Electrochemical Analysis of Polyamino Acids and Proteins

Marko N. Živanović[1,2*]

[*] [1]*Department for Biology and Ecology, Faculty of Science, University of Kragujevac, Serbia*
[2]*Institute of Biophysics, Academy of Sciences of the Czech Republic, Brno, Czech Republic*
e-mail: zivanovicmŽkg.ac.rs

The aim of this review is the research of basic polyamino acids, such as polylysine, polyarginine, and polyhistidine as model systems of more complex proteins. The special emphasis is given to electrochemical contribution of individual amino acids in catalytic hydrogen evolution reaction (CHER), as well as investigation of CHER of proteins and carbohydrates.

In examining of polyamino acids, we concluded that they are satisfactorily good model systems to better understand the protein CHER. The electrochemical measurements were performed on mercury electrodes. It was shown that basic polylysine, polyarginine and polyhistidine produce chronopotentiometric peak H, while acidic polyglutamic acid gave no catalytic response.

In this presentation electrochemistry of DNA, proteins and osmium complex labeled polysaccharides and 3'-end ribose of RNA was shortly reviewed. Chronopotentiometric analyses enable precise determination of above-mentioned biomacromolecules at nano- and femtomole level.

For the first time histones were electrochemically measured. Investigation was performed at hanging mercury drop electrode (HMDE) and on glassy carbon electrode. It was shown that using constant current chronopotentiometric stripping analysis (CPSA) and cyclic voltammetry it is possible to detect histones at subnanomolar level. Also, for the first time electrochemistry has been used to investigate the interaction of histones with DNA. Above mentioned results show that electrochemistry is useful tool for precise determination of polyamino acids, proteins, nucleic acids, polysaccharides and histone proteins. This is a small contribution to a better understanding of role of individual amino acid in CHER at mercury electrodes. A very important part of so far unpublished results tell us that the CPSA can be used in research of interaction of basic histones with DNA in order to study of DNA packaging in chromatin. Findings from this review can also be used for testing of other nuclear non-histone and histone-like proteins, such as protamines and transcription factors.

Introduction – Bioelectrochemistry, Promising Tool for Research of Biomacromolecules

This review is based on a long-term research of peptides and proteins in our laboratory [1-11] and is predominantly focused on investigation of CHER of basic poly(aa)s, as model systems of histone proteins, on HMDE. Most of the proteins studied in our laboratory have acidic character, such as Riboflavin-binding protein (theoretical pI 5.1), Thioredoxin (theoretical pI 4.7), MutS protein (theoretical pI 5.5), α-Synuclein (theoretical pI 4.7), Serum albumin (theoretical pI 5.9), Concanavalin (theoretical pI 5.5), tumor suppressor p53 (theoretical pI 6.3), and many others. At the beginning of research represented in this work, we started to develop this research towards investigation of highly basic histone proteins (theoretical pI 10.3 – 11.4). To find suitable experimental conditions for histones study many factors were needed to be taken into consideration, such as purity, concentration and stability of dissolved tested proteins; purity, ionic strength and pH of supporting electrolyte; nature of protein adsorption on the electrode, the possibility of partial denaturation of proteins on the electrode, applied current/voltage, temperature, the presence/absence of oxygen in the system. Basic poly(aa)s as model systems for basic histones appeared to be suitable for better understanding of electrochemical behavior of histones. Thus our focus turned towards the studies of poly(aa)s as structurally simpler model of more complex histones. It was important to investigate how basic model systems of poly(aa)s adsorb at electrode and how they take part in the catalytic evolution of hydrogen. Using of poly(aa)s contributions of individual amino acid in CHER were studied. It is already well known that CHER is due to so-called *labile protons* of the tested analyte [12]. Basic amino acids, such as Lys, Arg and His, and sulfur containing Cys possess labile protons, so it was expected that polymers of these amino acids would catalyze CHER in a great extent. To our knowledge, we first showed the CHER of poly(aa)s on HMDE at weakly acidic and neutral pH [11]. Under our experimental conditions we concluded that acidic polyGlu gives no catalytic signal, as it was expected because of absence of labile protons, while polyArg,Trp yielded smaller catalytic signal compared to more basic polyLys. These findings give us an additional question: *How individual basic amino acid relatively contributes to the CHER on HMDE?* We investigated different homo poly(aa)s namely polyLys, polyArg and polyHis. Further we showed, for the first time, the different impact of Lys, Arg and His residues on catalysis [13]. Lys residues in polyLys give peak at the most negative potentials as compared to peak potentials of Arg residues in polyArg while His in polyHis yielded peak H at the least negative potentials. Also our preliminary results show that exchange of Arg to Lys in 14-mer bombesin peptides shifted of E_p to more negative values. Further study of influencing of E_p by aa residues is necessary. This type of study could be utilized in histone analysis, because individual histones H1-H4 have various aa composition (especially Lys and Arg content). Also we showed that besides CPS the cyclic voltammetry is suitable for study of poly(aa)s.

Diversity of histones – H2A, H2B, H3, H4 and linker histone H1 influence electrochemical response due to structure and amino acid composition. We showed that Cys-containing

histone H3 we differ from other histones because H3 yielded Brdička catalytic reaction in agreements with previous results [14,15]. Also other non-Cys-containing histones can be discriminated on the peak potential, which depends mainly on aa residues. As it was mentioned above, experimental conditions strongly influence catalytical response. Further work is necessary for finding the better conditions for non-Cys-containing histones. But our work could be more difficult due to different structure of individual histones, what could strongly influence orientation of histone molecules at electrode surface and also catalytic process.

Protein–DNA interactions

DNA-protein binding plays a central role in many molecular processes in organisms and cells, such as replication, transcription, DNA repair and packaging. Research into the processes of DNA-protein binding and the nature and properties of complexes formed between DNA and proteins is therefore of utmost importance. Histones are proteins, which sequence specifically and also sequence nonspecifically interact with DNA. Non-specific interactions appear between positively charged helixes in H2B, H3, H4 with negatively charged phosphate groups [16,17]; histone chain amide groups form hydrogen bonds with DNA backbone [18]; histones form nonpolar interactions with deoxyribose of DNA [19]; lysine and arginine form salt bridges and hydrogen bonds with oxygens from DNA phosphate groups[19]; and where histones interact with DNA minor groove [20,21]. Sequence specific interactions were described in studies [22-24]. However, low DNA sequence specificity was estimated for the histone octamer [25].

Various methods including X-ray crystal analysis, NMR, fluorescence anisotropy, ultracentrifugation and other methods have been used to investigate DNA-protein interactions [26,27]. Only in recent years, methods of electrochemical analysis have been applied in DNA-protein interaction studies [28-30] dealing predominantly with aptamers (reviewed in [31-33]) mostly using labeled DNA. Recently label-free methods have been introduced and are based on changes in the electrode capacity (as detected by EIS) resulting from adsorption of a DNA-protein complex [34,35]. Preliminary results from our laboratory show that CPS analyses in combination with thiol-Hg-modified electrodes are suitable for study of protein-DNA interactions between p53 and DNA. Large differences in CPS responses of the sequence specific p53-DNA complex as compared to weaker non-specific p53CD binding to DNAs not containing the CON sequence were observed. To our knowledge, there are no records of using the electrochemical methods in investigation of histone-DNA interactions, and thus we conclude that electrochemistry might provide a contribution to this research.

Study of basic proteins

In addition to histones, there is still a whole range of other basic nuclear proteins, such as topoisomerases (theoretical pI 9.3), proteins of replication and transcription apparatus, gene repressors and activators, protamines (theoretical pI 12.1) and many other DNA interacting

nuclear proteins. The *histone-like* protamine proteins play an important role in DNA stabilization and packaging. Protamines are important, small, Arg-rich nuclear proteins that act as histones in sperm head condensation of DNA. It is believed that protamines bind to DNA more tightly than histones [36].

Nowadays it is known that every human cell contains many different proteins [37,38]. Many of these proteins have significant mutual structural analogy, which in turn depends on the function they perform. It is also known that proteins interact with their *structural domains* with other biomacromolecules and with low-molecular compounds. For example, protein-binding proteins possess domains *cadherin repeats* [39], *immunoglobulin domains* [40], *phosphotyrosine-binding domains* [41]; DNA-binding proteins possess *basic leucine zipper domain* [42], *zinc finger domains* [43]; Ca^{2+} binding *EF hand* domain [44]; ATP-binding proteins domains [45,46], and many others depending on the function. The examination of a large number of different proteins, finding conditions for their determination, research the behavior of proteins in solution, at interfaces and in various complexes may allow easier testing of, so far, electrochemically non-investigated proteins. In this sense, testing of homopoly(aa)s brought us some of the answers on easier determination of basic histones. Detailed examination of histones promises that it would be much easier to electrochemically investigate other basic histone-like proteins.

