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Electrochemical Behaviour of Native and Denatured β-Sheet Breaker Prion Protein

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Prion diseases are fatal neurodegenerative and infectious disorders of humans and animals, characterized by structural transition of the host-encoded cellular prion protein (PrP^{C}) into the aberrantly folded pathologic isoform PrP^{Sc}. The conformation change, from the α-helix in the natural protein form (PrP^{c}) to the β-sheet of the modified protein form (PrP^{Sc}), significantly influence the protein function. The mutated form (PrP^{Sc}) is extremely resistant to the cell degradation processes and may bind other PrP^{c} molecules inducing the conformation change to the PrP^{Sc}. The insufficiency of the physiological PrP^{c} and toxic incidence of PrP^{Sc} participate on the genesis of prion neurodegenerative diseases. The main aim of this study was to suggest electrochemical methods for detection of β-sheet breaker prion protein. For this purpose cyclic voltammetry (CV), differential pulse voltammetry (DPV), differential pulse voltammetry Brdicka reaction and chronopotentiometric stripping analysis (CPSA) were used. Primarily, the basic electrochemical behaviour of prion and its redox system was observed using CV where 3 various peaks at potential -0.5 V (peak 1), -1.2 V (peak 2) and -1.8 V (peak 3) were detected. Under the optimal conditions (phosphate buffer, pH 7.38 and time of accumulation 100 s), detected by DPV prion was characterized using different techniques and their limits of detection were found. Adsorptive transfer stripping technique coupled with the abovementioned methods offers very lower detection limits. The lowest limit detection were determined by CPSA AdTS i.e. 25 pmol in volume of 5 μl. CPSA is therefore a very sensitive tool for the studying of prion behaviour. Moreover, the influence of heat denaturation was observed. It clearly follows from the results obtained that signals of prion decreased linearly depending on the duration of the heat treatment at 99°C for various time intervals: 0, 15, 30, 45, and 60 min. The correlation coefficients of the measured dependencies as 0.9929, 0.9973, 0.9965 and 0.9957 were determined by CV, DPV, DPV Brdicka reaction and the most sensitive CPSA, respectively.

Keywords: Prion protein (PrP^{Sc} – scrapie); Adsorptive transfer stripping technique; cyclic voltammetry (CV); differential pulse voltammetry (DPV); Brdicka reaction; Chronopotenciometric stripping analysis (CPSA)

1. INTRODUCTION

It has been generally concluded that every infectious disease may be produced only by such agents with a nucleic acid. Professor Stanley B. Prusiner formulated the prion theory in 1982 for which he praised Nobel Prize in Physiology or Medicine in 1997. This theory assumes that there is a pathogenic agent of prion (proteinaceous infectious particle), an infectious protein. The other Nobel Prize was awarded to professor D.C. Gajdusek for his work focused on Kuru, the first human prion disease demonstrated to be infectious, which was associated with the practice of funerary cannibalism by the South Fore people of New Guinea in 1950s [1].

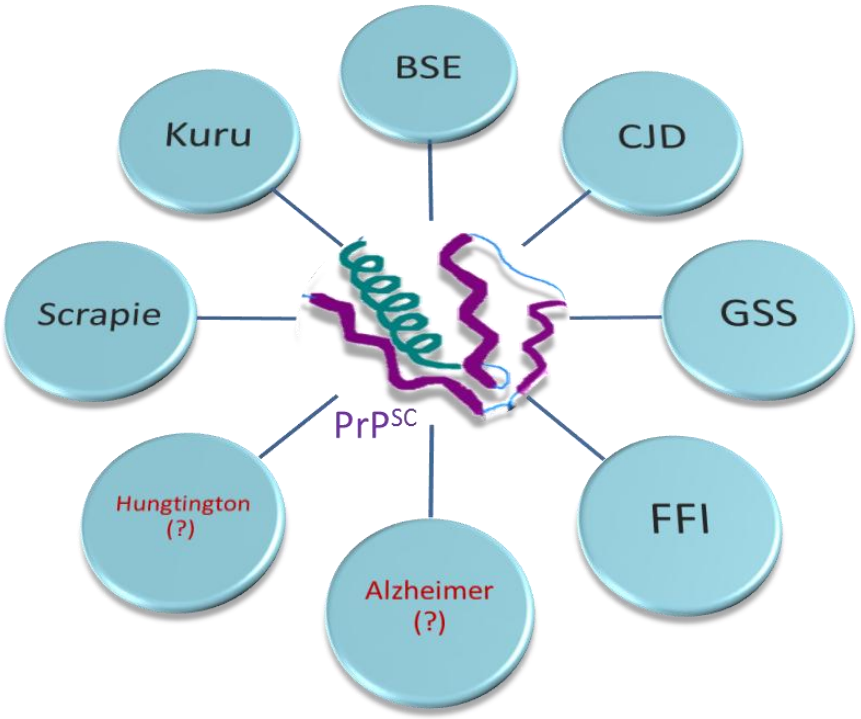

Figure 1. Prions (PrP^{SC}) have been held responsible for a number of degenerative brain diseases, including scrapie (a fatal disease of sheep and goats), kuru, bovine spongiform encephalopathy (BSE, commonly known as "mad-cow" disease), Creutzfeldt-Jacob disease (CJD), an unusual form of hereditary dementia known as Gertsmann-Straeussler-Scheinker disease (GSS) and fatal familial insomnia (FFI). Moreover, Alzheimer and Huntington disease are recently connected with protein-folding diseases.

Prion protein is a biomolecule naturally occurring in the animal cells. This protein is present in all mammal cells and occurs primarily in neural cells and immune system cells. Its physiological function is not completely clear, however it is assumed to participate on synaptic transfer and cell differentiation. It may become infectious. Natural and infectious forms differ only by the spatial

conformation. The conformation change from the α-helix in the natural protein form (PrP^{c}) to the β-sheet of the modified protein form (PrP^{Sc}), significantly influence the protein function [2]. The infectious form (PrP^{Sc}) is extremely resistant to the cell degradation processes and may bind other PrP^{c} molecules and change them to PrP^{Sc} [3]. The insufficiency of the physiological PrP^{c} and toxic incidence of PrP^{Sc} participate on the genesis of prion neurodegenerative diseases (transmissible spongiform encephalopathies, TSE) [4]. Nine of the main diseases caused by prions are known. Four of these diseases occur in human and five in animals. The most frequent human prion disease (85 %) is Creutzfeldt-Jakob disease (CJD) [5], which may be due to consumption of the food contaminated by the prions of diseased cow [5,6]. There are hypothesis relating prions to the other neurodegenerative diseases such as Alzheimer's or Huntington disease [7]. In the animal kingdom, bovine spongiform encephalopathy (BSE) is the most known, so called "the disease of mad cows". With the outbreak of epidemics and discovery of BSE case almost everywhere in Europe, the question arises of how to improve screening methods and the possibility of detection of prions.

Detection and quantification of prion is not simple due to the low amounts in biological samples especially during the early stages of disease. For this purpose, the method Protein-misfolding cyclic amplification (PMCA) for providing sensitivity improvement has been used [8]. Method is based on converting additional normal prion protein to the sample with infectious prion. PMCA involves repeated cycles on incubation and sonication. These repeated cycles can amplify the amount of prion protein present in the sample from four to 40 times in two weeks [9,10]. Analytical methods, which are used for prion detection are usually based on: (I) conformation dependent immunoassays, which are currently the most used assays for routine screening in plasma [11]. (II) Western blot assays [12] (III) CE – based methodology [13] and (IV) spectroscopic method [14].

One of the current research directions is the increasing of the method sensitivity enabling of detection of extremely low amounts of prions in short time [15]. Suitable method seems to be electrochemistry. Square-wave voltammetry and cyclic voltammetry are commonly used for protein determination and provide very low limits of detection [16]. Other electrochemical method used for the determination of proteins is Brdicka reaction in connection with differential pulse voltammetry (PDV). Brdicka reaction has been used for the study of physiological concentration of proteins in the wide range of organisms and is widely applied as a diagnostic method in the clinical medicine and pharmacology [17]. Another electrochemical method based on monitoring of catalytic signals is chronopotentiometry stripping analysis (CPSA). CPSA in the combination with adsorptive transfer stripping technique is able to determine femtomolar concentrations of analyte in a very low sample volume (5 μl) [18]. Voltammetric methods were also used for analysis of prion proteins [19], however, their detailed characterization has not been done yet.

The main objective of our work was to carry out the electrochemical behaviour of prion protein PrP^{Sc} on mercury drop electrode. For these purpose fundamental electrochemical techniques like cyclic voltammetry (CV), differential pulse voltammetry (DPV), differential pulse voltammetry with Brdicka reaction (DPV – Brdicka reaction) and chronopotentiometric stripping analysis (CPSA) were used. To reach very low limits of detection adsorptive transfer stripping technique (AdTs) was used. Moreover, we focused our attention on monitoring the signal change of native and denatured form of prion.

2. EXPERIMENTAL PART

2.1 Chemicals

Prion protein β – sheet breaker peptide fragment (Asp-Ala-Pro-Ala-Ala-Pro-Ala-Gly-Pro-Ala-Val-Pro-Val; FW = 1597.9) was purchased from Sigma Aldrich (St. Louis, USA) and dissolved by 8.77 M trifluoracetic acid bought from the same supplier. Sodium phosphate and other used chemicals were purchased from Sigma Aldrich.

Working standard solutions were prepared daily by dilution of the stock solutions. The pH value was measured using WTW inoLab Level 3 with terminal Level 3 (Weilheim, Germany), controlled by personal computer program (MultiLab Pilot; Weilheim, Germany). The pH-electrode (SenTix- H, pH 0–14/3M KCl) was regularly calibrated by set of WTW buffers (Weilheim, Germany).

2.2 Electrochemical measurements

Electrochemical measurements were performed with AUTOLAB Analyser (EcoChemie, Netherlands) connected to VA-Stand 663 (Metrohm, Switzerland), using a standard cell with three electrodes.

The working electrode was a hanging mercury drop electrode (HMDE) with a drop area of 0.4 mm^2. The reference electrode was an Ag/AgCl/3M KCl electrode and the auxiliary electrode was a graphite electrode. The supporting electrolyte was prepared by mixing buffer components except sodium chloride.

For smoothing and baseline correction the software GPES 4.4 supplied by EcoChemie was employed.

2.2.1 Adsorptive transfer stripping technique

Principle of the AdTS is based on the strong adsorbing of the studied analyte on the electrode surface at an open electrode circuit. The excess of analyte is rinsed from the surface of the working electrode in the buffer. The adsorbed analyte is finally detected in the presence of indifferent electrolyte. Volume of studied sample was 5 μl in all methods. Time of adsorption was tested in range from 10 to 120s.

