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A novel biosensor for sterigmatocystin constructed by multi-walled carbon nanotubes (MWNT) modified with aflatoxin–detoxifizyme (ADTZ)

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Abstract

Sterigmatocystin, ST, is carcinogenic mycotoxin with toxicity second to aflatoxins, contaminated in foods- and feeds-stuff widely. A three-electrode system was employed to examine the response character of the covalently united ADTZ–MWNTs electrode to ST, and the results indicated that an oxidation peak of ST was observed at about +400 mv, the linear detection range of ST was from 4.16×10^{-5} mg/ml (0.13 μM) to 1.33×10^{-3} mg/ml (4.29 μM) with the detection limit at 0.13 μM. Compared to the corresponding results obtained from the MWNTs modified electrode that ADTZ was directly sediment (adsorbed) on it, the sensitivity of ours had been improved by two orders of magnitude, which could provide some important data to further research.

Keywords: Sterigmatocystin; Multi-walled carbon nanotubes; Aflatoxin–detoxifizyme; Biosensor

1. Introduction

Sterigmatocystin(ST), a mycotoxin with a toxicity second to aflatoxins, is a precursor of aflatoxin B_1, with which its has a similar 8,9 double bond structure [1]. Closely correlated to the occurrence of gastric carcinoma, hepatocellular carcinoma, and esophagus carcinoma [2–4], the ubiquitous ST is commonly found as contaminants of foods and crops such as wheat, corn, peanut barley, soybean, coffee bean, ham, and cheese, especially the former three enumerated above [5–7].

Since the introduction of Multi-walled carbon nanotubes (MWNTs) in 1991, they have demonstrated a strong predominance in the manufacture of enzyme biosensors. This is because of MWNTs unique physical and chemical characters and its considerable specific superficial area which can increase the quantities of enzymes immobilized, enlarge the reacting areas between enzyme and substrate, high electrical conductivity, and enhance the biosensor responsibility [8–11]. Recently, several important investigations have been directed to the attachment of natural proteins and DNA immobilization onto nanotubes to construct biosensors [12–16]. Davis et al. [17,18] have reported the high surface area possessing multiply acidic sites that may make an offer of special opportunities for the immobilization of enzymes. MWNTs can be activated in acid oxidation conditions due to the residues such as –COOH, –COH, and –OH introduced on the surface of MWNTs [11]. Shim et al., Huang et al., Fu et al. and Sotiropoulou et al. [12–14,16] have described that BSA can be covalently attached to SWNTs and MWNTs by way of diimide-activated amidation under ambient conditions, while the majority of the protein in the nanotube–BSA conjugates remain bioactive.

In this paper, we reported a novel biosensor, whose working electrodes is constructed by enzyme modified MWNTs. Since oxidation is an effective way of opening the caps of nanotubes to increase the specific superficial area and to active nanotubes by acidic groups, which are both advantages for electron transporting and attachment to enzymes. To improve the sensitivity of the enzyme

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membrane, the oxidative multi-walled carbon nano-tubes (MWNTs), was used both as the electron transporter and the mediated to immobilize the enzyme.

The enzyme, aflatoxin–detoxifizyem (ADTZ), which was able to detoxify aflatoxin B_1 [19], was used as the biologic sensor to produce the biochemical response signal. The enzyme's NH_2 residues were immobilized on the oxidative MWNTs carriers via a covalent bond. This immobilization maintains enzyme activity, reduces the dosage of enzyme and losses and enhances the enzyme bioactive stability.

Previously, the authors have reported a preliminary study on the detection of ST with a triplet electrode enzyme-biosensor system [20], in which Ag/AgCl works as the reference electrode, while Pt and Au that has been modified and absorbed by Multi-walled carbon nanotubes (MWNTs) and ADTZ is used as counter and working electrode, respectively. The results show that the response signals are substrates correlative and the linear detection range of ST lays in $8.32\times10^{-5}\sim66.56\times10^{-5}$ mg/ml with the detection limit at 8.32×10^{-5} mg/ml (0.268 μM). However, the disadvantages were also clear: narrower linear range with lower sensitivity. In the presence paper, prior to immobilization on the surface of nanotubes-modified electrode covalently, ADTZ was saturated by its substrate (ST), in order to improve the detection sensitivity and widen the linear detection range.

