Full Paper

Zeptomole Detection of Streptavidin Using Carbon Paste Electrode and Square-Wave Voltammetry

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Abstract

We compared the suitability of avidin and streptavidin for avidin-biotin technology in view of the sensitivity of the analysis using square-wave voltammetry. We found out during our preliminary experiments that streptavidin gave approximately 100 times higher electrochemical response in comparison with avidin at the same experimental conditions and concentration. Thus, we used two approaches for streptavidin determination – analysis directly in electrochemical cell and analysis by adsorptive transfer technique (AdTS). Ten minutes long accumulation on carbon paste electrode surface was ascertained as optimal in both cases. Limits of detection were 0.3 aM (electrochemical cell) and 30 aM (AdTS).

Keywords: Streptavidin, Avidin, Modified electrode, Electrochemistry, Protein, Carbon paste electrode

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1. Introduction

Avidin, which was discovered in the 1920’s, was isolated from the bird egg white [1]. It is an alkaline glycoprotein which consists from four subunits with total molecular weight about 66 kDa (Fig. 1A; isoelectric point of the protein is about 9.5) [1]. Few years later after discovering of avidin, a protein, whose sequence is evolutionary related to that of avidin, was isolated from bacteria Streptomyces avidinii. This protein was called as streptavidin [2]. Streptavidin forms homotetrameric complexes as avidin with total molecular weight about 60 kDa (Fig. 1B; the isoelectric point is 6.1). There is a difference between the proteins, because streptavidin is not glycosylated unlike avidin [3].

Avidin takes its name from the avidity with which it binds biotin (vitamin H). Due to structural similarity between avidin and streptavidin, streptavidin itself have the affinity to biotin. The vitamin has a very high affinity to avidin/streptavidin (dissociation constant of 10^{-15} M). This interaction has been utilized in many types of avidin-biotin technologies, such as imunohistochemistry, electron microscopy, ELISA, DNA hybridization and construction of biosensors [4 – 13].

Suggesting of advantageous methods for the analysis of the avidin/streptavidin-biotin interaction is need. Electrochemical sensors and biosensors utilizing various kinds of working electrodes and detection procedures is suitable tool for this purpose [10, 14 – 29]. One of the most promised working electrodes are carbon paste electrodes (CPE) due their easy-to-use, no toxicity, easily and fast modifications, low cost and high sensitivity [30 – 39]. On the other, metal electrodes such as gold [40 – 43] or mercury [8] can be utilized for the analysis of the avidin/streptavidin-biotin interaction. The aim of the work was the utilizing of carbon paste electrode for ultra sensitive determination of streptavidin in the solution and also in the very low sample volume (5 µL drop).

2. Experimental

2.1. Chemicals

Streptavidin (from Streptomyces avidinii, essentially salt-free; molecular weight ca. 60 kDa), avidin (from egg white; molecular weight 66 kDa, subunit molecular weight 14 kDa), carbon powder, sodium acetate, acetic acid, and mineral oil were purchased from Sigma Aldrich Chemical Corp. (St. Louis, USA). Solutions were prepared using ACS water from Sigma Aldrich. The stock standard solutions of streptavidin at 16.7 nM were prepared and stored in the dark at 4 °C. All solutions were filtered through a 0.45 µm Teflon membrane filters (MetaChem, Torrance, CA, USA) prior to an electrochemical analysis.

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2.2. pH Measurements

The pH was measured using a WTW inoLab Level 3 instrument (Weilheim, Germany), controlled by a personal computer program (MultiLab Pilot; Weilheim, Germany). The pH-electrode (SenTix H, pH 0 – 14/0 – 100 °C/3mol L⁻¹ KCl) was regularly calibrated using a set of WTW buffers (Weilheim, Germany).

2.3. Electrochemical Measurements

Electrochemical measurements were performed using an AUTOLAB analyzer (EcoChemie, The Netherlands) in connection with a VA-Stand 663 (Metrohm, Switzerland). The electrode system consisted of a carbon-paste working electrode, an Ag/AgCl/3 M KCl reference electrode, and a platinum wire counter electrode. Acetate buffer (0.2 M CH₃COOH + 0.2 M CH₃COONa, pH 4.0) was used as the supporting electrolyte. Square-wave voltammetry (SWV) was performed using the following parameters: initial potential = 0.1 V, end potential = 1.3 V, amplitude = 25 mV, step potential = 5 mV, and frequency = 200 Hz. All experiments were carried out at 25 °C. The raw data were treated using the Savitzky and Golay filter (level 2) and a moving average baseline correction (peak width = 0.05) of the GPES software. Other details can be found in the paper by authors Kizek et al. [10].

