FROM DC POLAROGRAPHIC PRESODIUM WAVE OF PROTEINS TO ELECTROCHEMISTRY OF BIOMACROMOLECULES

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Dedicated to the memory of Professor Jaroslav Heyrovský on the occasion of the 50th anniversary of the Nobel Prize for polarography.

History of electrochemistry of proteins and nucleic acids is briefly reviewed. The ability of proteins to catalyze hydrogen evolution at Hg electrodes was discovered almost 80 years ago in J. Heyrovský's laboratory. This phenomenon was not sufficiently appreciated for several decades. Recently it has been shown that using constant current chronopotentiometric stripping (CPS) with hanging mercury drop, solid amalgam or Hg-film electrodes the CPS peak H is obtained with nanomolar concentrations of peptides and proteins. This peak is derived from the presodium wave but it has some new properties useful in protein research. It is sensitive to changes in protein structures and to protein redox states, representing a new tool for protein analysis applicable in biomedicine. Electroactivity of nucleic acids was discovered about 50 years ago. Electrochemistry of DNA and RNA is now a booming field because of its potential use in sensors for DNA hybridization and DNA damage. Quite recently it has been shown that electrochemistry can be applied also in polysaccharide analysis. A review with 99 references.

Keywords: Electrochemistry; Proteins; Polarography; Presodium wave; Chronopotentiometry; Nucleic acids.

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1. INTRODUCTION

Since 1958 electrochemistry of nucleic acids was studied for several decades in a handful of laboratories publishing on average about 10 papers per year. Progress in genomics and particularly in the Human Genome Project greatly affected electrochemistry of nucleic acids[1,2], which focused on the development of sensors for DNA hybridization (DNA nucleotide sequencing)[3,4] and for DNA damage[5]. After 1990 the number of papers dramatically increased[6] and in 2008 it mounted to about 700 papers per year.

To describe the genome protein complement, the word "proteome" was coined in the middle of the 1990s[7] giving rise to a new discipline of "proteomics". Proteomics represents a large-scale study of protein properties (expression level, interactions, post-translational modifications, etc.) that would help to understand complex biological processes at molecular level in different cell types and their alterations in various diseases. Present proteomics and biomedicine require new sensitive and simple methods for analysis of proteins. This challenge to electrochemistry of proteins has not been met in the way as in the case of nucleic acid electrochemistry. Since the beginning of the 1970s electrochemistry of proteins focused on electroactivity of non-protein components of relatively small molecules of conjugated proteins producing reversible electrode processes[2,8]. This important field was thus limited to a tiny protein fraction among thousands of proteins occurring in nature.

Recently it has been shown that practically all proteins produce at submicromolar concentrations a specific signal when constant current chronopotentiometric stripping (CPS) is used in combination with mercury electrodes. This signal is due to the catalytic hydrogen evolution[9] and it was denominated as peak H (as H in Hydrogen evolution, High sensitivity and Heyrovský[10]). In fact the ability of proteins to catalyze hydrogen evolution at the dropping mercury electrode (DME) was discovered in J. Heyrovský's laboratory about 80 years ago[11]. The history of this discovery was already

reviewed[9] but here we wish to summarize some aspects, which now appear interesting in relation to new trends in protein electrochemistry related to peak H.

2. PROTEINS CATALYZE HYDROGEN EVOLUTION AT DROPPING MERCURY ELECTRODES

In the 1920s, when polarographs were not yet commercially available, in order to make polarography known among potential users, Jaroslav Heyrovský was inviting young scientists to try solving their problems with the instrument in his own laboratory. Medical doctors Herles and Vančura[12] used this offer to study polarographic activity of various human tissue liquids. In blood serum, in urine and in bile a cathodic "wave" always appeared in the region of potentials by about 0.3 V more positive than the polarographic reduction wave of sodium ions, hence the authors called it "presodium wave" (in the Czech original referred to as "prenatrium wave") and ascribed it tentatively to proteins. Their research was continued by Heyrovský and Babička[11] who found that the "presodium wave" was caused by simultaneous presence of albumins and ammonium ions in the solution resulting in catalytic hydrogen evolution.

