Journal of Electroanalytical Chemistry xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Journal of Electroanalytical Chemistry

journal homepage: www.elsevier.com/locate/jelechem

Changes of electrocatalytic response of bovine serum albumin after its methylation and acetylation

Veronika Ostatná[a,*], Veronika Kasalová[a], Katarína Kmeťová[a], Ondrej Šedo[b]

[a] Institute of Biophysics of the Czech Academy of Sciences, Královopolská 135, 61265 Brno, Czech Republic
[b] Central European Institute of Technology, Masaryk University, Kamenice 753/5, 62500 Brno, Czech Republic

A R T I C L E I N F O

Keywords:
Bovine serum albumin
Protein acetylation
Protein methylation
Electroactive residues
Catalytic hydrogen evolution reaction
Mercury electrode

A B S T R A C T

Post-translational modifications play the crucial role in biological systems and the identification of novel post-translational modifications and study of their role are gaining much attention in proteomics research. For the first time, we compared methylated and acetylated bovine serum albumin to its native and denatured form using constant current chronopotentiometric stripping analysis, phase sensitive alternating current voltammetry and matrix assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS). Our results showed that acetylation of bovine serum albumin resulted in decrease of chronopotentiometric peak H due to modification of electroactive residues, while predominantly non-electroactive residues were modified by methylation of serum albumin. Nevertheless, both modifications altered adsorption of protein at surface and therein influenced chronopotentiometric peak H. MALDI-TOF MS analysis confirmed modifications of serum albumin and was in good agreement with electrochemical results. The present results show the capability of label- and reagent-free electrochemical methods to detect post-translational modifications in proteins.

1. Introduction

Post-translational modification (PTM) is one of the major mechanisms to increase diversity and functional specificity of proteins in the cell, which control protein activity, function, conformation, localization and interaction with other cellular molecules [1,2]. PTM is a modification of proteins by the covalent addition of functional groups enzymatically or non-enzymatically [3]. Among the prominent PTMs, such as phosphorylation, glycation, ubiquitination, are also acetylation and methylation.

Protein acetylation at the ε-amino group of lysine (Lys) residues is a reversible posttranslational modification controlled by two groups of enzymes: Lys acetyltransferases and Lys deacetylases [4]. Lys acetylation was first discovered on histones 50 years ago [5] and has been associated with chromatin remodeling, playing a crucial role in regulating of gene [6]. 30 years later, tumor suppressor protein p53, was identified as the first nonhistone Lys acetylated protein [7]. In the last years, Lys acetylation was described in a large number of proteins involved in almost all cellular processes [8]. Lys acetylation is closely linked to the aging, transcriptional regulation, cell signalling, metabolic function [9,10] and is connected with several diseases such as neurodegenerative disorders, cardiovascular diseases and cancer [11–13].

Other type of PTM is methylation, in which the methyl group is covalently bound to protein amino acids (AA) residues. By the early 1980s, it was known, that histidine (His), Lys, arginine (Arg) and dicarboxylic amino acids can be methylated [14]. Protein Arg and Lys methylation is a common PTM involved in various cellular functions [15–17].

Different approaches such as nuclear magnetic resonance [18,19] or methods based on antibodies [20,21] are used for the detection of protein acetylation or methylation. Very useful methods for detection of protein modification are different variants of mass spectrometry (MS) [22–24], which is currently the most universal and significant experimental platform in proteomics. However, new methods for suitable and fast diagnosis of PTM determination are still developed. Electrochemical methods have been utilized in analysis of protein PTM only rarely [25–27]. Electrochemical approach was used to evaluate the effect of acetylation of p53 C-terminal domain on its DNA binding ability [27]. C-terminal domain peptides with single or multiple acetylated Lys residues were immobilized on the electrode surface. After that, ferrocene labelled double stranded DNA probe was bound to the p53 C-terminal peptides. The ferrocene peak increased corresponding to the acetylation degree of peptides.

Other example of using of EC methods is application of the constant current chronopotentiometric stripping (CPS) analysis in detection of protein glycation [28,29]. The glycation of BSA was followed as the decrease of CPS peak H [28]. This peak H was described in Palecek's

* Corresponding author at: Czech Acad Sci, Inst Biophys, Královopolská 135, 61265 Brno, Czech Republic.
E-mail address: ostatna@ibp.cz (V. Ostatná).

https://doi.org/10.1016/j.jelechem.2017.11.044
Received 21 August 2017; Received in revised form 9 November 2017; Accepted 14 November 2017
1572-6657/ © 2017 Elsevier B.V. All rights reserved.

Please cite this article as: Ostatná, V., Journal of Electroanalytical Chemistry (2017), https://doi.org/10.1016/j.jelechem.2017.11.044

laboratory 20 years ago [30]. Peak H, due to catalytic hydrogen evolution reaction (CHER), appears at highly negative potentials, but is well separated from the background. Selected CPS method offers several advantages compared to voltammetric methods, such as better resolved peaks and electrode polarization in a very short time [25]. Active role in CHER play amino acids residues with exchangeable protons: cysteine (Cys), His, Arg and Lys [30,31]. Peak H was used in protein analysis for monitoring of protein denaturation [32,33], aggregation [34], oxidation damage [35], activity of enzymes [36] and also for investigation of transmembrane proteins [37], protein-protein [38,39] or DNA-protein [40,41] interactions.

In this paper, we applied CPS analysis for label-free detection of protein acetylation and methylation of bovine serum albumin (BSA). We observed differences in peaks H of native, denatured, methylated and acetylated protein. Our CPS results were combined with phase sensitive alternating current (AC) voltammetry and matrix assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS). CPS analysis seems to be method sensitive to acetylation and methylation.

2. Experimental

2.1. Materials

Bovine serum albumin (BSA, 66 kDa), acetylated BSA and methylated BSA were purchased from Sigma-Aldrich. Stock solutions were prepared in 0.1 M tris-HCl, pH 7.4. All other chemicals were of analytical grade.

2.2. Apparatus

The experiments were performed with a three-electrode cell connected to a μAutolab III potentiostat (Metrohm-Autolab) in connection with VA-stand 663 (Metrohm-Autolab). As the working electrode was used a hanging mercury drop electrode (area 0.4 mm^2), Ag|AgCl| 3 M KCl as a reference electrode and Pt wire as a counter electrode. All experiments were carried out open to air at temperature 25 °C. Data were collected using GPES version 4.9.

2.3. Procedures

300 nM BSA was adsorbed on electrode surface usually at accumulation potential, E_A − 0.5 V, during an accumulation time, t_A 60 s from 50 mM Na-phosphate pH 7 followed by chronopotentiogram recording with stripping current, I_{str} − 50 μA or alternating current voltammogram with amplitude 50 Vrms, scan rate 9.2 mV/s, frequency 223 Hz and phase angle 90°. Accumulation was done under stirring.

2.4. BSA acetylation

BSA was diluted in 50% solution of saturated sodium acetate in concentration of 20 mg/ml. During 1 h 1.85 μl of 1% solution of acetic anhydride was added to the 100 μl of BSA sample every 15 min. Acetic anhydride was added totally five times [42]. Samples were then purified by using Microcon centrifugal filter devices (ultracel YM-30 regenerated cellulose). Device was first spin-rinsed with deionized water. 100 μl of BSA sample was then pipetted into sample reservoir and spun for 12 min at 9500 × *g*. After that, sample reservoir was placed upside down in a new vial and spun 3 min at 1000 × *g* to transfer protein concentrate to vial.

2.5. Protein denaturation

14.2 μM BSA was denatured in 8 M urea at 4 °C overnight. Electrochemical measurements with the denatured protein were performed with freshly diluted protein solution in the presence of 170 mM non-denaturing urea concentration.

