The determination of avidin in genetically modified maize by voltammetric techniques

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ABSTRACT

Quality assurance is a major issue in the food industry. The authenticity of food ingredients and their traceability are required by consumers and authorities. Plant species such as barley (*Hordeum vulgare*), rice (*Oryza sativa*), sunflower (*Helianthus annus*), wheat (*Triticum aestivum*) and maize (*Zea mays*) are very common objects of interest of genetic modification (GMO); therefore the development of specific assays for their specific detection and quantification of GMO are needed. Furthermore, the production and trade of genetically modified lines from an increasing number of plant species brings about the need for control within research, environmental risk assessment, labeling-legal, and consumers' information purposes. Electrochemical sensors and biosensors based on modification of working electrode could be suitable tools for these purposes. Here, we report using of an avidin-modified carbon paste electrode for rapid and sensitive determination of avidin in plant extract solution and in a transgenic maize extract. The process could be used to determine avidin concentrations up to 3pM in solution and 170nM in a maize seed extract. Moreover, we applied the method to analyze different maize flours.

Keywords: avidin; maize; square-wave voltammetry; carbon paste electrode; GMO

Avidin is a minority component of egg white at reptiles, amphibians and birds. At neutral pH it is a glycosylated, positively charged protein occurring in tetramer form (Green 1975, Wilchek and Bayer 1990). It selectively binds biotin with high affinity (the dissociation constant is 10^{-15}M). This interaction is used in many types of avidin-biotin technology like immunohistochemistry, electron microscopy, ELISA, DNA hybridization and biosensors construction (Wilchek and Bayer 1990, Wang et al. 1996, Masarik et al. 2003, Havran et al. 2004). To ensure the nutriment for expanding human population, it is necessary to use the new technologies, which could help to increase the productivity of farming industry. The production of the quality foods is often suppressed by microbial and insect pests. This unwanted processes can be avoided by using the pesticides (Silman 1993). On the other hand the use of these compounds presents a risk of environment contamination and

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input of harmful compounds into food chain. The new molecular biology methods help to research the new natural pesticides produced directly by plants to protect themselves against the insect pests (Glicka and Skofb 1986). Recently, it was found out that avidin is toxic to broad spectrum of *Lepidoptera*, *Coleoptera* and *Diptera* (Morgan et al. 1993, Kramer et al. 2000, Markwick et al. 2001, Kramer 2002, Flinn et al. 2006). In order to protect plants without chemicals, the transgenic plants biosynthesizing avidin were proposed. To study the expression of avidin the new analytical methods were suggested. Some of them are electro-analytical methods. It is known that the carbon paste electrode (CPE) is suitable for biosensors construction (Wang 1985, Wang et al. 1997, Wang et al. 1999, Wang et al. 2000, Kizek et al. 2003). Moreover, the carbon paste electrode can be modified by addition of various compounds in order to increase the sensitivity, selectivity and rapidness of the determination (Wang and Lin 1988, Kubiak and Wang 1989, Masarik et al. 2003, Kizek et al. 2005). In our work we used the carbon electrodes (CPE and modified CPE) coupled with square-wave voltammetry to measure nanogram amount of avidin.

MATERIAL AND METHODS

Chemicals. Avidin, carbon paste, sodium acetate, acetic acid and mineral oil were purchased from Sigma Aldrich Chemical Corp. (St. Louis, USA). Other reagents of ACS purity were from Sigma Aldrich. The solutions were prepared by ACS water from Sigma Aldrich. The stock standard solutions of avidin 1 μg/ml were stored in dark at 4°C. All the solutions were filtered through 0.45 μm Teflon membrane filter (MetaChem, Torrance, CA, USA). The pH was measured by using the WTW inoLab Level 3 instrument (Weilheim, Germany), controlled by software (MultiLab Pilot; Weilheim, Germany). To calibrate the pH-electrode (SenTix H) the WTW buffers set (Weilheim, Germany) was used.

Electrochemical measurement. The electrochemical measurement was carried out on the AUTOLAB analyzer (EcoChemie, Netherlands) connected with the VA-Stand 663 (Metrohm, Switzerland). Three-electrode system consisted from working electrode (carbon paste electrode or modified carbon paste electrode), referent electrode (Ag/AgCl, 3M KCl) and platinum electrode as auxiliary. The measurement was carried out in 0.2M acetate buffer (0.1M CH_3COOH + 0.1M CH_3COONa, pH 4.0). The parameters of square-wave voltammetry were as follows: initiation 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.

Preparation of CPE and avidin-modified CPE. The carbon paste (about 0.5 g) was made from graphite powder (Aldrich) and mineral oil. The ratio of mineral oil and carbon powder was tested. This paste was inserted into Teflon body with disc

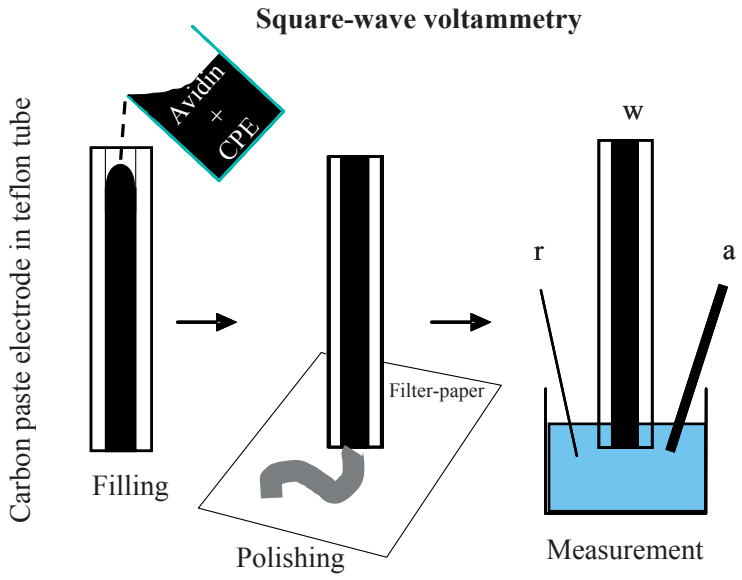

Figure 1. The scheme of measurement technique for avidin determination

diameter of 2.5 mm (Figure 1). The surface of the electrode was polished prior to the measurement with soft filter paper for analysis purpose. The CPE modified with avidin was prepared by adding of avidin or plant extract to the carbon paste during preparation.

The preparation of samples from transgenic plants. The construction of transgenic plants was described by our American colleagues (Hood et al. 1997). The samples of both transgenic and non-transgenic (control) maize kernels were ground for one minute in a coffee grinder. The powder was extracted for 1 h at 4°C with stirring in 50 ml of buffer containing 50mM sodium carbonate (pH 11.0), 500mM NaCl, 5mM EDTA and 0.05% (*v*/*v*) Tween-20. The extraction mixture was centrifuged at 16 000 g for 15 min (Jouan MR 23i) at 4°C. The supernatant was removed and filtered. The filtrate was centrifuged at 14 500 g for 15 min at 4°C. The pH of the supernatant was adjusted to pH 10.5 and then was centrifuged at 14 000 g for 30 min (Eppendorf 5402) in 4°C (Hood et al. 1997, Masarik et al. 2003). The resulting supernatant was then used to prepare the modified CPE.

RESULTS AND DISCUSSION

Square-wave voltammetry of avidin on carbon paste electrode. From electrochemical point of view, only tyrosine (Y) and tryptophan (W) were found to be electro-active using a variety of electrodes. Square-wave voltammetric analysis at solid carbon electrodes is not very sensitive. However, by using a CPE and sophisticated base line correction, we obtained well-defined voltammetric signals for both Y and W at 0.78 and 0.92 V vs. Ag/AgCl/3M KCl, respectively. The peaks were obtained at 150 µg/ml of avidin by using the square-wave voltammetry. The technique is based on the measurement with the electrode in the electrolyte containing no avidin in bulk solution where electrochemical measurements were performed (Figure 1). The detection limit was about 500 pg/ml.