Brdička[13] characterized the "presodium wave" in a greater detail – he studied it in various buffer solutions showing that the presence of ammonium ion is not essential for the reaction. Influenced by his previous discovery of the "double wave", a catalytic hydrogen evolution from solutions of thiols and cobalt ions[14], he concluded that the catalysis in the "presodium wave" is due to -SH groups contained in proteins. Jurka[15] compared the two cases of polarographic protein effects, showed that the "presodium wave" is much higher than the Brdička's "double wave" and made sure that it does not need the presence of cobalt ions in the solution. From the work of many later polarographic researchers (e.g. refs[16–19]) it follows that the "presodium current" does not depend specifically on the presence of -SH or another group in the catalyst molecule, but rather on its structure or conformation, on its adsorbability and interfacial reactivity, which all are functions of the type and composition of the solution, the electrode potential and the rate of its change. Thus this kind of catalytic current is of general nature and has great potentialities in bioelectrochemistry, beyond classical polarography with the dropping mercury electrode.

3. OSCILLOGRAPHIC POLAROGRAPHY OF AMINO ACIDS AND PROTEINS

In 1941, i.e. almost twenty years after invention of polarography, Jaroslav Heyrovský arrived at a new electrochemical method, the so-called "oscillographic polarography at controlled AC" (OP, AC chronopotentiometry according to the present nomenclature[20]). In this method the DME was polarized by alternating current at 50 Hz, and changes of the electrode potential were measured in dependence on time[21–23]. Usually derivative curves, such as dE/dt versus E, were recorded. Two striking bright points were observed on the derivative oscillogram (Fig. 1): the left point marking the dissolution of mercury at positive potentials, and the right one due to discharge of the cation from the background electrolyte. Presence of an electroactive substance was manifested by an indentation (incision) in the oscillogram (Fig. 1). The method was fast and simple with its cyclic mode, at present utilized in cyclic voltammetry. Commercially available instrument Polaroskop P 524 was produced in Czechoslovakia already at the beginning of 1950 in contrast to the instruments for cyclic voltammetry, which became commercially available almost twenty years later. Polaroskop P 524 displayed the curve with extreme positive potentials at the left side of Fig. 1, in agreement with the way of presentation of DC polarograms at that time. On the other hand the oscillopolarogram of ssDNA in Fig. 2 is shown with negative potentials at the left marginal point to conform with DNA pulse-polarographic and voltammetric curves in Fig. 3.

In 1954 three authors of this paper (D. K., Z. P. and E. P.) worked on their diplomas under supervision of Prof. V. Morávek at the Department of

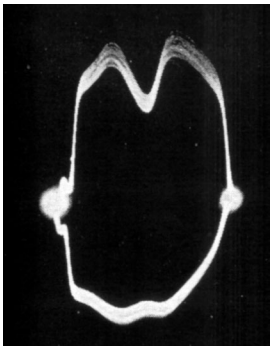

FIG. 1
Oscillopolarogram dE/dt versus E of 1 mM tryptophan in 1 M NaOH; dropping mercury electrode. For more detailed explanation of the oscillographic curves see the legend to Fig. 2. Reproduced from ref.[31] with permission. Copyright Springer-Verlag, Berlin–Heidelberg 1963

Biochemistry, Faculty of Science, Masaryk University in Brno, using polarography and other methods (e.g., paper chromatography and electrophoresis) to analyze proteins from animal blood sera fractionated on $Ca_3(PO_4)_2$. Comparing the results of DC polarography with OP we noticed that some compounds, which were DC polarographically inactive (e.g. amino acids) produced indentations in OP d*E*/d*t* versus *E* curves (Fig. 2). This finding attracted attention of Prof. J. Heyrovský and his close collaborator Dr. R. Kalvoda, who asked one of us (D. K., whose diploma task was analysis of protein fractions by OP) to visit their laboratory and to determine unknown samples of amino acids. This visit to Prague was successful