2. Materials and methods

2.1. Materials

Sterigmatocystin (ST), N (3-dimegthylaminopropyl)-N'-ethyl-carbodiimid hydrochlorid (EDC), and N-Hydroxysuccinimide (NHS) were obtained from Sigma-Aldrich; Multi-walled carbon nanotubes (MWNTs) were purchased from Nano Port Co. (China), other analytical pure reagents was domestically produced.

2.2. Preparation of MWNTs-modified electrode

After refluxed in aqua regia, mixture of nitric acid (HNO_3) and sulfuric acid (H_2SO_4) solution (v/v=1:3), for 8 to 12 h, MWNTs were centrifuged at 10,000 ×g for 15 min. Prior to desiccation in an oven the deposits were washed twice with double-distilled water. The dried MWNTs were then suspended in N,N-dimethylformamide (DMF) to 0.1 mg/ml following ultrasonically homogenize. The gold electrode polished with toothpaste and diatomite was ultrasonically cleaned orderly in acetone, sodium hydroxide (0.5 mol/L) and concentrated nitric acid (1:1) for 3∼5 min each time. The nanotubes films were prepared by dropping 10 μL of the suspension of MWNTs on the gold electrode and then evaporating the solvent in oven at 60 °C [21–23].

2.3. Immobilization of ADTZ on the MWNTs-modified electrode via covalent bond

The preparation of ADTZ followed a similar procedure according to Liu [24], and the concentration of ADTZ used was 0.189 μg/μL with the activity at 300 U (1 U was equal to the amount of ADTZ that can decrease one unit of the ultraviolet (UV) absorbance at 230 nm of ST).The MWNTs-modified electrodes were immersed orderly in 100 g/L EDC and 100 g/L NHS solution (both EDC and NHS were resolved in 0.01 M p H7.4 PBS), for 30 min, respectively. After dropping 20 μL ADTZ (containing 2 μL PEG-200 and tittles of ST) on its surface, the electrode was laid for 30 min at room temperature. The enzyme electrode of Au/MWNTs–ADTZ was therefore formed [19,24]. Before using, the electrode should be washed in 0.01 M pH7.4 PBS for three times.

2.4. The electrochemical response of the Au/MWNTs–ADTZ electrode to ST

A bioanalytical system BAS100 (BAS Co., USA) was used for amperometric measurements. The Au/MWNTs–ADTZ electrode described above, Ag/AgCl and a platinum wire were acted as working electrode, reference electrode and counter electrode, respectively. The electrochemical characters of ST were studied in a 0.1 g/L KCl aqueous solution buffered with 0.1 mol/L PBS (pH 6.0) by using cyclic voltammetry (CV) and different pulse voltammetry (DPV), respectively. The potential range was from −1000 to +1000 mV. All measurements were performed at 25 °C.

3. Results and discussion

3.1. The detection of oxidative MWNTs by TEM and IR

The morphology of carbon nanotubes before and after acidic oxidation was explored by using transmission electron micrograph (TEM). From TEM side view image, as shown in Fig. 1 after (b) and before (a) the aqua regia refluxed, the nanotubes were broken and the caps were opened after acid oxidation treated. Comparing the MWNTs with and without an acid refluxing by using EQUINOX 55 infrared (IR) spectrograph (Bruker Co., Germany) analyzing, the data indicated that a large number of carboxylic acid groups had been introduced into MWNTs after the refluxing process, as shown in Fig. 2. Compare with Fig. 2 (a) and (b), the novel peak near 3135–2928, 1718, 1179 and 1119 cm^{-1} (a double peak) have shown the carbon oxidative group such as carbonyl and carboxyl group absorbance. When comparing with Fig. 2 (b) and (c), the peak near 3000 cm^{-1} was significantly decreased, while the peaks at 1718 and 1218 cm^{-1} were increased. This implied that cyclic anhydride groups probably existed in the carbon nanotubes after the longer oxidation process.

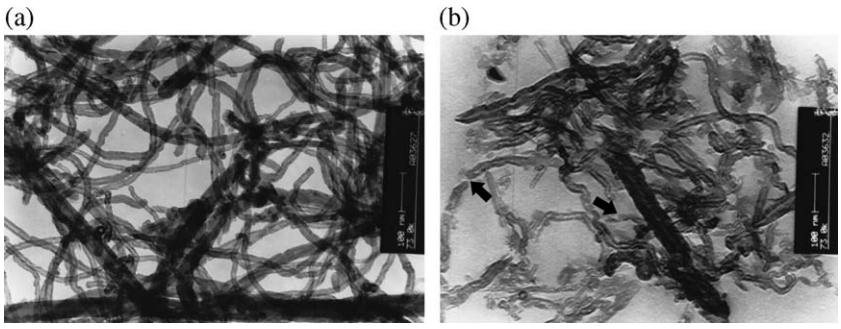

Fig. 1. Transmission electron micrograph view of multi-walled carbon nanotubes before (a) and after (b) aqua regia refluxing. After acid oxidation treated, the nanotube caps were opened (viewed with the arrows).

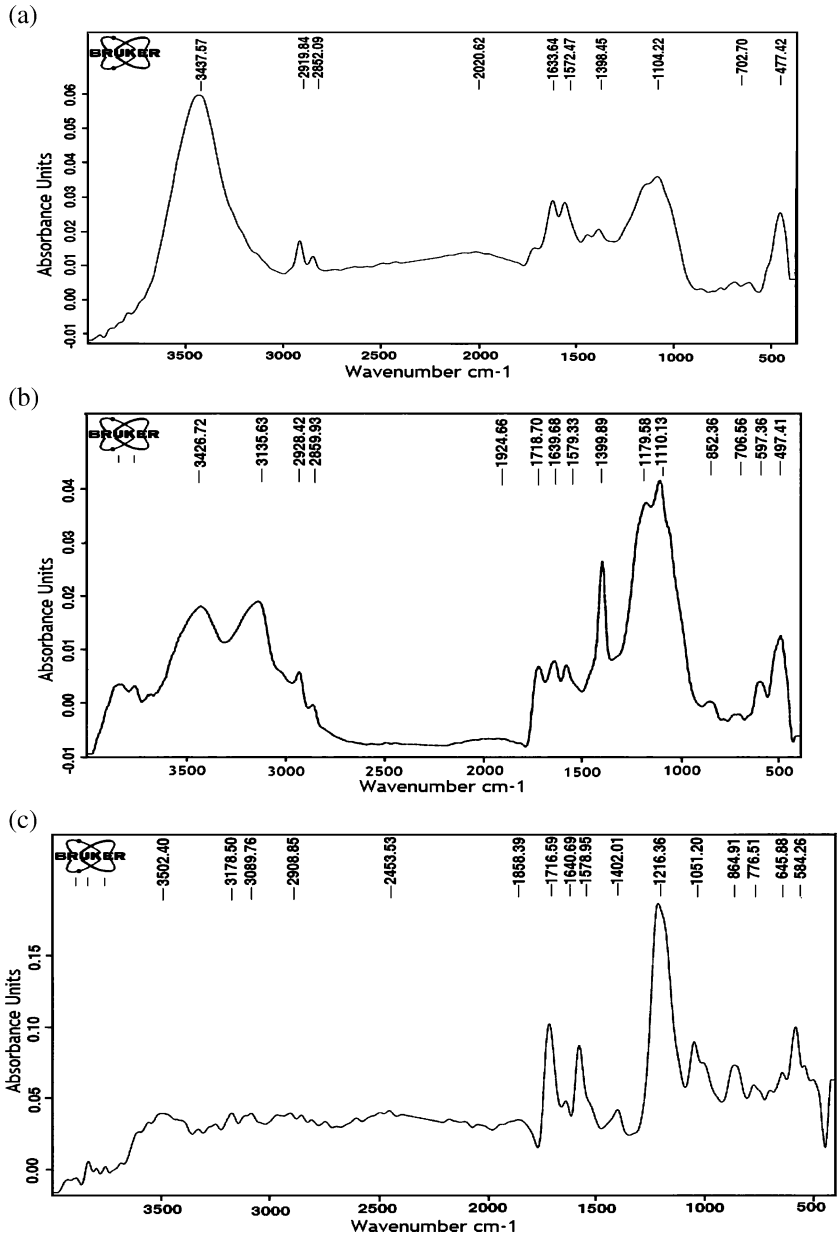

Fig. 2. The IR spectrum of the MWNTs with or without acid reflux. Compared with MWNTs that had not been refluxed (a), the results showed that the absorption at 1718 nm contributed by the carboxylic acid groups of the eight-hour-refluxed ones (b) was clearly observed, for the longer twelve-hour-refluxed ones (c), an enlarged absorption at this wavelength could be seen.

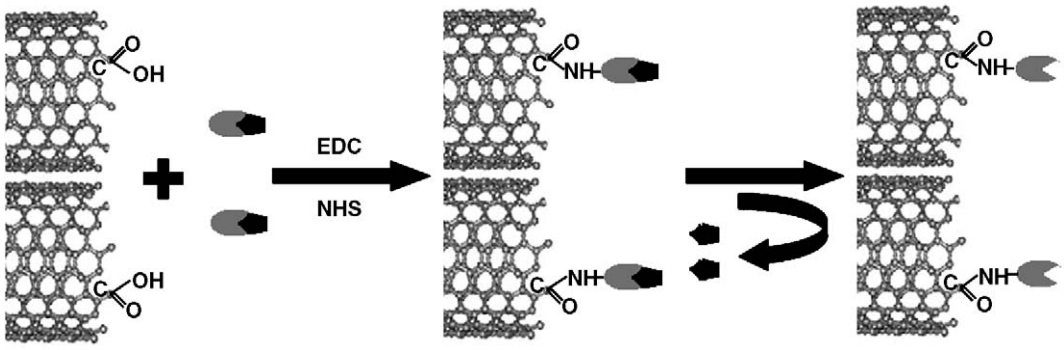

Fig. 3. Schematic illustration of the assembly of ADTZ onto MWNTs with EDC and NHS via covalent bond. ADTZ, which was combined with the substrate ST covalently linked to the carboxyl group on oxidative MWNTs via acid amide bond. After removing the substrate, the active-remained ADTZ was immobilized onto the MWNTs-modified gold electrode, the ST biosensor was then assembled.

3.2. Immobilization of enzyme on the oxidative MWNTs

Since covalent bond will possibly impair the enzyme activity during the immobilization process, a strategy of active-center-protection was employed to maintain the "right" conformation of the enzyme to compensate for the negative influence of the covalent linkage. This strategy was found to work well. As shown in Fig. 3, the enzyme ADTZ was saturated with its substrate ST prior to the immobilization into the oxidative MWNTs. The gold electrode was primarily modified with the acidic oxidative MWNTs, and then the ST-saturated ADTZ was cross linked to the carboxyl group on the nanotubes via an amide bond. After removing the substrate ST, a working electrode was developed. Because the enzyme active site was protected by the substrate, the covalently immobilization remain the enzyme bioactive well. Without this substrate protection strategy, only a weaker electro-signal can be produced on the ADTZ immobilized MWNTs biosensor (the results not shown).

3.3. A surface observation of the MWNTs-based enzyme electrode by Atomic force microscopy (AFM)

Morphological information gives a physical picture of how the ADTZ molecular are assembled on a biosensor

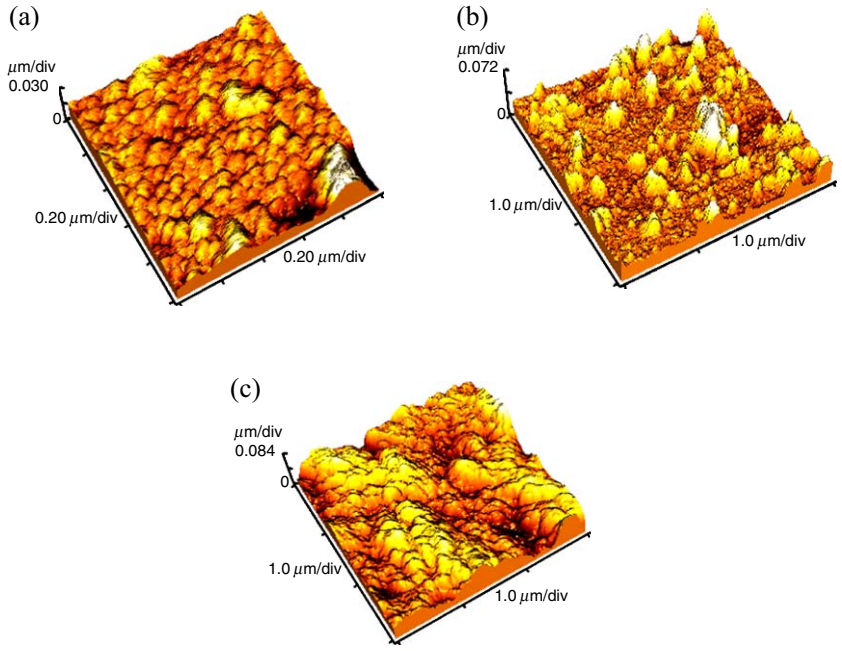

Fig. 4. Atomic force micrograph (AFM) of the surface 3D view of the only MWNTs modified electrode surface (a), the MWNT-based ADTZ covalently modified electrode (b), and the MWNT-based ADTZ adsorbed electrode surface (c). Scanning by Atomic force microscopy (AUTOPROBE CP RESEARCH THERMO Co., USA), in the AFM 3D view images of the MWNT-based enzyme electrode surface, two layers can be seen of the ADTZ-MWNTs film The bottom layer is MWNTs that distributed equably on the electrode matrix; while the top layer is ADTZ, whose molecules strewed at random over the MWNTs film. Compared to MWNTs modified electrode that ADTZ was immobilized via random adsorption (c), the covalently immobilized one had a more uniformed ADTZ-MWNTS film (crystal state can be seen).

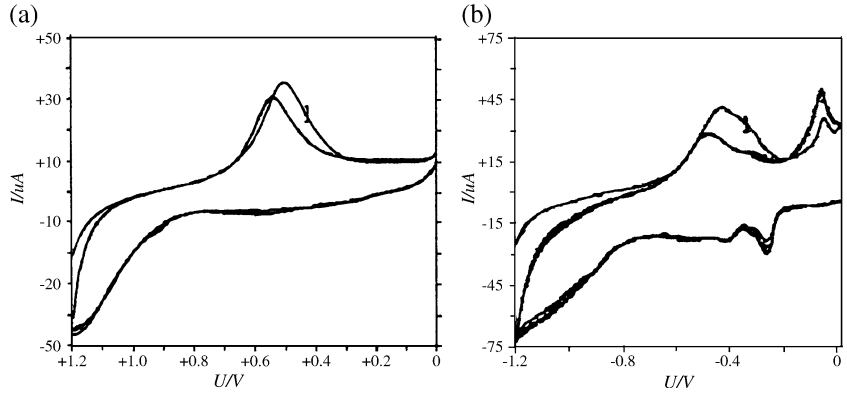

Fig. 5. The cyclic voltammetry of the ADTZ-MWNT electrode to KCl. solution (a): showed the stability of the electrode and to 13.4 μM ST solution (b): an oxidation peak of ST was observed at about +400 mV with a good recurrence (the cycle number was twenty).

surface. The mean roughness value (RMS) of images can also be analyzed and related to the properties of surfaces. Fig. 4-b and c has shown the AFM 3D views of ADTZ attached to MWNTs covalently and adsorptive (deposited), respectively. The only MWNTs modified surface (Fig. 4-a) is quite homogeneous with a mean roughness of 2 nm, and the ADTZ adsorptive surface changes and the increase of the mean roughness value up to 14 nm indicates the adsorption of ADTZ (ADTZ deposited on the electrode surface). Morphological view of the covalent immobilized ADTZ on the MWNTs surface which the mean roughness value is up to 10 nm, shown steadier and consistent properties compare with the adsorption method. The crystal state of protein can be observed due to the directional immobilization results.

3.4. The cyclic voltammetry character to ST of the covalently modified, MWNTs–ADTZ electrode

The stability of the MWNTs–ADTZ electrode was examined by using cyclic voltammetry in 0.1 g/L KCl solution at pH 6.6 buffered with 0.2 mol/L phosphates. The scanning rate was 100 mv/s while the cycle number was eight. The current was prone to be steady after the second cycle; it showed the stability of the electrode (Fig. 5-a). The reason of the slightly shift might come from the unbalance between the solution and the sensor surface, after the second cycle, when the balance established the signal become stable. The cyclic voltammetry response character of the MWNTs–ADTZ electrode to ST was studied in PBS described above with a three-electrode system. The concentration of ST added into PBS was 4.15×10^{-3} mg/ml (13.4 μM), and the cycle number was twenty. Compare with Fig. 5-a, in Fig. 5-b the oxidation peak of ST can be observed at about +400 mV with a valid recurrence.

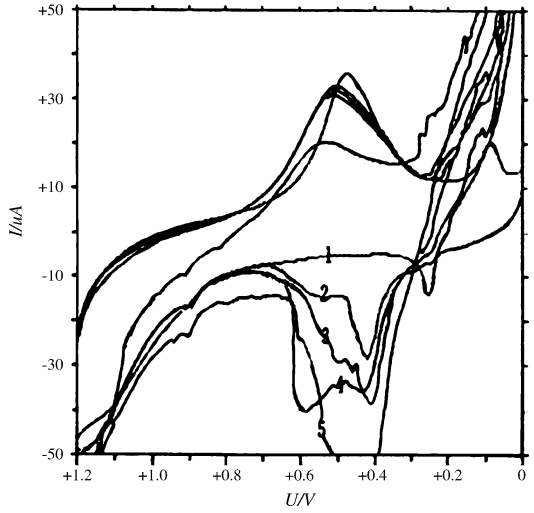

Fig. 6. The response character of the MWNTs-ADTZ electrode to ST of various concentrations (1:PBS, 2~5:ST solutions with an increasing concentration).

3.5. The dependence of response current on ST concentration for the MWNTs–ADTZ electrode

A series of experiments were performed to determine the dependence of response current on ST concentration for the MWNTs–ADTZ electrode. The scanning rate was 100 mv/s and the potential range was 0 to 1200 mv. The results showed that the response current existed positive correlations to ST concentration (Fig. 6). By summarizing this and

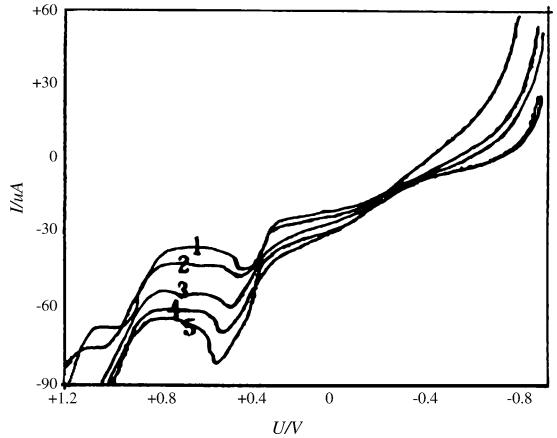

Fig. 7. The cyclic voltammetry of ST at various scanning rate. (1:100 mv/s, 2:200 mv/s, 3:300 mv/s, 4:400 mv/s, 5:500 mv/s).

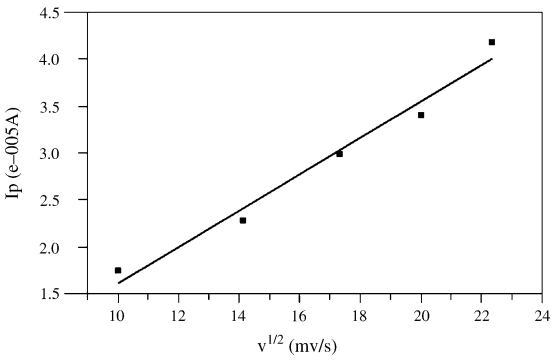

Fig. 8. The linear relationship between the response currents and the square root of scanning rates.

the upper results, the correlative signal to ST lie at +400 mV due to the substrate oxidation.

3.6. The dependence of response current on scanning rate for the MWNTs–ADTZ electrode

In this experiment, the MWNTs–ADTZ electrode was scanned in the 4.15×10^{-3} mg/ml ST solution at the rate of 100, 200, 300, 400, and 500 mv/s, respectively using cyclic voltammetry (Fig. 7). Fig. 8 showed the linear relationship between the response currents and the square root of scanning rates. Analyzing by SPSS (statistical package for the social science), the data showed a correlative coefficient of 0.987 and $P=0.02<0.05$, which meant a fine linear relationship between the response currents and the square root of scanning rates. According to the Randles–Sevcik equation, this relationship suggested that the transfer of the substrate ST on ADTZ film surface was mostly affected by linear diffusion, while the effect of sacrificial absorption on the electrodes surface was much less.

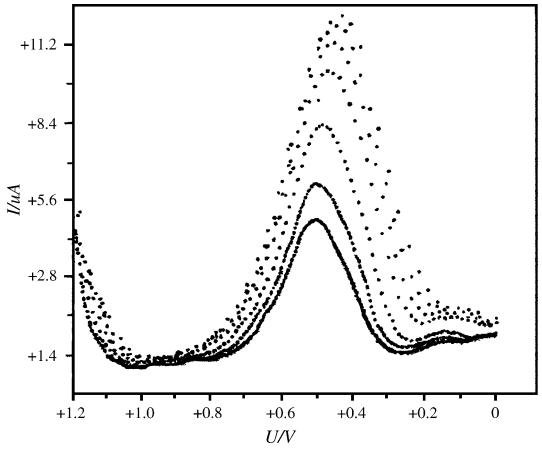

Fig. 9. The different pulse voltammetry (DPV) character of the MWNTs-ADTZ electrode to ST solutions of various concentrations (0.134 μM, 0.535 μM, 1.07 μM, 1.61 μM, 2.15 μM, 4.29 μM, 8.58 μM, 10.74 μM, 16.10 μM, 21.48 μM, 26.84 μM). The electro signals have shown increasing response to the ST conc.

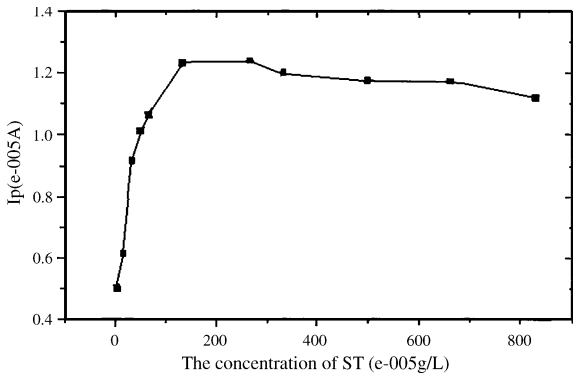

Fig. 10. The relationship between the response current and ST concentration The linear relationship between electric current to ST conc. lies between 0.134 μM and 4.29 μM. The sensor has shown passivation towards a higher concentration.

3.7. The different pulse voltammetry (DPV) character of the MWNTs–ADTZ electrode to ST solutions of various concentrations

The MWNTs–ADTZ electrode was scanned in a series of ST solutions of various concentrations (0.134~26.84 μM) by using DPV (Fig. 9). Fig. 10 displayed the relationship between the response current and the ST concentration, it can be seen that when the ST concentration ranged from $4.16 \times 10^{-5} \sim 2.66 \times 10^{-3}$ mg/ml, the response current increased with the increase of ST concentration. Moreover, the response current and the ST concentration had a valid linear relationship in ST solutions whose concentrations ranged from $4.16 \times 10^{-5} \sim 1.33 \times 10^{-3}$ mg/ml, and the linear equation was $ip=0.03C+0.497$, $R^2=0.9023$. When ST concentration was between 1.33×10^{-3} and 2.66×10^{-3} mg/ml, the response current was changeless, while when ST concentration went higher than 2.66×10^{-3} mg/ml, the response current appeared saturated with a slight decreasing.

4. Conclusions

In this paper we have shown a ST biosensor developed by MWNTs modified gold electrode, which was immobilized an aflatoxin–detoxification enzyme, ADTZ. Since ST is some how a stable compound, neither the naked nor the MWNTs modified gold electrode can detected any signal of ST. This behavior indicated that ADTZ catalyzed ST oxidation. If we compare the chemical structure of aflatoxin B_1 and ST, the similarity between them is easy to find. That implies that the enzyme ADTZ reaction site is at the bi-furan-ring part of them. This suggestion is consistent with our previous investigation findings [19,24].