PolyLysine-Catalyzed Hydrogen Evolution at Mercury Electrodes

Poly(aa)s and peptides can be considered as model systems of proteins. Here, we studied electrochemical behavior of polyLys and two other poly(aa)s namely polyarginine,triptophane (polyArg,Trp) and polyglutamic acid (polyGlu) on mercury electrode [11]. At the first time we showed the role of individual amino acid residue in poly(aa)s in the CHER. At pH 6 polyLys and also polyArg,Trp yielded a peak H, in agreement with their ionization state, while polyGlu gave no catalytic response. Investigation of CHER of polyLys contributes to better understanding of electrochemical determination of peptides and proteins.

CHER of poly(aa)s was performed on hanging mercury drop electrode (HMDE) by methods of voltammetry and constant current chronopotentiometric stripping (CPS). 22.8 μM polyLys (related to the monomer content) at an accumulation potential E_A=−1.5 V and an accumulation time t_A=60 s in 0.1 M McIlvaine buffer, pH 6.0 produced well-developed CPS peak (peak H) at −1.88 V clearly distinguishable from the electrolyte discharge. Under the same conditions CPS of polyArg,Trp produced as about 20-fold smaller peak H than polyLys, while polyGlu yielded no catalytic signal (Figure 1). Chronopotentiograms confirmed that CHER needs so-called *labile protons* in amino acid side chain groups. Square wave voltammetric peaks of polyLys at −1.9 V were less separated from the background discharge compared to that obtained by CPS.

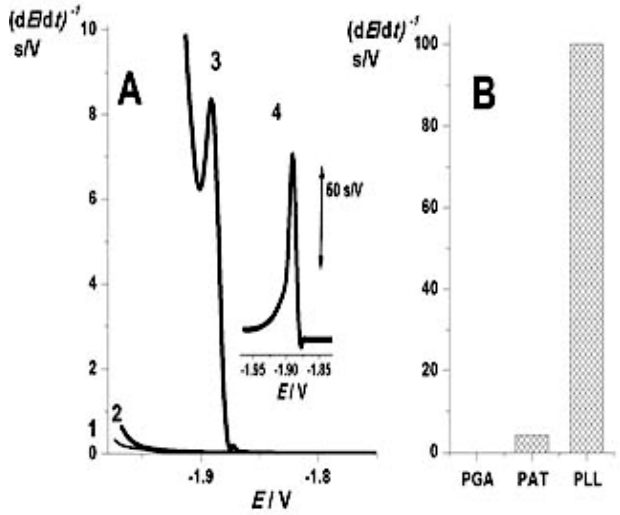

Figure 1 – A) AdS chronopotentiograms of 22.2 μM polyGlu (PGA) (2), 22.8 μM polyArg,Trp (PAT) (3) and 22.8 μM polyLys (PLL) (4, Inset) at HMDE recorded at I_{str}=–30 μA, t_A=60 s, E_A=–1.5 V, E_i= –0.1 V, E_f=–2 V in stirred solution. 1, background electrolyte 0.1 M McIlvaine buffer, pH 6. **B)** Bar graph showing relative intensities of peak H expressed as percent of polyLys signal intensity.

Investigation of catalysis shows that polyLys catalyzes HER in its adsorbed state. Moreover, by measuring of the effect of accumulation potential, E_A we can conclude that polyLys molecules could be absorbed by electrostatic or hydrophobic interactions with electrode surface.

In this work it is also noted that peak H is suitable and sensitive tool for studies of DNA-protein interactions. For this conclusion we tested the mixture of polyLys (5.7 μM) and DNA (5 μM) on HMDE (E_A=–0.5 V; t_A=120 s) in 0.1 M McIlvaine buffer pH 6.0. The peak of DNA/polyLys was of about 7-fold smaller than signal (peak H) of polyLys alone and by >100 mV more positive. These results show that peak H appears to be a new sensitive tool for studies of DNA-protein interactions. Probably similar approach might be used to investigate histone-DNA interactions.

Catalysis of Hydrogen Evolution by Polylysine, Polyarginine and Polyhistidine at Mercury Electrodes

Since the electrochemistry of peptides and proteins made significant progress in the past few decades, we believe that it is especially important to study the impact of each amino acid residue on the CHER. In this paper we studied contribution of polyLys, polyArg and

polyHis to CHER on mercury electrode [13]. In previous work with proteins, CPS was used predominantly, because voltammetric methods yielded poorly developed cathodic signals showing no relevance to changes in protein structures. Herein we showed that poly(aa)s produced well-developed voltammetric peaks.

Under experimental conditions shown on figure below, voltammograms are obtained in the presence of poly(aa)s and exhibit well-defined cathodic peaks at −1.89 V (polyLys, M_W of 4-15 kDa), −1.86 V (polyArg, M_W of 5-15 kDa) and at −1.70 V (polyHis, M_W of 5-15 kDa).

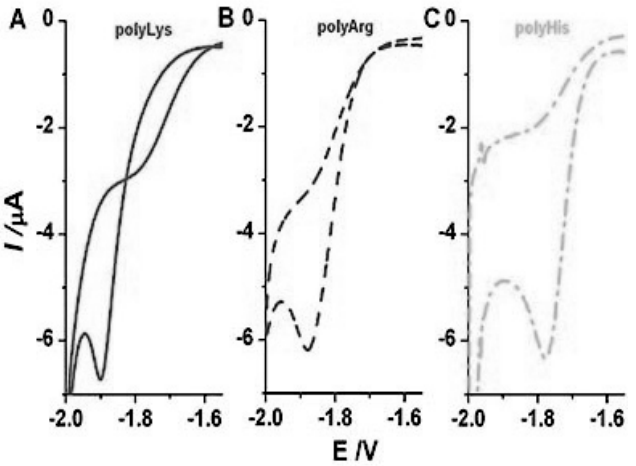

Figure 2 – **A-C.** Cyclic voltammograms of **A.** polyLys, **B.** polyArg and **C.** polyHis in 1/4 McIlvaine buffer, pH 7. 40 μM poly(aa) was adsorbed at HMDE at accumulation potential, E_A of −1.4 V for accumulation time, t_A of 120 s.

Investigation of I_{pc} dependence of polyLys of various lengths (M_W of 4-15 and 70-150 kDa) showed that shorter polyLys molecules move faster towards the electrode, so they earlier reach the electrode surface saturation. At electrode saturation voltammetric responses of those polyLys's were as of same extent and explanation is that the poly(aa) s chain lengths have no significant influence on the organization on electrode surface at full coverage. AC voltammetry has shown that these three poly(aa)s are adsorbed at the electrode in a different manner. Available literature suggests that under applied measuring conditions polyHis and polyLys adsorb as α-helix, while polyArg as random coil structured chain. Furthermore, in this study it is investigated the dependence of CHER on scan rate (ν). At low ν's, i.e. up to 50 mV/s, catalysis of poly(aa)s was as of the same extent. Greater difference is observed at higher scan rates, which suggests that relatively slow processes, such as diffusion of protons from the solution (to recombine with the aa residues in which reduction to hydrogen took place) could take place at slow ν, in agreement with the notion that electrode process coupled with chemical reaction

dominated at slow ν's. At greater ν's charge transfer more influenced the CHER. In CPS similarly to voltammetry at pH 7, polyLys peak H is the most negative (E_p −1.93 V) as compared to polyArg and polyHis. E_p of polyArg is −1.89 V, whereby polyHis peak is by 100 mV less negative as polyLys. The heights of poly(aa) peaks depended not only on the rate of potential changes but probably also by unequal length-distribution and structure of molecules in individual poly(aa)s.

This work shows that similarly as Arg in polyArg and Lys in polyLys also His residues in polyHis contribute to the catalysis of hydrogen evolution under the given conditions.

Electrochemical Sensing of Proteins and Carbohydrates

This work shortly reviewed about electrochemistry of DNA, proteins and peptides at mercury electrodes done in our laboratory [10]. Using of CPS enables us to investigate peptides and proteins at femtomole level. Also, in this paper it is presented the method of labeling of polysaccharides and 3'-end ribose of RNA by some osmium complexes and detected by CPS directly in reaction mixture at nano- and femtomole level.

First papers on electrochemistry of NAs were published about 50 years ago [47]. From that time until today electrochemistry of NAs was intensively developed including development of DNA biosensors capable of detecting even a single-base mismatch in DNA sequence. Recently, electrochemists turn their attention towards developing the methods for microRNAs estimation (shown to be potential tumor biomarkers).

Constant current chronopotentiometry in combination with mercury containing electrodes was successfully applied for study of peptides and proteins in distinguishing between native, denatured, aggregated, reduced/oxidized, mutant and chemically modified form of proteins by so-called peak H due to CHER. Besides the sensitivity to local and global protein structure, CPS analysis is suitable for detection of proteins at pico- to femtomolar concentrations.

Glycomics is started to be booming field. Alterations in protein glycosylation have been found for example in a variety of tumors. Using of electrochemical analysis in glycomics is a new approach in carbohydrates investigation. Carbohydrates contain neither chromophoric groups that strongly absorb UV/visible nor redox group for reversible electrochemistry. Therefore new approaches for carbohydrates detection are sought. We used complexes of six-valent osmium with nitrogenous ligands (L), Os(VI)L, for modification of some polysaccharides, and subsequently following electrochemical detection at mercury electrodes.

We hope that new electrochemical approaches described in this paper become useful in biomedicine, including neurodegenerative diseases and cancer research.

Catalysis of hydrogen evolution by histone H2A at mercury electrodes

Histones are small basic proteins containing a great number of basic amino acids (aa's), such as Arg and Lys. They are responsible for DNA stabilization and packaging into chromatin. To our knowledge, our work represents the first attempt of electrochemical investigation of histones. We focused on studying of histones H2A and H3. Also, we

performed some measurements with histone H1 and H4 (data not shown), but in much lesser extent as compared to H2A and H3. Our results show that histones H2A and H3 in neutral and slightly acidic pH produce catalytic hydrogen evolution reaction (CHER) at hanging mercury drop electrode (HMDE). CHER of histones, in particular, was performed by methods of CV and CPS analysis. Monitoring of irreversible oxidation of tyrosines and tryptophanes by means of square-wave voltammetry at glassy carbon electrode was also done.

First it was measured Brdička catalytic responses (BCR) in cobalt-containing solutions showing that 1.5 μM histone H3 (containing one Cys residue) produce a well-measurable catalytic double wave, while 1.5 μM H2A (containing no Cys residues) only suppressed maximum of catalytic peak of cobalt (Figure 3A) without showing a double wave. Besides measuring of BCR, we turned our attention to investigation of chronopotentiometric peak H, for which Cys and also Arg, Lys and His residues are responsible for CHER at HMDE. Histone H2A and H3 are rich in basic aa's (č 23-25% of total aa content) and 1.5 μM histones in 50 mM phosphate buffer, pH 7.3 produced a well-developed peak H for both H2A and H3 histones. Peak H of H2A was 2-times smaller than peak H of H3 and shifted to more negative potentials by about 60 mV (Figure 3C). Investigating these histones by CV we obtained similar results as with CPS analysis, i.e. peak potential of histone H2A was as about 70 mV more negative than peak potential of histone H3 (Figure 3B). Up to now CPS was predominantly used in protein study. Here we show for the first time that also CV is applicable for this purpose.

Our investigations showed that CV is suitable for distinguishing of histones by their different peak potentials (E_p), which greatly depend on Arg and Lys content. Our results show a relationship in E_p observed in CV measuring of polyArg and polyLys with peak potentials obtained for various histone proteins depending on their aa content.

CPS analysis proved to be a powerful tool for the examination of histone H2A in subpicomolar concentrations as well as for investigation of H2A-DNA interaction. Our results show a significant change in the size and potential of the peak H of H2A and H2A-DNA complexes at different ionic strength. Thus, these results suggest that the CPS analysis with HMDE could be suitable for investigation of protein-DNA interactions (especially for histone-DNA). Even though these interactions are intensively investigated by various non-electrochemical methods, electrochemical analysis has been only little utilized for this purpose. Further work will be necessary for better understanding protein-DNA interactions as detected at electrode surfaces.

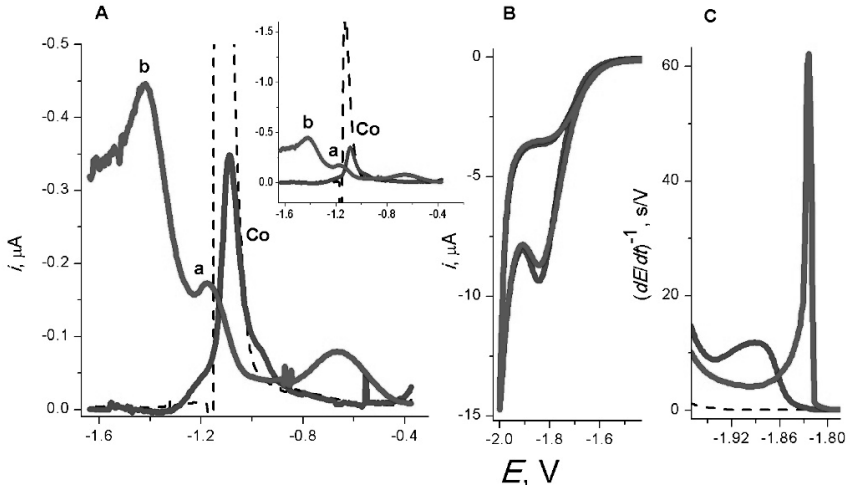

Figure 3. Stripping voltammograms (**A, B**) and chronopotentiogram (**C**) of 1.5 µM histone H2A (blue line) and histone H3 (red line) at HMDE in background electrolyte (dashed line) with adsorption time, t_A 60 s at a given accumulation potential. A) AdS differential pulse voltammetry (DPV): Background electrolyte of 0.1 M ammonium buffer with 1 mM ŠCo$(NH_3)_6$ĆCl_3, pH 9.25 (BCR), frequency 223 Hz, step potential 5 mV, potential limit −1.85 V. a and b: catalytic protein peaks; Co: cobalt reduction peak. Inset A: The whole cobalt reduction peak range. B) AdTS cyclic voltammetry (CV): Background electrolyte 0.05 M sodium phosphate, pH 7.3, scan rate 0.1 V/s, initial potential −0.1 V, switching potential −2.0 V, step potential 2 mV, purging with argon 1 min before each measurement. C) *AdTS CPS*: Background electrolyte 0.05 M sodium phosphate, pH 7.3, stripping current −12 µA, potential limit −1.99 V.

Conclusion

Electrochemical analysis of proteins has recently entered a new phase based on contribution of CPS and bare and thiol-modified mercury electrodes which allow structure-sensitive analysis of various proteins, including those important in biomedicine.

In addition recent studies as well as some results contained in these presentation show that using CPS, interaction of proteins with DNA can be investigated. The basis of the above research lies on the ability of proteins to catalyze hydrogen evolution on mercury electrodes, discovered by J. Heyrovský et al in 1930. The outlooks of electrochemical analysis of proteins in 21th century appear great.

Summarized

This review focuses primarily on studying of poly(aa)s as a model systems of histone H2A. We tried to develop a new approach to the study of basic proteins and for the first time showed that histones and poly(aa)s are electroactive at HMDE, as well as that different amino acids make different contribution to CHER at HMDE. Studies were based on the methods of CPS and voltammetry.

- Poly(aa)s contribute to CHER at mercury electrodes by producing CPS peak H or voltammetric peak
- For the first time it was shown that individual amino acid residue in protein/peptide has its own role, regarding the influence on catalytic response directly by its own contribution to CHER or indirectly by influencing adsorption of the molecules
- Basic poly(aa)s produce peak H, while acidic poly(aa)s (such as polyGlu) give no electrochemical response under applied measuring conditions
- PolyLys catalyzes hydrogen evolution in its adsorbed state. PolyLys adsorbs at HMDE by electrostatic and also by hydrophobic interactions (even at potentials negative to the potential of zero charge)
- PolyLys can be considered as a model system of histones, which are rich in basic Lys and Arg residues
- PolyLys is useful tool for explanation of some principles important in analyzing of DNA interaction with basic histones
- Peak H potentials of individual poly(aa)s are different and depend on type of amino acid residues
- This work is the very first attempt for histone detection by means of CPS at HMDE
- CPS at HMDE can be used for investigation of histone-DNA interaction.

The results of this work indicate that it is possible and useful to use poly(aa)s as intermediate model systems between peptides and proteins for obtaining useful information about CHER on HMDE. It was especially important to use poly(aa)s for better understanding of the factors affecting CHER. Homopoly(aa)s proved to be beneficial for our attempts to clarify the role of individual amino acids in CHER, as well as for the simple approach to the study of the interaction of basic proteins with DNA using peak H.

Acknowledgements

The author would like to say many thanks to prof. RNDr. Emil Paleček, DrSc., dr. Veronika Ostatn, prof. dr. Mara Aleksić and to doc. dr. Snežana Marković for the mentorship and valuable advices regarding my scientific work.
This work was done at the Institute of Biophysics, Academy of Sciences of the Czech Republic, Brno.

References

1. Bartosik, M., Ostatna, V. & Palecek, E., (2009). Electrochemistry of Riboflavin-Binding Protein and its Interaction with Riboflavin. *Bioelectrochemistry* **76**, 70–75.
2. Dorcak, V. & Palecek, E., (2009). Electrochemical Determination of Thioredoxin Redox States. *Anal. Chem.* **81**, 1543–1548.
3. Masarik, M., Cahova, K., Kizek, R., Palecek, E. & Fojta, M., (2007). Label-free voltammetric detection of single-nucleotide mismatches recognized by the protein MutS. *Anal. Bioanal. Chem.* **388**, 259-270.
4. Masarik, M. *et al.*, (2004). Sensitive Electrochemical Detection of Native and Aggregated α-Synuclein Protein Involved in Parkinson's Disease. *Electroanalysis* **16**, 1172–1181.
5. Ostatna, V., Kuralay, F., Trnkova, L. & Palecek, E., (2008). Constant Current Chronopotentiometry and Voltammetry of Native and Denatured Serum Albumin at Mercury and Carbon Electrodes. *Electroanalysis* **20**, 1406–1413.
6. Ostatna, V. & Palecek, E., (2008). Native, Denatured and Reduced BSA. Enhancement of Chronopotentiometric Peak H by GuanidiniumChloride. *Electrochim. Acta* **53**, 4014–4021.
7. Ostatn, V. & Paleček, E., (2006). Self-assembled monolayers of thiol-labelad DNA at mercury electrodes. *Langmuir* **22**, 6481-6484.
8. Palecek, E. & Ostatna, V., (2007). Electroactivity of Nonconjugated Proteins and Peptides. Towards Electroanalysis of All Proteins. *Electroanalysis* **19**, 2383–2403.
9. Palecek, E., Ostatna, V., Cernocka, H., Joerger, A.C. & Fersht, A.R., (2011). Electrocatalytic monitoring of metal binding and mutation-induced conformational changes in p53 at picomole level. *J. Am. Chem. Soc.* **133**, 7190–7196.
10. Paleček, E. *et al.*, (2010). Electrochemical Sensing of Proteins and Carbohydrates. *IEEE SENS J.*
11. Zivanovic, M., Aleksic, M., Ostatna, V., Doneux, T. & Palecek, E., (2010). Polylysine-catalyzed hydrogen evolution at mercury electrodes. *Electroanalysis* **22,** 2064-2070.
12. Frumkin, A., (1963). Hydrogen overvoltage and adsorption phenomena. In: Delahay P, Tobias CW, editors. Advances in Electrochemistry and Electrochemical Engineering, vol. 3. New York: Interscience Publishers Inc; p. p.65-122.
13. Vargov, V., M. Zivanović, V. Dorčk, E. Paleček, and V. Ostatn, (2013). Catalysis of hydrogen evolution by polylysine, polyarginine and polyhistidine at mercury electrodes. *Electroanalysis, in press.*
14. Tomschik, M., Havran, L., Fojta, M. & Palecek, E., (1998). Constant Current hronopotentiometric Stripping Analysis of Bioactive Peptides at Mercury and Carbon Electrodes. *Electroanalysis* **10**, 403–409.
15. Palecek, E., Modern Polarographic (Voltammetric) Techniques in Biochemistry and Molecular Biology', in Topics in Bioelectrochemistry and Bioenergetics, ed. *G. Milazzo, Wiley, London, 65–155 1983.*
16. Hong, L., Schroth, G.P., Matthews, H.R., Yau, P. & Bradbury, E.M., (1993). Studies of the DNA binding properties of histone H4 amino terminus. Thermal denaturation studies reveal that acetylation markedly reduces the binding constant of the H4 "tail" to DNA. *J. Biol. Chem.* **268**, 305-314.
17. Wang, X. & Hayes, J.J., (2007). Site-specific Binding Affinities within the H2B Tail Domain Indicate Specific Effects of Lysine Acetylation. *J. Biol. Chem.* **282**, 32867-32876.