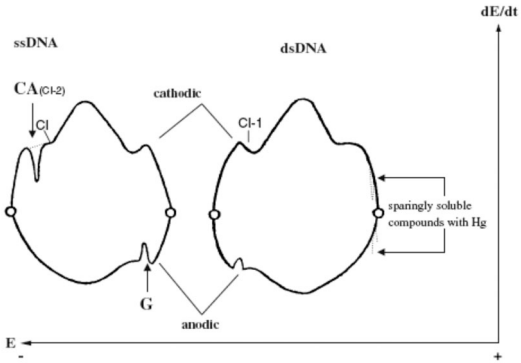

FIG. 2

DNA oscillopolarographic curves d*E*/d*t* versus *E*. The upper part of this curve showed the course of the cathodic polarization in the range from about 0 to about –2 V (depending on the background electrolyte composition). The lower part displayed the anodic polarization from –2 V back to zero. Two striking bright points were observed on the oscilloscope screen: one, marking the most positive potentials (on the left), was due to mercury dissolution and the other one was due to discharge of the cation of the background electrolyte. Presence of an electroactive substance (depolarizer) in the solution was manifested by indentations (incisions). Potentials of these indentations corresponded to half-wave potentials in DC polarography. Comparison of the potentials of the indentations provided an information about reversibility of the given electrode process. The area or depth of the indentations depended on concentration of the analyte, similarly to the height of the DC polarographic wave. Cathodic indentation CI-2 (due to reduction of A and/or C) was characteristic for denatured ssDNA while capacitive CI-1 was produced by both ss and native dsDNA. Both ss and dsDNA produced peak G but the peak produced by dsDNA was much smaller. In this Figure the oscillogram of ssDNA is shown with negative potentials on the left (in agreement with x-axis), i.e. in the way used in electrochemistry at present. In opposite, the oscillogram of dsDNA is displayed with negative potentials on the right, i.e. in the way used in the first decades of the existence of polarography. Adapted from ref.[1]; reprinted with permission from Elsevier. Copyright Elsevier 2002

and represented the beginning of our future contacts with the Heyrovský's laboratory. Soon we realized that most of the OP indentations of amino acids were of capacitive nature.

Indentations at highly positive potentials (Fig. 1) were attributed to the formation and reduction of mercury compounds on the indicator electrode[22]. The amino acids were best observed in 1 M NaOH showing oscillograms characteristic of a given substance[24]. Here we summarize our early OP studies of some amino acids, peptides and proteins. Some of these OP indentations were very sharp in 12 M NaOH[22,25]. These signals were demon-

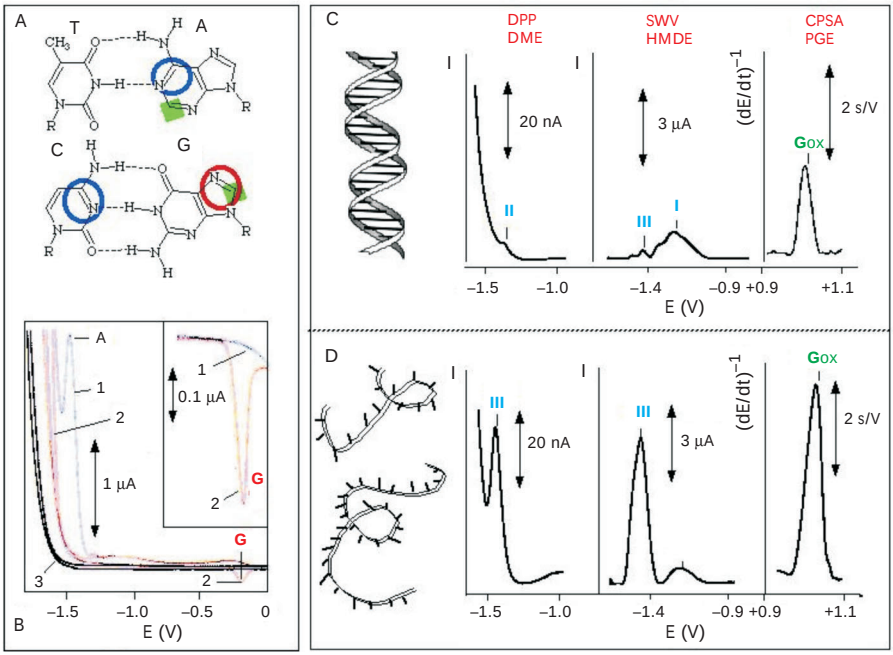

FIG. 3

Reduction and oxidation of single-stranded and double-stranded nucleic acids at electrodes. Schematic representation of Watson–Crick base pairs and electroactive groups (A); primary reduction and oxidation sites at mercury (rectangles) and carbon electrodes (circles) are shown. Cyclic voltammograms of biosynthetic polyribonucleotides (100 mg/ml) measured at the HMDE (B); polyadenylic acid (1), polyguanylic acid (2), background electrolyte (3), inset: detail of peak G. Redox signals obtained by differential pulse polarography (DPP) at the static DME, by adsorptive stripping square-wave voltammetry at a HMDE, and by constant current CPS analysis at a pyrolytic graphite electrode (PGE) with 100 mg/ml of native (double-stranded) (C) or with 50 mg/ml of denatured ssDNA (D). Adapted with permission from ref.[3]. Copyright American Chemical Society 2001

strated[25] with the use of DME and of electrolytically mercury-plated platinum indicator electrode as well. (Hg-plated Pt electrodes were recently greatly improved and applied in lipid[26] and protein analysis[27]) Similar responses were obtained with some dipeptides, such as alanyl-glycine, alanyl-alanine, β-alanyl-histidine (karnosine) and mainly the tripeptide prolyl-leucyl-glycinamide[24,25,28]. The above amino acids and peptides were also analyzed in saturated aqueous solutions of LiCl or KCl[28]. Some proteins, such as insulin, pepsin, trypsin, urease, haemoglobin, casein, edestine, lysozyme, ovalbumin, serumalbumin and human or animal sera, were detectable at the concentrations down to 0.01% in 0.1–1 M ammonia–ammonium chloride buffer containing 1 mM Co(II) or Ni(II) ions[28–30]. These results allowed later detection of proteins in large excess of DNA[29]. Protein–protein interactions were studied by OP[30], e.g., interaction of serum antiglobulinum humanum with gamma globulin resulted in a marked change in the proteins oscillograms, reflecting the antigen– antibody reaction. The OP was applied also in photochemistry of some amino acids, proteins, nucleic acid constituents and UV-irradiated nucleic acids[31–34]. Different processes were studied, e.g., destruction of UV irradiated compounds, denaturation, hydration and dimerization of significant constituents of nucleic acids and nucleic acids themselves[24,33,34]. Results of the experiments mentioned above were reviewed[33,35]. Practical applications in microbiology, related to metabolism and enzyme actions in microbial cultures and some tissue cultures, were reported[36–38]. The early experiments mentioned above pointed to new possibilities in application of electrochemical methods.

The above studies of peptides and proteins by OP were thus limited to Brdička's type analyses and signals due to capacitive effects[22]. Studies of the catalytic hydrogen evolution related to the DC polarographic presodium wave were practically impossible because the expected indentation was too close to the shining marginal point at negative potentials (Fig. 1).

4. POLAROGRAPHY OF NUCLEIC ACIDS AND THEIR COMPONENTS

After the discovery of the DNA double helical structure in 1953[39] attention was paid to the development of new physical and chemical methods for the DNA study. At the beginning of 1960 the mechanism of DNA denaturation was elucidated and the ability of DNA to reform its double helical structure (renaturation) was discovered[40]. This finding intensified attempts to develop new methods of DNA analysis. To our knowledge, H. Berg was the first one who attempted to analyze DNA electrochemically. In 1957 he pub-

lished a paper[41], where he concluded that DNA and RNA were not electroactive. At the same time one of us (E. Paleček) was graduate student at the Institute of Biophysics in Brno and he showed that DNA and RNA produce reduction and oxidation signals using OP[42], that is by the same method used by Berg. Moreover, he showed that also monomeric components of nucleic acid are electroactive[43] (Table I). The difference between Berg's and Paleček's results were in different ionic conditions used by these authors. Berg used alkaline pH with cobalt ions (Brdička's solution) while Paleček used acidic, alkaline and neutral pH. It turned out that adenine, cytosine and guanine are reduced in their protonated state and therefore their electrode processes do not take place at alkaline pH. Negative results of Berg were recently discussed[6]. Soon it was shown that electrochemistry reflects changes in DNA structure and clearly distinguishes denatured (single-stranded) from native (double-stranded) DNA[42,44–46]. This was an important finding evoking great interest of DNA investigators in DNA electrochemistry, resulting in invitation of Paleček to top DNA laboratories. The peculiar story describing adventures of Paleček in the USA was already published[1].

4.1. Why Oscillographic Polarography?

In difference to DNA experts some electrochemists were reluctant to admit that DNA could be analyzed by means of electrochemistry. In addition, many of them did not like OP, trying to persuade Paleček to use DC polarography instead. However, DC polarography was poorly sensitive in DNA analysis requiring DNA concentrations mostly not acceptable for biochemical experiments[47]. Better sensitivity of OP was related to several reasons, such as (i) DNA was adsorbed at DME at open circuit potential followed by very fast AC polarization of the DME, while in DC polarography the negatively charged (polyanionic) DNA had to be adsorbed at DME at about –1.4 V, where the reduction wave of denatured DNA appeared; (ii) OP inherently included principles of adsorptive stripping methods, because DNA could accumulate at DME for several seconds (before application of the AC polarization), depending on the chosen drop time. The cyclic mode of OP made it possible to measure specific signals of guanine and its residues in DNA and RNA[42,45,48]. These signals were due to oxidation of the guanine reduction product back to guanine (see below for details). OP was thus very useful for about eight years, representing at that time the method of choice for DNA analysis. Later AC and pulse polarography entered the field and became useful in DNA studies[49]. More detailed survey of electrochemistry

of nucleic acids in the first thirty years (1958–1989) was recently published[6]. Many of the findings made in that period of time, such as DNA modified electrodes[50–52], DNA labeling[53,54], DNA unwinding at the electrodes surface[55,56], etc., have been utilized in the present development of the sensors of DNA damage and DNA hybridization[2].

5. POLAROGRAPHY OF NUCLEIC ACID COMPONENTS

The early excitement with the applications of OP and related techniques in the study of nucleic acids is still palpable. After graduating from the Masaryk (later J. E. Purkyně) University in Brno, Paleček had not only the foresight of pioneering the work in this area but also of collaborating with some of his former student colleagues either in other institutions (D. Kaláb, Z. Pechan) or with those who joined him directly (B. Janík and V. Vetterl) in the Institute of Biophysics, Czechoslovak Academy of Sciences. The excitement was understandable since nucleic acids were becoming the macromolecules of the future (and you cannot study them completely without understanding the proteins), and the application of OP promised insight into nucleic acids at the primary-secondary-tertiary structural levels. Al-

TABLE I
Oscillopolarographic activity of nucleic acids and their components

Substance	Supporting electrolyte				
	H_2SO_4	HCOOH	$HCOONH_4$	KCl	NaOH
Adenine	+	+ (0.4 μg)	+		+
Guanine	+	+	+ (5 μg)		+
Cytosine	+	+	+		+
Thymine	–	–			+ (3 μg)
Uracil	–	–			+ (3 μg)
Adenylic acid	+	+	+	+	+
Guanylic acid	+	+	+	+	
Deoxycytidylic acid	+	+	+	+	+
DNA	–	–	+ (2 μg)	+ (15 μg)	+
RNA	–	–	+	+	+

+, produced one indentation; –, does not react. The quantity of substance in 1 ml, at what the indentation was just visible, is given in parentheses. Reproduced according to ref.[99] with permission from Springer-Verlag, Berlin–Heidelberg.

though OP could produce signals of differing origins, only the basic DC polarography could unambiguously pin-point the reduction components of the signal.

The early work[57] demonstrated that cytosine and its nucleoside and nucleotide analogues, to the contrary of previous report[58], indeed produced a DC polarographic reduction wave. Details on this process and elucidation of the reduction process observed in DC polarography came shortly afterwards[59]. Cytosine, certain 5-derivatives and corresponding nucleosides and nucleotides were electrolyzed in their protonized form at the DME in an overall 4 e process. As result, the C-4 amino group was cleaved off and the 3–4 double bond was saturated. Further details and the existence of intermediate steps were studied later[60].

A fairly comprehensive review of polarographic behavior of biologically interesting compounds, purine, pyrimidine, pyridine and flavine bases, nucleosides and nucleotides further expanded our knowledge of the reduction processes, the mechanisms and the chemistries involved[61]. Without any surprises, pyrimidine, cytosine, purine and adenine were polarograhicaly reducible in aqueous media at DME. Details were also given on electrochemical reduction of 6-substituted purines with the reduction of 1,6 N=C bond[62]. Similarly, adenine nucleosides and nucleotides were reduced at the same position[63]. The reduction of di- and oligonucleosides of cytosine and adenine was similar to the monomers except the intervening and complicating adsorption at the DME [64]. The next logical step was elucidation of the polarographic reduction of polynucleotides, poly(A), versus their macromolecular characteristics[65]. Three distinct forms of poly(A) were postulated[66,67]. At certain conditions the polarographic reduction wave was distorted by desorption and repulsion of poly(A) from the electrode[68].

6. CONSTANT CURRENT CHRONOPOTENTIOMETRY OF PROTEINS

The ability of proteins to catalyze hydrogen evolution at mercury electrodes was discovered almost 80 years ago (see above) but the utilization of this finding in the analysis of proteins was negligible. DC polarographic presodium wave was too close to the background discharge and difficult to measure. Application of OP did not help because the indentation of proteins was too close to the shining end point (Fig. 1) at negative potentials. CPS proved to be very useful technique in the analysis of DNA and RNA at carbon[4,69] and mercury electrodes[70].

About ten years ago we attempted to apply CPS in studies of peptides[71] and we observed peaks of vasopressin well separated from the electrolyte

background which we denominated as peak H. In several years peak H turned out to be a very useful tool in the analysis of peptides and proteins[10,72–81].

In the beginning of the 1980s it was found that tyrosine and tryptophan residues in proteins are oxidized at carbon electrodes[82,83]. Later it has been shown that using square wave voltammetry and CPS [4,10,79,83–87], better developed peaks can be obtained than with linear sweep voltammetry. However, oxidation responses of proteins did not appear sufficiently sensitive to changes in protein structure[75] and in peptide and protein redox states[73]. In contrast, CPS peak H displayed sensitivity to local and global changes in protein structure at mercury electrodes[4,10,72,73,79].

6.1. High Current Densities are Important for the Sensitivity of Peak H to Changes in Protein Structure

Because of its catalytic nature involving a large number of electrons per protein molecule high current densities can be used to obtain peak H. At such current densities very fast potential changes are induced in absence of an electrode process. These changes are by several orders of magnitude faster than those in usual linear sweep voltammetry (LSV). It was shown that fast potential changes are of critical importance in electrochemical detection of changes in protein structure[4,10,76,78]. At pH 9.3 and around neutral pH we observed large differences in peak H height of native and denatured forms of bovine serum albumin (BSA) and some other proteins (Fig. 4). Native proteins usually produce small signals while their denatured forms yield peaks which are ten to fifty times higher (Figs 4a and 4c). Using high stripping currents is critical for observing such large differences between peaks of native and denatured proteins[74,76,78,87].

6.2. Proteins are not Denatured Upon Adsorption at HMDE at Potentials Close to p.z.c.

Very small peaks of native proteins suggested that no significant denaturation of the protein took place at the mercury electrode surface. Such results were in contradiction with the long time held belief that routinely used metal electrodes such as gold, platinum, mercury and silver, lead to denaturation and irreversible adsorption of the proteins[88]. It was also believed[89] that proteins were denatured upon adsorption at mercury electrodes producing adsorbed layers of uniform thickness[90]. However, conclusions of some papers did not agree with the results claiming the protein

surface denaturation at mercury electrodes[91,92]. Recently we have studied the problem of protein surface denaturation at mercury electrodes in a greater detail[75,76,78,79]. We found that in a wide pH range proteins are not denatured when adsorbed at the mercury electrode surface at the potential of zero charge (p.z.c.) and at potentials positive of p.z.c. However, we showed that the denaturation of proteins can take place at negatively

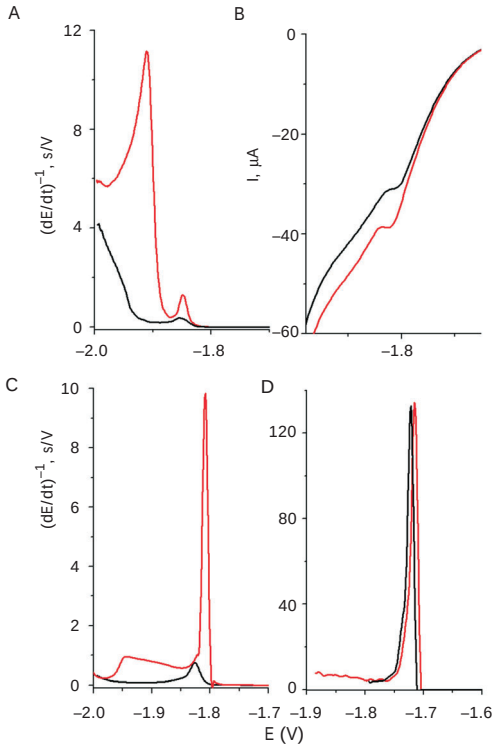

FIG. 4

Peaks H of native and denatured BSA. 100 nM native BSA (black) and denatured BSA (red) with 70 mM (A, B) or 56 mM urea (C, D) in 50 mM sodium borate, pH 9.3 (A) or 50 mM (C) and 200 mM sodium phosphate, pH 7 (D); I_{str} –35 μA (A) or –30 μA (C, D). Linear sweep voltammograms (LSV) of 120 nM native (black) and urea-denatured BSA (red) in 50 mM sodium borate, pH 9.3; step 2 mV, scan rate 2 V/s (B). Denaturation of 14.4 μM BSA in 0.1 M Tris–HCl, pH 7.3 with 8 M urea was performed at 4 °C overnight. The protein solution was then diluted by the background electrolyte to the final protein concentration. BSA was adsorbed at HMDE for accumulation time t_A 60 s at accumulation potential E_A –0.1 V with stirring at 1500 rpm. Reprinted from ref.[75] (A, B) and from ref.[78] (C, D) with permission. Copyright Elsevier 2008 and Royal Society of Chemistry 2009.

charged mercury surface at higher ionic strengths, at neutral pH [78] and at acid pH (e.g. pH 4.5 [79]) even at ionic strengths which cause no significant harm to proteins at neutral and alkaline pH[10,78].

6.3. Determination of Peptide and Protein Redox States

Oxidized and reduced forms of peptides and proteins were studied using carbon electrode and hanging mercury drop electrode (HMDE)[72,73,75]. We found that reduced peptides adsorbed at positively charged HMDE produced substantially higher peak H than their oxidized form. Similar behavior was observed with thioredoxin (TRX, general protein disulfide reductase with a large number of biological functions). 1 mM TRX produced well developed peak H [73]. Peak was used not only for determination of TRX redox state but also to study interactions of this protein with the product of lipid peroxidation 4-hydroxy-2-nonenal. Using carbon electrodes it was not possible to discriminate between reduced and oxidized TRX [73] and peptides[72]. Using peak H redox states of other peptides and proteins can be determined. For example we were able to follow changes of the redox state of the tumor repressor protein p53 and insulin[93].

6.4. Application of CPS Peak H in Biomedicine

Understanding better the changes in protein structure at the mercury electrode surface helped us to apply electrochemistry in studies of proteins important in biomedicine. For example, we studied α-synuclein (AS) protein which is involved in Parkinson disease[94]. We showed that both oxidation signals at carbon electrodes and peak H at HMDE can be used to study this protein[80]. HMDE was particularly sensitive to pre-aggregation changes, detected at short incubation time periods preceding AS oligomerization observable by dynamic light scattering. Our preliminary data obtained with mutant AS suggest that electrochemical techniques have a good chance to become of great value in studies of some neurodegenerative diseases[77]. Application of electrochemistry in cancer research and particularly studies of CPS responses of the tumor suppressor protein p53 and its mutants appear particularly interesting. The CPS responses of wild and mutant proteins agreed well with changes of the X-ray crystal structures resulting from single amino acid exchange in these proteins.

6.5. Electrochemistry of Biomacromolecules

Both electrochemistry of proteins[2,8] and nucleic acids are at present important scientific fields. Electrochemistry of lipids is closely related to membrane research and electrochemical studies of lipids have been widely reviewed[26,95] (and references therein). On the other hand literature on electrochemistry of polysaccharides (PS) is scarce. Only very recently we have shown that electroactive labels based on Os(VI) complexes can be introduced in PS and determined at carbon[96] and mercury electrodes[97]. Moreover, we have found that sulfated PS, such as carrageenans and dextran sulfate produce CPS [98] peaks H_{PS} which, similarly to protein peak H, are due to the catalytic hydrogen evolution[98]. No such peak was observed in PS not containing sulfur. It can be expected that electrochemical methods will become important tools in the PS research very soon. Thus at present electroanalysis can be applied to all main classes of biomacromolecules, i.e. nucleic acids, proteins, lipids and polysaccharides.

7. CONCLUSION

There is no doubt that at present electrochemical reactions of proteins and nucleic acids are booming fields. Increasing number of papers in these fields are closely related to the progress in genomics and proteomics providing important information about basic life processes forming a solid foundation for new medicine of the 21st century. Sensors and detectors for DNA and protein analysis prefer solid electrodes to liquid mercury. On the other hand this paper shows that mercury electrodes are very convenient in biomacromolecule analysis. Fortunately, it has been shown that solid amalgam and mercury film electrode[26] can yield similar voltammetric and CPS responses of biomacromolecules as HMDE. We can thus expect that Hg-containing electrodes will be more and more applied in bioelectrochemistry.

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