt} = \frac{I_1}{nFS} \tag{4}$$

$$\frac{d\Gamma(B)}{dt} = -\frac{I_1}{nFS} - k_f\Gamma(B) \tag{5}$$

$$\frac{d\Gamma(C)}{dt} = \frac{I_2}{nFS} + k_f\Gamma(B) \tag{6}$$

$$\frac{d\Gamma(D)}{dt} = -\frac{I_2}{nFS} \tag{7}$$

C. For EECat mechanism (redox mechanism III)

$$\frac{d\Gamma(A)}{dt} = \frac{I_1}{nFS} \tag{8}$$

$$\frac{d\Gamma(B)}{dt} = -\frac{I_1}{nFS} + \frac{I_2}{nFS} + k_{cat}\Gamma(C) \tag{9}$$

$$\frac{d\Gamma(C)}{dt} = -\frac{I_2}{nFS} - k_{cat}\Gamma(C) \tag{10}$$

Eqs. (1)–(10) have been solved under the following initial and boundary conditions:

$$t = 0; \quad \Gamma(A) = \Gamma(A)^*; \quad \Gamma(B) = \Gamma(C) = \Gamma(D) = 0 \tag{11}$$

$$t > 0; \quad \Gamma(A) + \Gamma(B) + \Gamma(C) + \Gamma(D) = \Gamma(A)^* \tag{12}$$

We assume that at the beginning of the experiment there is only compound A present in the cell, and it is strongly adsorbed at the electrode surface. The compound C is generated chemically via chemical transformation of B with Y (for ECE mechanism), or electrochemically via redox transformation of B (for EE and EECat mechanisms). The compound B is generated via electrochemical transformation of compound A (for all considered mechanisms), but also via catalytic regeneration reaction of the electrochemically generated product C with the substrate Sub (for EECat mechanism). The term "catalytic" describes that the chemical (catalytic) reaction regenerates B from C by the EECat mechanism (III). The symbols $\Gamma(A)$, $\Gamma(B)$, $\Gamma(C)$ and $\Gamma(D)$ represent the surface concentrations of the species A, B, C and D, respectively, while $\Gamma(A)^*$ is the total surface concentration of all species. Γ is a symbol of the surface concentration of particular specie in the considered mechanisms that is function of the time t. I is the symbol of the current, S is the electrode surface area, F is the Faraday constant, while n is a number of exchanged electrons in an act of electrochemical transformation (it is assumed that n are equal for both electrochemical steps in reaction mechanism I–III). The solutions of Eqs. (1)–(10) were obtained by help of Laplace transformations. The solutions for the surface concentrations of the electroactive species A, B, C and D in their integral forms for EE, ECE, and EECat read: