Fourier Transform and Cyclic Loop for Distinguishing Catalytic Metallothionein Signals at Patient with Malignant Tumours

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The goal of this topic deal with analysis catalytic metallothionein signals at patient with malignant tumours. In the analysis is used Fourier transform for noise filtering and cyclic loop for detect peaks on the signal.

1 Introduction

Electrochemical analysis of proteins and nucleic acids is at the beginning of 21st century actual and may broaden our knowledge or may be used in routine diagnostics [1,2]. More than eighty years ago, catalytic signals of proteins in ammonium medium with presence of cobalt ions were described. Free sulfhydryl groups of proteins are responsible for these catalytic signals [3,4] . Even if principle of this catalytic reaction is still unknown, this way of electrochemical analysis is very suitable for proteins with high content of –SH groups [5]. Thermostabile metallothionein is one of these proteins [6,7]. Metallothionein (MT) is a small protein [8] of which primary function is in keeping of metals homoeostasis in living organisms (Fig. 1).

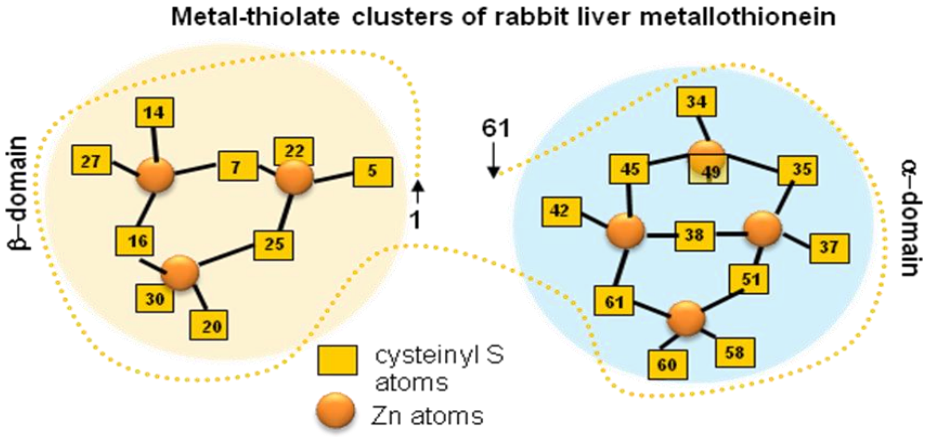

Fig 1. Scheme of the metallothionein clusters domains.

Synthesis of human metallothionein may be induced by increasing metals concentrations. Researches associate significant relation of MT concentration with carcinogenesis, spontaneous mutagenesis, and participation in mechanisms of action of anti-tumour drugs and pharmaceutical products [9-11]. Overexpression of metallothionein is under investigation as a new prognostic marker in many types of malignant tumours [12,13]. The main aim of this paper is processing of electrochemical catalytical signals of metallothionein.

2 Methods

Differential pulse voltammetric measurements were performed with 747 VA Stand instrument connected to 746 VA Trace Analyzer and 695 Autosampler (Metrohm, Switzerland), using a standard cell with three electrodes and cooled sample holder (4 °C). A hanging mercury drop electrode (HMDE) with a drop area of 0.4 mm^2 was the working electrode. An Ag/AgCl/3M KCl electrode was the reference and glassy carbon electrode was auxiliary. For data processing, GPES 4.9 supplied by EcoChemie was employed. The analyzed samples were deoxygenated prior to measurements by purging with argon, saturated with water for 120 s, for measurement the Brdicka supporting electrolyte containing 1 mM $Co(NH_3)_6Cl_3$ and 1 M ammonia buffer (NH_3(aq) + NH_4Cl) was used. The supporting electrolyte was exchanged after each analysis. The parameters of the measurement 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, volume of injected sample: 20 µl (100 × diluted sample with 0.1 M phosphate buffer). All experiments were carried out at temperature of 4 °C by thermostat. Samples of serums of patients suffering from malignant tumour disease were obtained from Department of Paediatric Haematology and Oncology of Charles University in Prague – Motol. Prior to temperature denaturation, samples were stored at -20°C. In order to remove ballast proteins and peptides, which could influence electrochemical response, samples were kept at 99°C in a thermomixer for 15 min with shaking. The denatured homogenates were centrifuged at 4°C for 30 min.

For operation with measured data, it is necessary to adjust these data using suitable mathematical method. Pre-treatment of these data concludes elimination of undesirable components of signal (noise). In the case of repeated measurement of one sample, its ergodicity can be used. First, noise filtering was performed by averaging. Then, noise suppression was done in frequency domain. After application of discrete Fourier transform, selected Fourier coefficients were zeroed and filtered signal was restored by inverse discrete Fourier transform. Sampling period of the measured signals was 2mV, then sampling "frequency" f_s=1/0.002=500 V^{-1}. Cut-off frequency of the used filter was 25 V^{-1} (0.05 f_s). Individual peaks within signals were detected using simple method of comparison three neighborging signal samples. The method was applied repeatedly sample-by-sample while a sample with one preceding and one following sample of lower value was searched. Multiple peaks in a signal were allowed.

3 Results and Discussion

Metallothionein as biomarker of malignant tumour disease was monitored in patients suffering from medulloblastoma, neuroblastoma, osteosarcoma, Ewing's sarcoma, and anaplastic ependymoma (Fig. 2).