2.4. Preparation of CPE

The carbon paste (about 0.5 g) was made of 70% graphite powder (Sigma-Aldrich) and 30% mineral oil (Sigma-Aldrich; free of DNase, RNase, and protease). This paste was housed in a teflon body having a 2.5-mm-diameter disk surface. Prior to measurements, an electrode surface was renewed by polishing with a soft filter paper. Then, the supporting electrolyte. Square-wave voltammetry (SWV) was performed using the following parameters: initial potential = 0.1 V, end potential = 1.3 V, amplitude = 25 mV, step potential = 5 mV, and frequency = 200 Hz. All experiments were carried out at 25 °C. The raw data were treated using the Savitzky and Golay filter (level 2) and a moving average baseline correction (peak width = 0.05) of the GPES software. Other details can be found in the paper by authors Kizek et al. [10].

Fig. 1. Amino acids composition of avidin (A) and streptavidin (B). SWV CPE voltammogram of avidin and streptavidin (1.52 and 1.67 μM, respectively; tₜ 120 s). The voltammetric signal corresponds to the oxidation of W and Y residues in avidin/streptavidin molecule. All measurements were performed in acetate buffer, pH 4. The square-wave voltammetric method was used with the following parameters: initial potential: 0.1 V, end potential: 1.3 V, amplitude: 25 mV, step potential: 5 mV, and frequency: 200 Hz. Mₛ corresponds to a subunit of avidin or streptavidin.
surface was ready for measurement of a sample volume of 5 μL.

2.5. Statistical Analysis

Precision of the measurements expressed as relative standard deviation was evaluated with standard solutions of avidin and/or streptavidin at concentration of 0.15 pM and/or 16.7 fM, respectively. Intraday precision was tested during 9 hours \((n = 4)\). Interday precision was tested for four days \((n = 9)\). STATISTICA Cz (StatSoft, Inc. (2005), version 7.1.) was used for statistical analyses. Value of \(p < 0.05\) was considered significant. Limits of detection are expressed to one significant digit.

3. Results and Discussion

A great attention of number of scientific groups is devoted to the proteomic research now (more than 2000 articles contains proteomic in article titles, keywords, or abstracts according to Web of Science database from years of 2000 to 2006). Utilizing of carbon paste electrodes (CPE) instead of the mercury and solid ones \([8, 44, 45]\) is very promising in the proteomic research. Possibility of CPE modification by various compounds eventually by extracts and homogenates is their excellent property and advantage \([35, 36, 38]\). The aim of such modification is to achieve maximal possible sensitivity and selectivity of the electrochemical measurement \([46]\).

3.1. Electrochemical Determination of Streptavidin

Recently we published the paper, where we described electrochemical behavior of avidin on CPE by square-wave voltammetry (SWV) \([10]\). We obtained limit of detection \((LOD; 3 S/N)\) for avidin 30 pM (100 amol of avidin in 3 μL) using this time-non-consuming technique (the measurement time was up to 3 min including the accumulation time of 120 s) \([10]\). As for avidin incorporation into the CPE, detection limit expressed as \(3 S/N\) for avidin was 0.7 nM (0.2 pmol in 250 μL solution added to 1 g of carbon paste). Relative standard deviation was up to 10%. We also detected biotin by avidin modified CPE with limit of detection 0.2 pmol in 5 μL (3 μM; 3 S/N). We also utilized this procedure for biotin analysis in pharmaceutical drug \([10]\).

The sensitivity of the abovementioned determination is insufficient if we want to utilize the technique for, e.g., monitoring of hybridization of DNA using biotinylated nucleotides. Due to this we were faced to a problem of another decreasing of the detection limit. As it is mentioned in Introduction section, streptavidin, the avidin-like protein, also effectively and selectively binds biotin. For that reason we were interested in the issues how do streptavidin behave on the surface of CPE.

During our preliminary experiments we found out that streptavidin gave approximately 100 times higher electrochemical response in comparison with avidin at the same experimental conditions and concentration (16.7 nM). This phenomenon relates probably with the different amino acid composition and also with absence of saccharide in streptavidin molecule, which results in dissimilar orientation of amino acid residues to the surface of the working electrode. More electroactive amino acid residues (tryptophan – W and/or tyrosine – Y) of streptavidin in comparison with avidin are able to be oxidized on the CPE surface and that leads to the higher current response. This presumption confirms the potential shift of peak \((Y_{\text{avidin}} 770 \text{ mV})/ (Y_{\text{streptavidin}} 730 \text{ mV})\) and \((W_{\text{avidin}} 910 \text{ mV})/(W_{\text{streptavidin}} 860 \text{ mV})\) (Fig. 1). These results show applicability of streptavidin as biological part of a biosensor in avidin-biotin technologies. Due to this we decided to study streptavidin as biotechnologically advantageous protein in comparison with avidin.