2.2.2 Cyclic voltammetry (CV) and PrP^{Sc}

Prion protein was studied using CV. Amount of adsorbed sample was 5 μl. Time of accumulation, pH and electrolyte type was optimized. Volume of supporting electrolyte was 5 ml in the electrochemical cell. CV parameters were as follows: an initial potential 0 V, an end potential -1.9 V, step potential 2.44 mV, scan rate from 20 to 640 mV/s. All experiments were carried out at room temperature (22-24 °C)

2.2.3 Differential pulse voltammetry and PrP^{Sc}

The amount of prion PrP^{Sc} was measured using AdTS DPV. Following supporting electrolytes were tested: 0.5 M sodium phosphate in pH range from 5.59 to 8.04, borate in pH range from 7.09 to 9.11 and acetate in pH range from 3.8 to 5.6. DPV parameters were follows: an initial potential of –0.2 V, an end potential –0.8 V, a modulation time 0.057 s, a time interval 0.2 s, a step potential of 1.05 mV/s, a modulation amplitude of 250 mV, E_{ads} = 0 V. All experiments were carried out at room temperature (22–24 °C). The analysed DPV samples were deoxygenated prior to measurements by purging with argon (99.999%) saturated with water for 120 s.

2.2.4 Differential pulse voltammetry - Brdicka reaction and PrP^{Sc}

DPV Brdicka reaction was also used for prion determination. The Brdicka supporting electrolyte containing 1 mM $Co(NH_3)_6Cl_3$ and 1 M ammonia buffer ($NH_3(aq)$ + NH_4Cl, pH = 9.6) was used and changed per one analysis. DPV parameters were as follows: initial potential of -0.7 V, end potential of -1.75 V, modulation time 0.057 s, time interval 0.2 s, step potential 2 mV, modulation amplitude -250 mV, E_{ads} = 0 V. All experiments were carried out at temperature of 4 °C (Julabo F12 cooler, Germany).

2.2.5 Peak H and PrP^{Sc}

Constant current CPSA was used for the determination of prion by recording the inverted time derivation of potential $(dE/dt)^{-1}$ as a function of potential E. CPSA parameters were as follows: potential limit -1.9 V, I_{str} of –1 μA, max. time of measurement 600 s, temperature 20 °C, supporting electrolyte phosphate buffer (pH 7.38).

2.3 Descriptive statistics and estimation of detection limit

Data were processed using MICROSOFT EXCEL® (USA). Results are expressed as mean ± standard deviation (S.D.) unless noted otherwise (EXCEL®). The detection limits (3 signal/noise, S/N) were calculated according to Long and Winefordner [20], whereas N was expressed as standard deviation of noise determined in the signal domain unless stated otherwise.

3. RESULTS AND DISCUSSION

3.1 Adsorptive transfer stripping technique

In the fact, this technique belongs to hyphenated ones because composes of two different processes: i) accumulation and ii) transfer. The analytical purpose of the accumulation process has been found more than fifty years ago when working with the sc. oscillographic polarography [21,22]

and this technique was termed as adsorptive stripping technique (AdS). The mercury dropping electrode was polarized with alternating current several seconds after the mechanical drop detachment- in this quiescent period the electrode was held at the "open circuit potential", during which certain compounds with adsorptive properties could be adsorbed on the working electrode surface [23,24]. Other historical remarks of this technique were reviewed by Kalvoda [21]. Even if this technique markedly enhance sensitivity of an electrochemical measurements, decrease detection limits for target molecules, the affecting by various interferences still remains. Thus, a real sample must be homogenized with respect to analyte, if the sample is measured by this technique.

To prevent interferences Palecek and his colleagues brilliantly improved the adsorptive stripping technique by so-called transfer step [25]. The main improving is based in electrode removing from a solution after accumulating of a target molecule on its surface, rinsing of the electrode and transferring to a pure supporting electrolyte, where no interferences are present. The scheme of adsorptive transfer stripping technique (AdTS) can be summarized to the following steps: (**1**) renewing of a surface of a working electrode; (**2**) adsorbing of target molecule in a drop solution onto the surface at open circuit and/or superimposed potential; (**3**) washing the working electrode in a solution; (**4**) transferring of the washed electrode to a supporting electrolyte and measurement of adsorbed target molecules (Fig. 2).

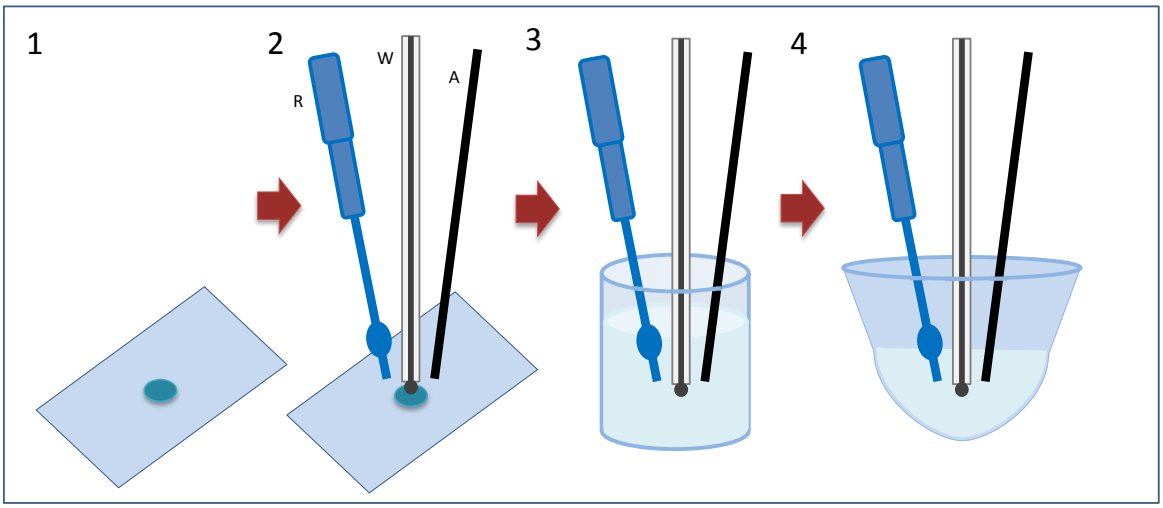

Figure 2. Scheme of using of adsorptive transfer stripping technique for study of prions by various techniques. Renewed surface of HMDE (1) is placed to drop containing prion standard (2) where Prion is bond only. Other low molecular substances are washed out in the following step (3) HMDE electrode is placed to supporting electrolyte (4) and analysed.