18. Luger, K., Mader, A.W., Richmond, R.K., Sargent, D.F. & Richmond, T.J., (1997). Crystal structure of the nucleosome core particle at 2.8 ”thinsp” A resolution. *Nature* **389**, 251-260.
19. Urnov, F.D. & Wolffe, A.P. Chromatin Structure and Modification. in *Encyclopedia of Physical Science and Technology (Third Edition)* (ed. Editor-in-Chief: Robert, A.M.) 809-829 (Academic Press, New York, 2003).
20. Richmond, T.J. & Davey, C.A., (2003). The structure of DNA in the nucleosome core. *Nature* **423**, 145-150.
21. Bewley, C.A., Gronenborn, A.M. & Clore, G.M., (1998). Minor groove-binding architectural proteins: structure, function, and DNA recognition. *Annu. Rev. Biophys. Biomol. Struct.* **27**, 105-131.
22. Kaplan, N. *et al.*, (2009). The DNA-encoded nucleosome organization of a eukaryotic genome. *Nature* **458**, 362-366.
23. Zlatanova, J. & Yaneva, J., (1991). DNA sequence specific interactions of histone H1. *Mol. Biol. Reports* **15**, 53-56.
24. Axel, R., Melchoir, W., Sollner-Webb, B. & Felsenfeld, G., (1974). Specific Sites of Interaction Between Histones and DNA in Chromatin. *Proc. Nat. Acad. Sci.* **71**, 4101-4105.
25. Jen-Jacobson, L., Engler, L.E. & Jacobson, L.A., (2000). Structural and thermodynamic strategies for site-specific DNA binding proteins. *Structure* **8**, 1015-1023.
26. Travers, A.A. & Buckle, M. DNA-protein Interactions: A Practical Approach. (ed. Press, O.U.) (Oxford 2000).
27. Dey, B. *et al.*, (2012). DNA-protein interactions: methods for detection and analysis. *Mol. Cell. Biochem.* **365**, 279-299.
28. Horakova, P. *et al.*, (2011). Tail-labelling of DNA probes using modified deoxynucleotide triphosphates and terminal deoxynucleotidyl transferase. Application in electrochemical DNA hybridization and protein-DNA binding assays. *Org. Biomol. Chem.* **9**, 1366-1371.
29. Nemcova, K. *et al.*, (2010). A label-free electrochemical test for DNA-binding activities of tumor suppressor protein p53 using immunoprecipitation at magnetic beads. *Anal. Chim. Acta.* **668**, 166-170.
30. Palecek, E. *et al.*, (2004). Sensitive electrochemical determination of unlabeled MutS protein and detection of point mutations in DNA. *Anal. Chem.* **76**, 5930.
31. Cho, E.J., Lee, J.W. & Ellington, A.D., (2009). Applications of aptamers as sensors. *Annu. Rev. Anal. Chem.* **2**, 241-264.
32. Paleček, E. & Bartošík, M., (2012). Electrochemistry of Nucleic Acids. *Chem. Rev.* **112**, 3427-3481.
33. Tombelli, S. & Mascini, M., (2009). Aptamers as molecular tools for bioanalytical methods. *Curr. Opin. Mol. Ther.* **11**, 179-188.
34. Tersch, C. & Lisdat, F., (2011). Label-free detection of protein–DNA interactions using electrochemical impedance spectroscopy. *Electrochimica Acta* **56**, 7673-7679.
35. Sontz, P.A., Mui, T.P., Fuss, J.O., Tainer, J.A. & Barton, J.K., (2012). DNA charge transport as a first step in coordinating the detection of lesions by repair proteins. *Proc. Natl. Acad. Sci. U S A* **109**, 1856-1861.
36. Balhorn, R., (2007). The protamine family of sperm nuclear proteins. *Genome Biol.* **8**, 227.
37. Beck, M. *et al.*, (2011). The quantitative proteome of a human cell line. *Mol. Syst. Biol.* **7**, 82.
38. Vogel, C. & Chothia, C., (2006). Protein family expansions and biological complexity. *PLoS Comput. Biol.* **2**, 26.

39. Chappuis-Flament, S., Wong, E., Hicks, L.D., Kay, C.M. & Gumbiner, B.M., (2001). Multiple cadherin extracellular repeats mediate homophilic binding and adhesion. *J. Cell. Biol.* **154**, 231-243.
40. Bork, P., Holm, L. & Sander, C., (1994). The immunoglobulin fold. Structural classification, sequence patterns and common core. *J. Mol. Biol.* **242**, 309-320.
41. McCleverty, C.J., Lin, D.C. & Liddington, R.C., (2007). Structure of the PTB domain of tensin1 and a model for its recruitment to fibrillar adhesions. *Protein Sci.* **16**, 1223-1229.
42. Glick, D.M., (1997). "Leucine scissors". Glossary of Biochemistry and Molecular Biology. *London: Portland Press*.
43. Klug, A. & Rhodes, D., (1987). Zinc fingers: a novel protein fold for nucleic acid recognition. *Cold Spring Harb Symp Quant Biol* **52**, 473-482.
44. Ban, C., Ramakrishnan, B., Ling, K.Y., Kung, C. & Sundaralingam, M., (1994). Structure of the recombinant Paramecium tetraurelia calmodulin at 1.68 A resolution. *Acta Crystallogr. D Biol. Crystallogr.* **50**, 50-63.
45. Hung, L.W. *et al.*, (1998). Crystal structure of the ATP-binding subunit of an ABC transporter. *Nature* **396**, 703-707.
46. Higgins, C.F. *et al.*, (1990). Binding protein-dependent transport systems. *J. Bioener. Biomem.* **22**, 571-592.
47. Palecek, E., (1960). Oscillographic polarography of highly polymerized deoxyribonucleic acid. *Nature* **188**, 656.