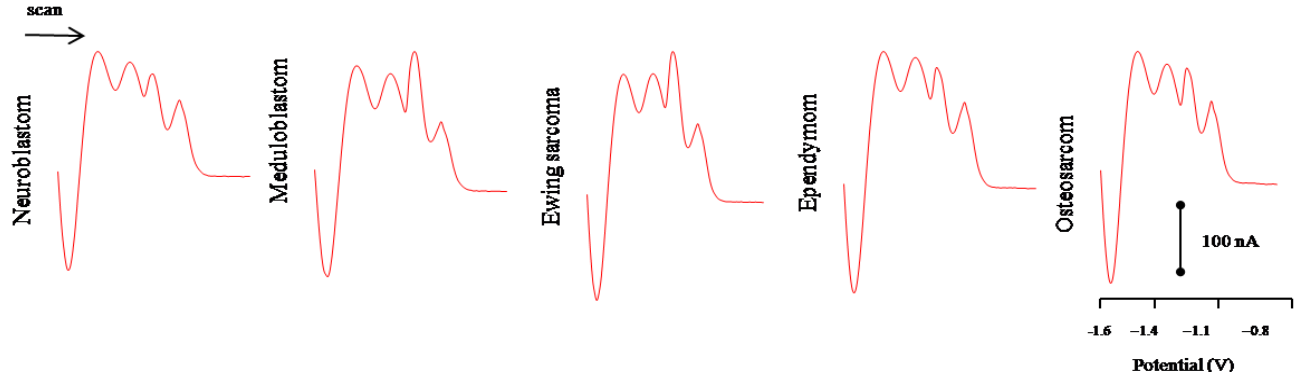

Fig 2. DPV voltammograms of blood samples of serums of patients suffering from malignant tumours.

In addition, we accentuated automatization of detection because of approximation of this method to needs of clinical practice to rapid and standardized laboratory examination methods. For this purpose, we used in middle Europe rare equipment arrangement, which enables this examination at low consumption of biological material. Current responses are results of electrochemical analysis using Brdicka reaction. Principle of this reaction is very difficult and its character is partially of catalytic origin. In obtained signals, following phenomenons are described (without protein presence): at potential of -0.25 V, cobalt(III) ions are reduced to cobalt(II) ions and at potential of -1.1 V, cobalt(II) ions are reduced to cobalt. In presence of proteins, dramatic changes are well evident. Signal of reduction of cobalt(II) ions is significantly reduced and at potential of -1 V, signal of originating complex of cobalt with–SH groups (RS_2Co) appears. After signal of this complex, in dependence on concentration and amount of free –SH groups, catalytic signals Cat 1 at potential about -1.25 V, Cat 2 at potential of -1.35 V, and Cat 3 at potential about -1.45 V appear. In addition, at very negative potential (about -1.7 V), catalytic signal marked as H peak appears. From our experimental results, it is well evident that Cat2 signal is directly proportional to level of MT in sample. By the help of Fourier transform of data proposed by us, maxima on voltammetric curve were determined. Individual maxima of signals were characterized by their position (potential) and height (current). This method simplified evaluation of very complicated electrochemical records. As the registered signals are noise-free with well recognizable peaks, reliability of the pean detector was high. Successfulness of the peak detector, applied on the MT signal, is in this case 100%. Detection of local maximum operates at highest value of middle component (Fig. 3).

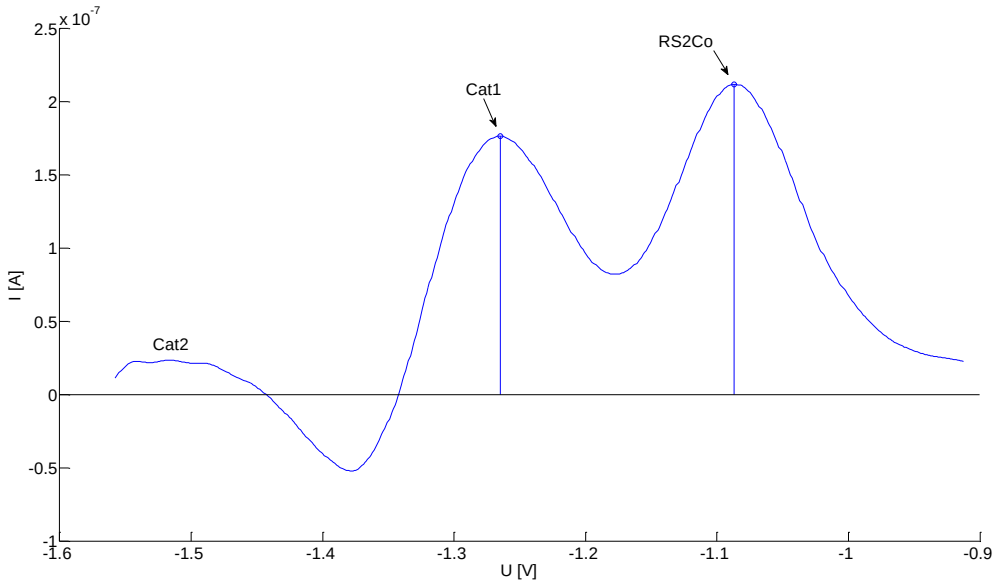

Fig 3. Output signal after filtration and detection of maximum.

4 Conclusions

Precise and rapid evaluation of biological signals enables enhance of efficiency of bioanalytical analysis and above all leads to reduction of random mistakes caused by human factor. For MT detection, we used in clinical practice uncommon electrochemical methods, which demonstrate high sensitivity, selectivity, and low costs.

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