3.2. Cyclic voltammetry

CV is perhaps the most widely used electrochemical technique, and is frequently used for the characterization of a redox system and is often the first experiment performed in an electroanalytical study. It can provide rapid information about the number of redox states of the electroactive species, as

well as qualitative information about the stability of these oxidation states and the electron transfer kinetics [26,27]. CV follows the potential scans from the starting potential to the end potential, then reverse from the end potential back to the starting potential. CV is suitable for protein detection as described already in the paper of Rezaei-Zarchi *et al.* [16]. For this purpose we decided to use CV for basic electrochemical characterization of prion protein.

It was observed using AdTS cyclic voltammetry that prions provided oxidations signals only. In oxidation part of cyclic voltammograms, three peaks at potential -0.5 V (peak 1), -1.2 V (peak 2) and -1.8 V (peak 3) were detected (Fig. 3A), which indicated three electroactive moieties in prion molecule. Based on the primary structure of the protein fragment, there were $–NH_2$ and –OH moieties, which can undergo oxidation.

However, detection of three peaks can be associated with some structural features of this protein as β-sheet conformation. Further, this method was optimized using various scan rates from 20 to 640 mV/s (inset in Fig. 3A). It was observed that the increasing scan rate enhanced the height of all detected peaks. Logarithms of peak height were plotted against logarithm of scan rate. The obtained dependencies revealed that peak 2 with the most linear regression line (R^2 higher than 0.9) is diffusion controlled process.

The other peaks (peak 1 and peak 3) are close to logarithmic dependence, which indicate some adsorption-desorption process and structural controlled processes.

Besides this, the resolution of the detected peaks decreased at highest used scan rates, which can be associated with the fact that redox processes are slower and, thus, structural dependent. In order to use one of scan rates for calibration of prion protein, the scan rate of 160 mV/s was used. As a peak suitable for calibration, peak 2 was employed due to the fact that the electrode process is diffusion controlled and well correlates with the concentration of prion protein. With increasing concentration the growing peak 2 was observed (Fig. 3B). The calibration curve was measured (R^2 higher than 0.9) and the detection limit 32 μg/ml was estimated as 3 S/N.

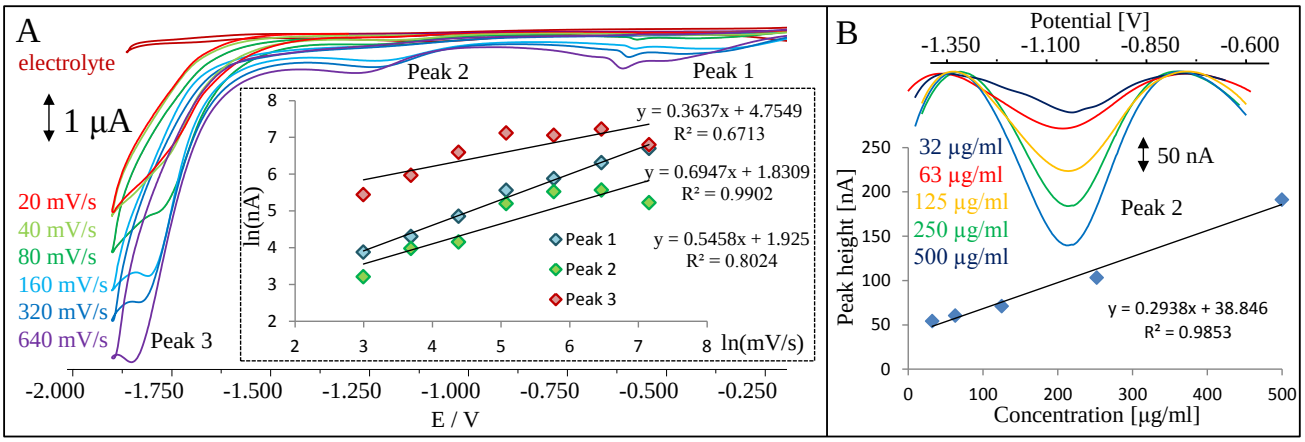

Figure 3. CV A) Dependence of scan rate on peaks height. The peaks heights enhance with increasing scan rate. B) Dependence on concentration. With increasing concentration enhance the peak height. Calibration curve

3.3. Differential pulse voltammetry

DPV is an extremely useful technique for measuring trace levels of organic and inorganic species. In DPV, fixed-magnitude pulses – superimposed on a linear potential ramp – are applied to the working electrode at a time just before the end of the drop. The current is sampled twice, just before the pulse application and again late in the pulse life. The first current is instrumentally subtracted from the second, and this current difference is plotted versus the applied potential. In addition to improvements in sensitivity and resolution, the technique can provide information about the chemical form in which the analyte appears [26].