Determination of avidin in transgenic avidin maize extract using avidin extract-modified CPE. Modern technologies of the genetic engineering bring new possibilities in preparation of plant species producing a number of value-added proteins

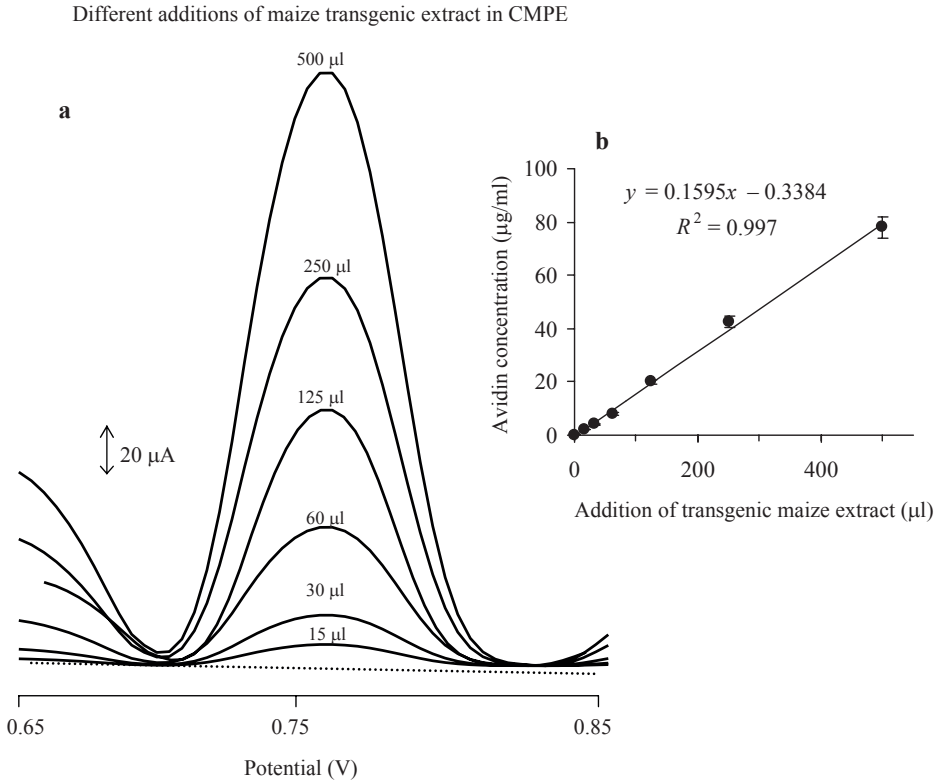

Figure 2. Concentration dependence of the voltammetric signal on the volume (a) of the transgenic maize extract in MCPE, and (b) corresponding calibration curve related to avidin concentration. The square-wave voltammetric method was used with the following parameters: initial potential = 0.1 V, end potential 1.3 V, amplitude 25 mV, step potential 5 mV, and frequency 200 Hz

(i.e. monoclonal antibodies, antigens for vaccines, etc.). Recently, transgenic maize, which produces avidin as an insecticidal agent against insect pests, was prepared. In our previous work, we prepared the avidin modified CPE by mixing the plant seed extract obtained from the transgenic maize with carbon powder. The height of resulting electrochemical signal refers to the concentration of avidin occurring in maize sample. We determined the peak height of avidin in different amounts of maize extract added to carbon powder. For analytical purposes we constructed a calibration curve showing a linear relationship between the avidin concentration and the volume of transgenic maize extract (Figure 2). The peak height of the voltammetric signal linearly increased with additions of transgenic seed extract into the avidin-modified CPE from 15 µl to 500 µl, the regression equation was $y = 0.1595x - 0.338$ with regression coefficient $R^2 = 0.997$, $n = 5$, R.S.D. = 5%. The lowest detectable volume of the plant extract was about 10 µl, which corresponds to 2 µg/ml of avidin in the sample. When we tested a non-transgenic avidin plant, only a very small signal was observed. To compare the avidin signal from the transgenic seeds with the non-transgenic ones, we prepared a set of extract samples from the commercial maize food (farina, grits, and grout). Voltammetric signals obtained from these samples were very low and their heights were about 2–8% according to the signal obtained from the transgenic sample extract. This procedure can be used for analyses of other transgenic products that contain avidin such as avidin apple, tobacco and rice (Markwick et al. 2001).

We constructed a protein-modified voltammetric bioelectrode by incorporating a protein into the CPE (avidin-modified CPE). This technology offers a broad spectrum of applications in the development of biosensors for GMO detection and avidin-biotin technology.

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