Particularly, we investigated changes in streptavidin electrochemical signal with its increasing concentration in the electrochemical cell filled by 2 mL of 0.1 M acetate buffer \((\text{pH } 4)\). We obtained well developed signal of streptavidin (with two peaks corresponding to amino acids W and Y) at highest tested concentration (167 nM, accumulation time of 120 s was used). Then, we decreased streptavidin concentration down to 167 fM. At this concentration, the electrochemical response was less noticeable at accumulation time of 120 s. Thus, we investigated the influence of accumulation time on streptavidin signal in order to improve sensitivity of streptavidin analysis. We used very low concentration of streptavidin in electrochemical cell \((167 \text{ fM})\) for this purposes and construed dependence of peak height of amino acid W on concentration of compound of interest. The highest signal of amino acid W was observed at 10 min (upper inset in Fig. 2A). We were interested in the issue how much sensitive is our analysis now. Typical Langmuir dependence of W signal height on streptavidin concentration (from 0.65 fM to 330 fM) has been obtained (Fig. 2A). The curve was strictly linear \((y = 8.1627x – 1.8917; R^2 = 0.9964)\) in the range from 0.65 to 11 fM (lower inset in Fig. 2A). Detection limit of streptavidin \((LOD; \text{expressed as } 3 S/N)\) in the electrochemical cell \((2 \text{ mL of background electrolyte and streptavidin solution})\) was 0.3 aM. Other details see in Table 1.

In the following experiments we aimed on detection of streptavidin by adsorptive transfer technique (AdTS) on CPE due to utilizing of the technique for analysis of low volumes of a sample \((a b o u t 5 – 10 \mu L)\). On the basis of our previous experimental results we choose a low concentration of streptavidin during optimization of accumulation time. Particularly, we choose the same concentration as in the case of analysis performed in electrochemical cell – 167 fM \((\text{it corresponds to } 835 \text{ zmol of the streptavidin in } 5 \text{ μL})\). The highest current response was obtained at 10 min. long accumulation time similarly to electrochemical cell determination (upper inset in Fig. 2B). We construed...
dependence of streptavidin peak height on its concentration in the range from 3 to 1660 zmol at the most optimal accumulation time (sample volume of 5 μL). The curve obtained shown the same manner as for analysis of streptavidin in electrochemical cell (Fig. 2B). The curve was linear \(y = 1.7351x + 18.695\; R^2 = 0.9906\) in the range from 3 to 60 zmol of streptavidin per 5 μL (lower inset in Fig. 2B). Limit of detection (LOD; expressed as 3 S/N) of...
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The detection limit of streptavidin determined by AdTS SWV was 30 aM (0.2 zmol in 5 μL). Other details are shown in Table 1.

During statistical data treatment, we investigated precision of the technique by five times repeated (n = 5), intraday (analysis after 3 hours up to 9; n = 4) and interday (analysis after 12 hours up to 96; n = 9) measurements. We found out that relative standard deviation (RSD) of the measurement is about 16% and/or 15% as for streptavidin determination in 2 mL of background electrolyte (accumulation in electrochemical cell for 120 s) and/or in 5 μL drop (adsorptive transfer technique), respectively (Table 1). The significant difference between RSD of the avidin (3.6%) and streptavidin (16%) analysis is probably due to the amount of determined analyte by our sensitive method. The concentration streptavidin is approximately million times lower versus avidin concentration [10]. This difference may result in higher deviations due to adsorption of the analyte on the electrochemical cell surface, test tube etc., which have much more significant effect at studied concentration. This higher relative standard deviation could be minimized e.g. by using of specially coated accessories. Due to an influence of the adsorption, we studied interday precision using on the one hand streptavidin working solution prepared from the stock solution (streptavidin concentration 16.7 nM) exactly before analysis (solution A) and on the other hand working streptavidin solution prepared before the first interday precision measurement (solution B). In the case of precision of interday measurement using solution A, electrochemical signal was equally distributed around the mean value. On the other hand, if we used solution B, marked decrease (about 60%) in the streptavidin current response appeared after the 12 hours. After that the signal decrease was more gradually (about 5%). It clearly follows from these results that adsorption of streptavidin has significant influence on measurement precision.

4. Conclusions
Carbon paste electrodes belong to promising tools for detection of biotechnologically important compounds. Here, we revised and evaluated the limits of detection and experiments, where we determined avidin. We shown that streptavidin gave approximately 100 times higher current response than avidin. Thus, detection limit of streptavidin was about 0.2 zmol per 5 μL, which was lower than as for avidin. We assume that streptavidin could be more suitable than avidin for avidin-biotin technological purposes.

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6. References