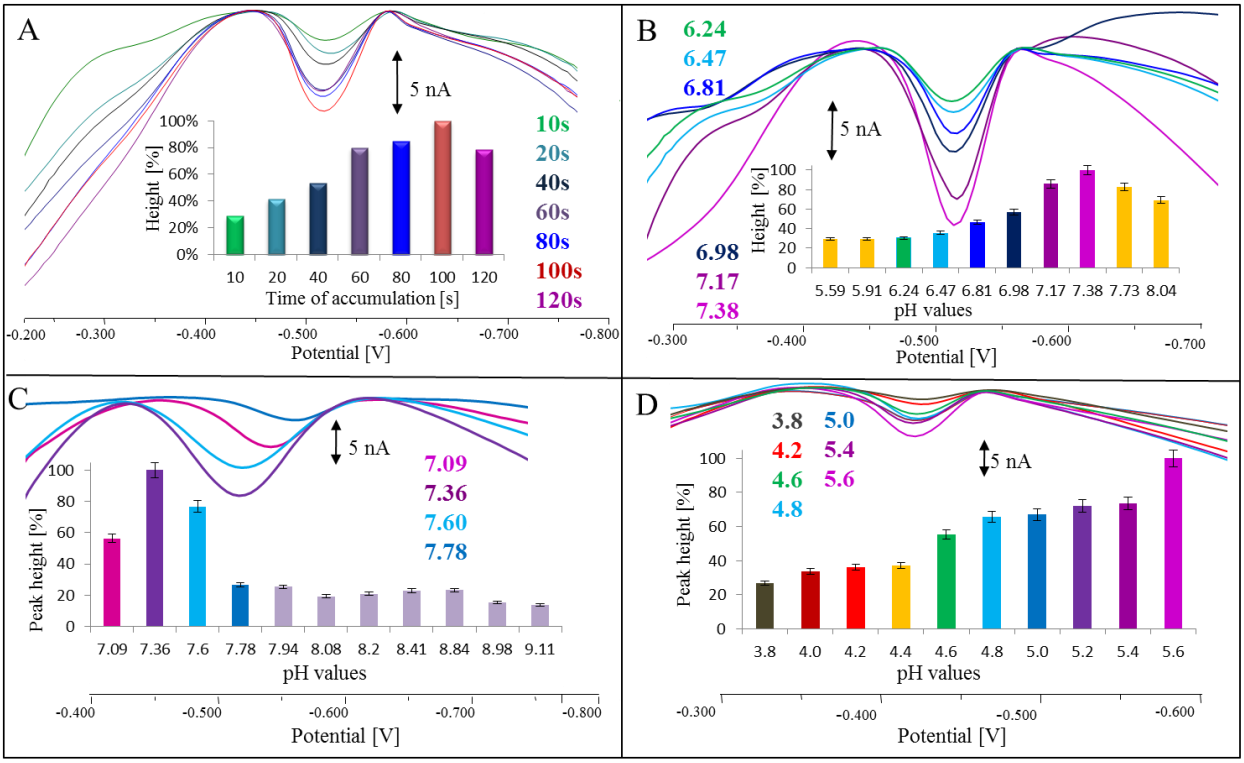

Figure 4. AdTS DPV. A) Dependence of peak height on time of accumulation. Current response enhanced with the increasing time of accumulation. The highest peak was determined under 100 s long accumulation. B) Phosphate buffer. Dependence of peak height on pH. Electrochemical response enhanced with the increasing pH. The highest electrochemical response was determined in pH 7.38. C) Borate buffer: Dependence of peak height on pH. The highest response was determined in pH 7.36. Higher values of pH decreased the peak height. D) Acetate buffer: Dependence of peak height pH [%]. The highest response was determined at pH 5.6.

In this study AdTS DPV was employed for prions electrochemical characterization as electrochemical method more sensitive compared to CV. Primarily, the method was optimized to achieve the best condition for prion detection. Particularly, we tested the following conditions as time of accumulation, buffer composition and pH of the supporting electrolyte. Determining dependence of peak height on time of accumulation showed that current response enhanced with increasing time of

accumulation (Fig. 4A). The highest peak was determined under 100 s long accumulation. Longer time of accumulation provided also sufficient responses but due to formation of multiple layers the signal slightly decreased (inset in Fig. 4A).

Moreover, the dependence of prion protein peak height on buffer composition and its pH was tested. As buffers phosphate, borate and acetate buffers were chosen. The pH range was from 3.8 to 5.6 in acetate buffer, pH from 6.24 to 8.04 in phosphate buffer and pH from 7.09 to 9.11 in borate buffer. Generally phosphate buffer provided higher signals of the same concentration of prion (125 µg/ml) compared to borate and acetate buffer. The highest electrochemical response was observed in pH 7.38 in the presence of phosphate buffer (Fig. 4B). Despite the fact that borate buffer of pH 7.38 also gave good results, the signal did not reach the phosphate buffer values (Fig. 4C). In addition, acetate buffer indicated increasing trend in peak height but the measured heights were not sufficient to other buffers. This phenomenon, the highest peak of prion determined at neutral pH, can be associated with the fact that structure and charges of amino acids moieties of prions is dependent on pH and physiological conditions are the most convenient.

Under the optimal conditions (phosphate buffer, pH 7.38 and time of accumulation 100 s), the calibration curve was measured with equation y = 0.162x – 0.9898 and regression coefficient R^2 higher than 0.99. This shows very good linearity of the electrochemical response on concentration of prion protein. Considering the fact that prions are still of interest of numerous scientists, DPV fulfils demand on the detection of this protein in real samples after pre-treatment. The detection limit as 16 µg of prion protein per ml was estimated (3 S/N) i.e. 50 pmol in 5 µl drop (Fig. 5A). The very well developed signals are shown in Fig. 5B.

