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Protein Electrochemistry: Application in Medicine. A Review

Elena V. Suprun*,[1], Victoria V. Shumyantseva, Alexander I. Archakov

Institute of Biomedical Chemistry, Russian Academy of Medical Science Pogodinskaya St. 10/8, Moscow, 119121 Russia

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ABSTRACT

This mini-review outlines the current state of knowledge in field of protein electrochemistry and its application in medicine. Protein electrochemistry is considered according to (i) its intrinsic redox activity as generated by prosthetic groups and/or amino acid residues as well as (ii) charge transfer or adsorption at interfaces between two immiscible electrolyte solutions (liquid-liquid interfaces). Despite protein electrochemistry already has experimental and theoretical basis, its potential applications in medicine–where proteins serve as the most valuable biomarkers–is not yet attended. Mediator-free and label-free protein detection, analysis of its denaturation and aggregation, measurement and regulation of enzyme activity, a search for new substrates and inhibitors–i.e. the major problems of protein research–are treated in this mini-review from the electrochemical viewpoint. Significance of protein electrochemistry for clinical, pharmacological and scientific medicine is discussed.

1. Introduction: medical aspects

Proteins have been the subject of intense interest from the very first steps of the electrochemistry [1] and continue to be one of the favorite molecules of electrochemists until now [2]. Nowadays the proteins attract renewed attention owing to their unique properties, wide variety, structural complexity, multiplicity of functions in living systems and, last but not least, their vital importance for medicine. The proteins can serve as drugs [3]; as biomarkers at the early stages of diseases [4,5]; as biorecognition elements (antibodies) [6,7] and as selective catalysts (enzymes) [8,9] in various assays; as well as targets for new drugs discovery [10,11]. Applications of proteins in medicine are based on molecular mechanisms of life processes [3].

It is necessary to note that the diversity of distinct covalent forms of proteins (the proteome) greatly exceeds the number of proteins predicted by gene coding capacities owing to directed post-translational modifications (PTM), such as protein phosphorylation, acetylation, methylation, and ubiquitylation. In human body, there are special enzymes (protein kinases, protein phosphatases, proteases) that are dedicated to such protein modifications. There are also non-enzymatic PTM of proteins: oxidation, nitration, nitrosylation etc., which arise mainly over oxidative stress. [12,13]

The intracellular level of oxidized protein reflects the balance between the rate of protein oxidation and the rate of the oxidized protein degradation. This balance is a complex function of numerous factors that lead to the generation of reactive oxygen species, on the one hand, and of multiple factors that determine the concentrations and/or activities of the proteases that degrade oxidatively damaged protein, on the other. [14] A number of publications reported that oxidative and nitrosative damage of proteins is involved in the aging process and in the pathogenesis of cardiac and vascular diseases, circulatory shock, local inflammation, in cancer, stroke and other forms of reperfusion injury, as well as in neurodegenerative disorders, and hormonal disorders such as diabetes and diabetic complications. [15,16] In the past years, many *in vitro* and *in vivo* studies, including the proteomic ones, were aimed at discovering proteins with PTM as biomarkers of different diseases. The main problem of identification of proteins with PTM is that only a small amount of molecules are changed and only one or a few amino acid residues are modified in each. [17,18] However, this area seems to be perspective, especially for neurodegenerative diseases. Thus, the plasma proteins, α-1-antitrypsin and fibrinogen γ-chain precursor, were found oxidized in plasma from Alzheimer's disease subjects, and their oxidation levels were significantly higher compared with normal, i.e. those proteins may serve as disease biomarkers [19]. Nitrated plasma albumin was shown to be a marker of neonatal encephalopathy in' perinatal asphyxia [20]. There was a significant increase in protein tyrosine (Tyr) nitration in brain under amnestic mild cognitive impairment (transitory clinical condition between normal and Alzheimer's disease) [21] and Alzheimer's disease [22]. In addition to PTM

* Corresponding author. Tel.: +7 499 246 58 20; fax: +7 499 245 08 57.
E-mail address: lenasuprun@mail.ru (E.V. Suprun).
[1] ISE member

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of amino acids the distinct protein aggregates that are found in Alzheimer's, Parkinson's, Huntington's and prion diseases seem to cause these disorders. Small intermediates–soluble oligomers–in the aggregation process can confer synaptic dysfunction, whereas large, insoluble deposits might function as reservoirs of the bioactive oligomers [23]. Inhibition of the protein misfolding into neurotoxic oligomeric aggregates is one of the drug design strategy for treatmet of neurodegenerative disorders [24].

Proteins, especially enzymes, are widely used in medicine as targets for developing new drugs, as well as for their selective determination. The cytochromes P450 (CYP) constitute the major enzyme family capable of catalyzing the oxidative biotransformation of most drugs and other lipophilic xenobiotics and are therefore of particular relevance for clinical pharmacology. [25] Thus, inhibitors of CYP19 for the treatment of breast cancer ushered in a new era in hormone ablation therapy for estrogen dependent cancers, and have paved the way for similar strategies (i.e., inhibition of CYP17) that combat androgen dependent prostate cancer. Identification of CYP involved in the inactivation of anti-cancer metabolites of vitamin D3 and vitamin A has triggered development of agents that target these enzymes. [11] It has been established that abnormal CYP activity is associated with several cardiovascular pathologies. In addition to production of vasoactive arachidonic acid metabolites, certain CYP also generate reactive oxygen species, such as superoxide anions, hydrogen peroxide and hydroxyl radicals. It is has been proposed that CYP2C9 is also able to play a pathogenic role in cardiac ischemia–reperfusion injury through the reactive oxygen species generation. Selective inhibitors of CYP2C9 can significantly attenuate ischemia-reperfusion mediated myocardial injury. [26] Another class of proteins, protein kinases, is associated with a large percentage of tumors. Some viral oncogenes produce protein kinases, mainly Tyr-type. [3] Protein kinases participate in pathways which control diverse cellular processes such as proliferation, survival, and motility. These pathways are often upregulated in human cancers and as such represent an attractive target for mechanism-based approaches to cancer treatment. [10,27]