2.6. MALDI-TOF MS

MALDI-TOF MS experiments were recorded on an Ultraflextreme instrument (Bruker Daltonics, Germany) operated in the linear mode (intact protein analysis) or reflectron mode (analysis after proteolysis) with detection of positive ions. Ferulic acid (12.5 mg/ml in water: acetonitrile: formic acid, 50:33:17 v/v mixture) or alpha-cyano-4-hydroxycinnamic acid (saturated solution in water: acetonitrile: trifluoroacetic acid, 47.5:50.0:2.5 v/v mixture) were used as the MALDI matrixes for intact protein or proteolytic peptide analysis, respectively. The spectra of intact 1 mg/ml BSA, native, acetylated and methylated BSA were obtained. Samples were also subjected to MALDI-MS/MS analysis after being cleaved by trypsin or chymotrypsin in 50 mM ammonium bicarbonate for 2 h at 40 °C or 37 °C, respectively. These analyses were carried out twice with or without the reduction (by using dithiothreitol) and alkylation (by using iodoacetamide) steps.

3. Results and discussion

3.1. Electrochemical behaviour of modified BSA

In last decades, researchers believed that adsorption of proteins at metal electrodes, such as gold, silver, mercury etc., resulted in their denaturation [43]. Using CPS analysis in combination with hanging mercury drop electrode (HMDE), we showed, that denatured proteins catalyse hydrogen evolution in larger extent than their native forms [32]. These large differences in CPS peak H heights of both forms were explained by better accessibility of electroactive residues in denatured protein [33]. Our results suggested that proteins are not completely denatured after their adsorption at mercury surface. Unfolding of native protein adsorbed at surface can appear after protein exposure to electric field at negative potential [25,44].

In this work, we compare the peaks H of native (nBSA) and urea-denatured BSA (dBSA) with peaks H of commercially available methylated (mBSA) and acetylated (acBSA) BSA forms. 300 nM BSA was adsorbed at HMDE at an accumulation potential, E_A of − 0.5 V during an accumulation time, t_A of 60 s from 50 mM phosphate buffer, pH 7 followed by chronopotentiogram recording at stripping current, I_{str} of − 50 μA. nBSA yielded a small peak H at a potential of − 1.86 V, 20-times smaller than the peak H of dBSA (Fig. 1.A). The difference in peak H heights of nBSA and dBSA was in good agreement with previous studies [35,45]. All BSA forms were studied under conditions where the peak H was not dependent on the surface concentration of the sample, as shown by the dependence on t_A (Fig. 1.B). At t_A of 60 s, mBSA produced a well-developed peak H, about 15-fold higher than the peak H of nBSA and 25% lower than the peak H of dBSA (Fig. 1.A). Under the same conditions, acBSA yielded only a very small, difficulty measurable peak H, shifted to the more negative potentials (Fig. 1.A Inset). These results suggest, that methylation of the BSA could result in changes of the protein structure, whereby AA residues, buried inside of the native protein, became more accessible to the electrode processes. Destabilization of BSA molecule by methylation made this protein more sensitive to the effects of the electric field. On the other hand, 5-fold lower peak H of acBSA compared to nBSA may be explained by modification of electroactive residues, leading to a dramatic decrease in CHER. We cannot exclude possibility that acetylated electroactive residues were located only on the surface of the BSA molecule, what did not have to lead to change in its structure. To verify this assumption, acBSA was denatured by 8 M urea, and peaks H of the acBSA and urea-denatured acBSA were compared. We did not observe any differences between these two peaks. The peak height H of the denatured acBSA was the same in comparison to peak H of non-denatured acBSA (Fig. 1.D). In addition to acBSA, we also compared the peaks H of mBSA and its denatured form (Fig. 1.C), where similar results were obtained; i.e. both forms of mBSA produced peaks H of similar size. Peak H heights of acBSA and mBSA did not change after denaturation by urea (Fig. 1.C, D), indicating that the both BSA

Download English Version:

https://daneshyari.com/en/article/6661645

Download Persian Version:

https://daneshyari.com/article/6661645

Daneshyari.com