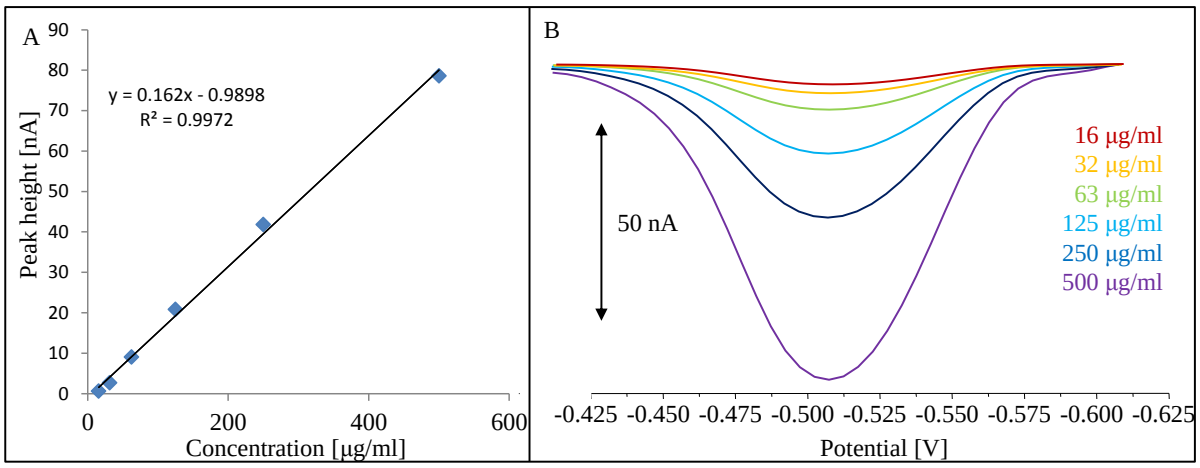

Figure 5. DPV. A) Calibration curve with regression coefficient R^2 = 0.9972. B) DP voltammograms of various prion protein concentrations. The peak height enhanced with the increasing concentration of prion proteins. Tested concentration range was from 500 µg/ml to 16 µg/ml.

3.4 Differential pulse voltammetry – Brdicka reaction

DPV-Brdicka reaction belong to the most common methods used for the determination of proteins. The method for polarographic determination of proteins containing sulfhydryl groups using

mercury electrode was developed by Brdicka in thirties of the last century [17,28,29] and several times optimized [30-37]. . The mechanism of the reaction is based on the catalytic evolution of hydrogen on mercury electrodes from solutions of protein containing –SH group in ammonia buffer and hexaamminecobalt(III) chloride complex ($Co(NH_3)_6Cl_3$) called Brdicka solution [38].

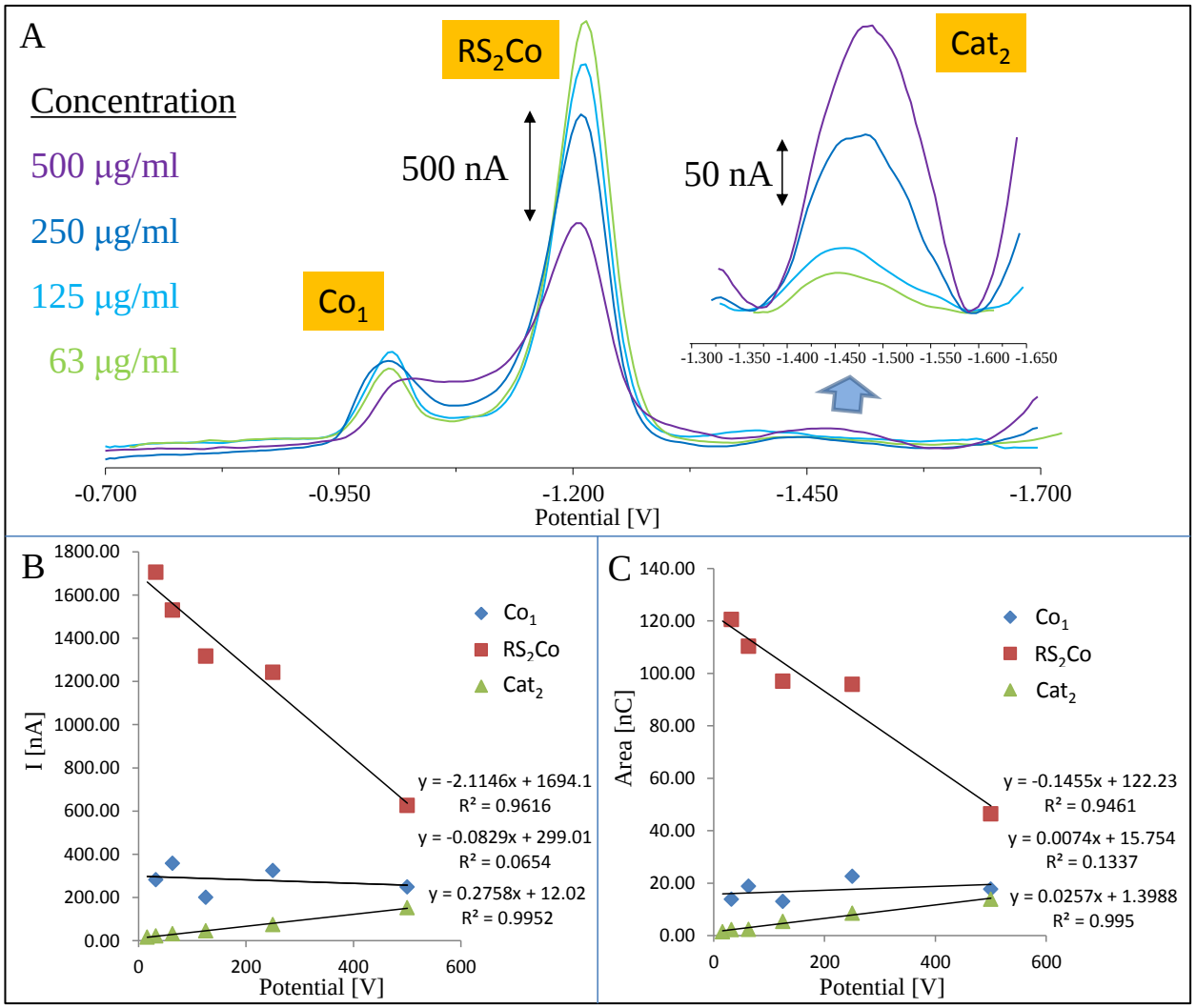

Figure 6. AdTS DPV Brdicka reaction. A) DP voltammograms of various prions concentrations. Cat_2 peak enhanced with increasing concentration. This signal corresponds to prion concentration. B) Dependence of Co_1, RS_2SH and Cat_2 peaks height on concentration. Peak 3 linearly increases with prion concentration in sample. C) Dependence of Co_1, RS_2SH and Cat_2 peaks height on peak area.

The mechanisms of the reaction is not elucidated in detail, but it is expected that the cobalt(II) complex with protein, peptide or basic nitro compounds play important role in catalytic process [33]. Interaction between cobalt(II) ion and protein causes decreasing of cobalt peak and occurring two new voltammetric peaks at potential area from -1.2 to -1.5 V. The reduction of complex $R(SH)_2$ and Co(II) at potential app. -1.35 V corresponds to the first catalytic signal (RS_2Co). Other two signals Cat_1 and Cat_2 correspond to the reduction of hydrogen at the mercury electrode and can be used for

quantification because their height is proportional to concentration of MT. In addition, the signal called Co_1 could occasionally result from reduction of $[Co(H_2O)_6]^{2+}$ [39].

It was observed formation of three prion signals – Co_1, RS_2Co and Cat_2 in potential -0.9 V, -1.2 V and -1.5 V, respectively, by AdTS DPV Brdicka reaction (Fig. 6A). The signal Cat1 was not observed, which can be related with the fact that this signal can be overlapped by higher and wider RS_2Co peak. It clearly follows from the results obtained that the character of the mentioned prion signals changes with different prion amount. Signal Co_1 decreased and shifted to more positive potential with higher prion concentration.

The signal RS_2CO markedly decreased with the increasing concentration of prion but the position of peak remained (Fig. 6C). The other mentioned prion signals of Brdicka reaction Cat_2 was getting well-developed and separated with the increasing prion concentration. As it has been published, the height of catalytic signal Cat_2 is directly proportional to the concentration of protein [17,35,36,40]. This signal was subsequently employed for the measurement of prion concentrations as follows 16, 32, 64, 125, 250, 500 μg/ml were used.

The peak height increased linearly with the increasing prion concentration $R^2 = 0.99$ and regression equation $y = 0.2758x + 12.02$ (Fig. 6B). Recalculation on the peak area gave the same results, which confirmed that the catalytic peak was well developed (Fig. 6C). The detection limit of DPV was estimated as 50 pmol in 5 μl drop (3 S/N).

3.5 Chronopotentiometric stripping analysis – peak H

CPSA measures the evolution of hydrogen from the supporting electrolyte catalysed by the presence of a protein. This method is a highly sensitive technique commonly used for the analysis of proteins with detection limits at subnanomolar and lower levels [41]. CPSA has been used for the detection of several biologically important peptides [42,43] and proteins such as metallothionein [36,44-49], α-synuclein protein [50], MutS protein [51], glutathione-S-transferase [52], thrombin [53]. Compared to voltammetry CPSA allows one to reach more negative potentials necessary to obtain a well-developed peak H, which in voltammetry is too close to the background discharge. In this paper, CPSA was therefore used to analyse prion at mercury.

For the determination of prion using adsorptive transfer stripping technique in connection with chronopotentiometric stripping analysis (AdTS CPSA) dependence of dt/dE on E was recorded. Prion gave a distinct, sharp chronopotentiometric signal at very negative potentials (about - 1.5 V), also called as the "peak H" (inset in Fig. 7). No baseline correction was necessary to measure this peak. Characteristic prion concentration dependence was obtained under 100 long time of accumulation measured in phosphate buffer with pH 7.38 and temperature-controlled supporting electrolyte at 25°C (Fig. 7). This dependence was linear with regression equation $y = 19.087x + 4277.8$ and coefficient $R^2 = 0.9919$). The estimated detection limit (3 S/N) was 25 pmol in 5 μl drop. CPSA is therefore a very sensitive tool for the study of prion behaviour.