Therefore, proteins present a vast area for studies. Electroactivity of proteins may have many different applications, especially in medicine. Electroanalysis with its numerous advantages–such as precision, rapidity, simplicity, low price, facility of miniaturization etc.–holds great promise for: (i) developing of new analytical approaches, suitable not only for laboratories, but also for point-of-care devices; (ii) monitoring of biochemical processes in living systems *in vivo* and *in vitro*; (iii) discovery of new drugs and many other goals. For all of this, we need close collaboration of electrochemists with medics as well as with other interfacing specialists.

2. Historical perspective of protein electrochemistry

2.1. Mercury electrode

History of protein electrochemistry started in early 1930s from the first work of Heyrovsky and Babicka with mercury drop electrode [1]. Thus, in the presence of protein in polarographic cell with ammonia-ammonium chloride buffer, a wave at −1.6 V (that is, by 0.2 V more positive than for reduction of the NH_4^+ ions) was registered. This wave was attributed to the electroreduction of hydrogen catalyzed by protein. A similar effect was observed by Bredicka [28]: double reduction wave of the protein was registered in ammoniacal cobaltous chloride solution. Bredicka found that sulphydryl groups of amino acids, cystine (CySS) and cysteine (Cys), serve as a source of hydrogen ions for this signal [29]. From now on, polarographic studies with proteins were based on catalytic waves, mainly in the presence of cobalt [30–32]. Further, in the 1960s, the polarographic reduction of CySS disulphide bonds of proteins was reported [33,34]. It was necessary to reach high sensitivity to observe waves with currents of about 0.1 μA, whereas the currents reported for catalytic waves were about 10-100 μA. Nowadays, Emil Palecek's group [35] continue to use hydrogen evolution catalyzed by proteins and peptides (peak H) at mercury electrodes for biomedical problems. In the 1970s a polarographic study of reducibility of hemeproteins revealed electron transfer between electrode and prosthetic group, giving start to direct electrochemistry of cofactor-containing proteins [36–38]. Unfortunately, today mercury electrode is passing into history of electrochemistry, because of the strong toxicity of mercury.

2.2. Direct electrochemistry of cofactor-containing proteins

In the beginning, the big size of protein molecules, protein degradation during electrochemical procedure, and protein adsorption on the electrode surfaces were causes of doubts of electrochemists. Proteins were studied dissolved in the electrochemical cell. Very important converging of protein and electrode took place in 1962 [39], when enzyme solution (glucoseoxidase or urease) was entrapped between two membranes on the electrode surface. This "enzyme electrode" grew to the concept of first biosensor. Over the years, it turned out that adsorption of proteins on the electrode surface is the main key to their direct electrochemistry and bioelectrocatalysis. First steps in this area were done with hemeprotein cytochrome *c* (cyt *c*). In 1972, reduction of cyt *c* from its solution at mercury, platinum, and gold electrodes without irreversible protein denaturation was demonstrated by S. R. Betso with coworkers [36]. The authors stated: "Adsorption of protein onto electrode surface has significant influence on the observed electrochemistry, but it does not cause electrode fouling or loss of the electrode's ability to transfer electrons". Then, in 1977 reversible electron transfer between cyt *c* and electrode was registered Eq. (1) [40,41]:

$$\text{Fe(III)-heme} + e \leftrightarrow \text{Fe(II)-heme} \quad (1)$$

In particular, tin-doped indium oxide electrode demonstrated diffusion controlled mechanism of the cyt *c* electrode reaction [40]. At the same time, cyt *c* was shown to be electrochemically inert on the planar disc gold electrode before the addition of 4,4′-bipyridyl in the solution [41]. Presence of 4,4′-bipyridyl (which in itself is not electroactive in this potential region) promoted the electron transfer between cyt *c* and the electrode. Among other possible effectors investigated, only 1,2-bis-(4-pyridyl)ethylene was as effective as 4,4′-bipyridyl, with 1,2-bis-(4-pyridyl)ethane having no effect. The authors assumed that 4,4′-bipyridyl modifies the double layer by interacting with the electrode and/or the protein. Thus, 4,4′-bipyridyl was the first compound which served as a "bridge" between protein and electrode for protein's proper orientation on the electrode surface and promotion of direct electron transfer. Later works confirmed the idea of importance of some "bridge" for protein direct electrochemistry. In particular, similar effect was observed with liquid crystal surfactant films as catalysts toward direct electron transfer of hemeproteins [42]. Further, by using different electrode materials and immobilization matrixes, direct electrochemistry have been demonstrated for a variety of mono- and multi-cofactor-containing proteins found in bacteria, fungi, plants and animals: peroxidase [43]; myoglobin (Mb) [44]; haemoglobin (Hb) [45]; CYP [46]; superoxide dismutase [47]; catalase; cellobiose and fructose dehydrogenases; sulphite, theophylline and bilirubin oxidases; ceruloplasmin, azurin etc. [48].

2.3. Direct bioelectrocatalysis of enzymes

Further investigations in this area led to protein (enzyme) immobilization on the electrode surface and discovery of bioelectrocatalysis. Bioelectrocatalysis is determined as an acceleration