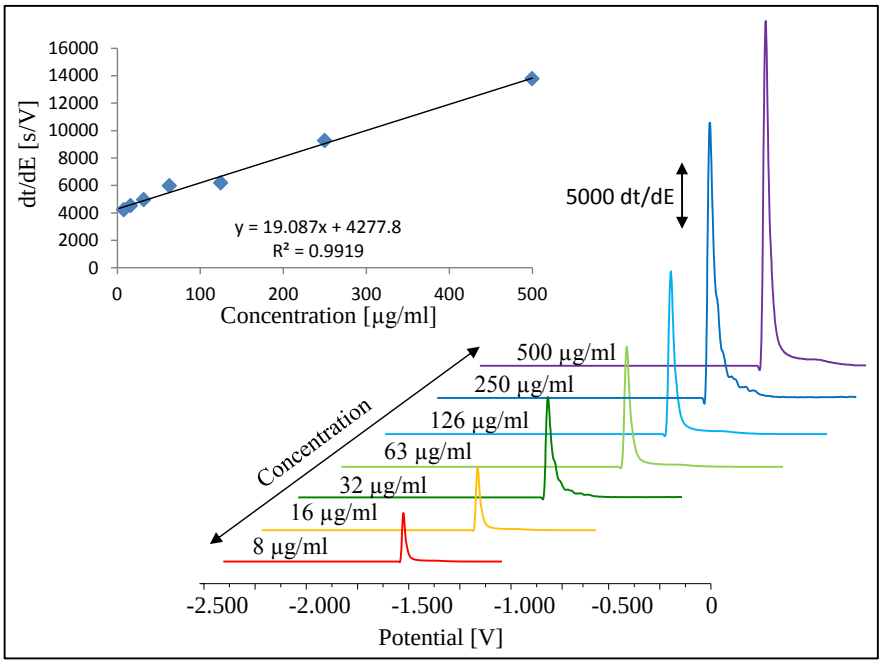

Figure 7. AdTS CPSA calibration curve of prion and their voltammograms in concentration range from 8 µg/ml to 500 µg/ml. The peak enhanced with the increasing concentration. The highest responses (13 770 s/v) was determined at concentration 500 µg/ml in potential -1.55 V.

3.6 Native and denatured prion

Protein denaturation is a complex process [54,55] and its complete understanding requires taking into consideration numerous aspects such as net charge, protein structure, environmental conditions (e.g. electrolyte pH and concentration) as well as equilibrium of folded and unfolded protein form. Elevated temperature is one of the most commonly used approaches for protein denaturation [56-59]. It is well known that at elevated temperatures α-PrP converted rapidly and irreversibly to the thermodynamically more stable β-sheet form [60,61]. Electrochemistry seems to be powerful tool for studying of protein denaturation and renaturation [50,57,58,62-65].

In the present study, the behaviour of native and denatured form of prion was studied using all abovementioned methods. It clearly follows from the obtained results that signals of prion decreased linearly depending on the duration of the heat treatment at 99°C for various time intervals as 0, 15, 30, 45, and 60 min. The correlation coefficients of the measured dependencies as 0.9929, 0.9973, 0.9965 and 0.9957 were determined by CV (Fig. 8A), DPV (Fig. 8B), DPV Brdicka reaction (Fig. 8C) and the most sensitive CPSA (Fig. 8D), respectively.

Electrochemical signals decreased with the increasing time of thermal treatment in all applied methods. The aggregation rate is more sensitive in case of DPV and CPSA where the prion amount decreased more than six times against native prion (0 min. of denaturation). This phenomenon suggests that the thermal treatment causes significant changes in the protein structure. Thermal denaturation is responsible for an irreversible precipitation leading to the aggregation of the denatured prion molecules and creating an undefined polymeric structure.

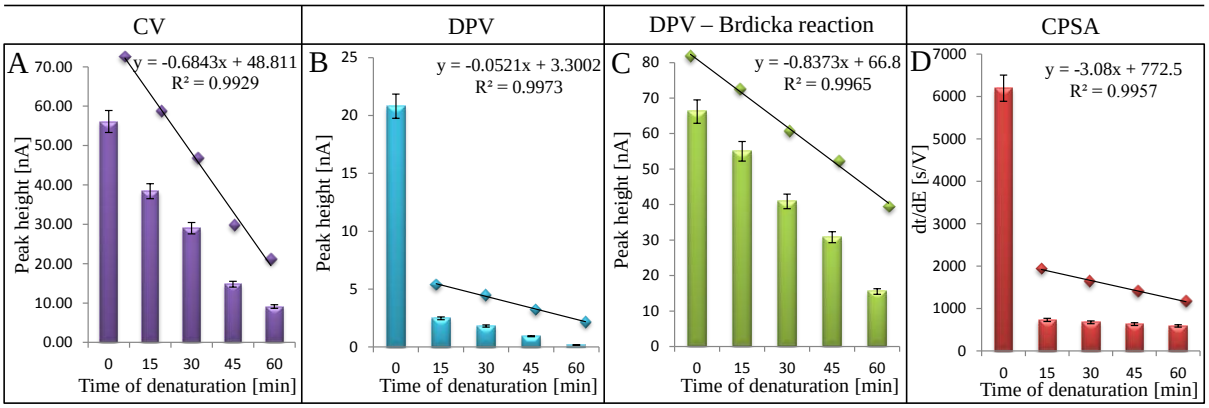

Figure 8. Dependence of peak height on time of heat denaturation at 99 °C for 0, 15, 30, 45 and 60 min. measured by A) cyclic voltammetry, B) Differential pulse voltammetry, C) Differential pulse voltammetry Brdicka reaction and D) Chronopotentiometric stripping analysis. With longer influence of height temperatures the signal significantly decreased.

4. CONCLUSIONS

A great attention on analytical determination of prion protein is paid. Electrochemical methods represent an excellent tool for such studies. As we report in the paper, adsorptive transfer stripping technique coupled with various methods represents powerful tool to detect very low concentration of specific protein. Moreover, the results of denaturation undergo to creation of more complex structures in prion molecule during heat treatment.

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