Carbon nanotubes, especially after oxidation, the specific area are increased due to the caps opening, and the surface property of them converted from the hydrophobic state to the hydrophilic state [11,25], and these properties will

enhance their attachment to enzyme hydrophilic sites. In addition, these properties offer an extra advantage to this ST biosensor assembling. When the hydrophilic sites of ADTZ linked to MWNTs, the enzyme's hydrophobic sites remain to its hydrophobic substrate. By coupled with the substrate protection strategy, the assembled biosensor worked well. Both TEM and IR spectrograph discoveries showed that acid oxidation is an effective way to open the tips of nanotubes.

In this study, both the linear detection range (0.134~4.29 μM) and the detection limit (0.134 μM) of covalently united ADTZ–MWNTs electrode are improved compared to the corresponding results of the random absorbed ADTZ–MWNTs electrode [16]. The improvement may be explained as follow: when the ADTZ molecules were directly united to MWNTs and protected by ST, the active center of ADTZ wouldn't be blocked and the correct conformation was holding, therefore in the detection, both the sensitivity and the stability were amended.

Although, when it was operated at optimized conditions, the biosensor's linear detection range was enlarged to two orders magnitude, and its detection limit (4.16×10^{-5} mg/ml) lower below the limit (4 mg/kg) detected in cheese when using TLC [26] (5 mg/kg) and HPLC-MS [27], there are several intricate factors that affect its practical usage. Sample temperature, ion intensity, and acidity affect the responsibility, sensitivity and stability of the detection in some sense. Thereby, further research should be focus on the effect of temperature, pH value and ion intensity on the detection, optimizing detection conditions, improving the detection sensitivity and applicability.

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References

[1] J. Xu, X.Y. Luo, Molecular biology of aflatoxin biosynthesis, Journal of Hygiene Research 32 (2003) 628–631.
[2] F.J. Ma, J. Misumi, W. Zhao, K. Aoki, M. Kudo, Long-term treatment with sterigmatocystin, a fungus toxin, enhances the development of intestinal metaplasia of gastric mucosa in *Helicobacter pylori*-infected Mongolian gerbils, Scandinavian Journal of Gastroenterology 38 (2003) 360–369.
[3] F.J. Ma, W.Y. Zhao, M. Kudo, K. Aoki, J. Misumi, Inhibition of vacuolation toxin activity of *Helicobacter pylori* by iodine, nitrite and potentiation by sodium chloride, sterigmatocystin and fluoride, Toxicology in Vitro 16 (2002) 531–537.
[4] X. Zhang, F. Wang, J. Wang, X. Yan, X. Huang, T. Xie, Z. Zhang, Experimental lung carcinoma induced by fungi and mycotoxins—a review, Journal of Peking University. Health Sciences 35 (2003) 4–6.
[5] F.N. Kristian, N. Fog, Mycotoxin production by indoor molds, Fungal Genetics and Biology 39 (2003) 103–117.
[6] D.S. Jayas, N.D.G. White, Storage and drying of grain in Canada: low cost approaches, Food Control 14 (2003) 255–261.
[7] M.H. Xie, X.M. Yang, X.F. Xin, B. Lv, Y.D. Chen, Analysis of sterigmatocystin in corn from Hubei province, Journal of Preventive Medicine (Hu Bei province, China) 12 (2001) 3–4.
[8] R. Garjonyte, A. Malinauskas, Amperometric glucose biosensors based on Prussian Blue and polyaniline-glucose oxidase modified electrodes, Biosensor & Bioelectronics 15 (2001) 445–451.
[9] M.D. Rubianes, G.A. Rivas, Carbon nanotubes paste electrode, Electrochemistry Communications 5 (2003) 689–694.
[10] J. Wang, A.N. Kawde, M.R. Jan, Carbon-nanotube-modified electrodes for amplified enzyme-based electrical detection of DNA hybridization, Biosensors & Bioelectronics 20 (2004) 995–1000.
[11] J.S. Ye, X. Liu, H.F. Cui, W.D. Zhang, F.S. Sheu, Tit Meng Lim, Electrochemical oxidation of multi-walled carbon nanotubes and its application to electrochemical double layer capacitors, Electrochemistry Communications 7 (2005) 249–255.
[12] M. Shim, N.W.S. Kam, R.J. Chen, Y. Li, H. Dai, Functionalization of carbon nanotubes for biocompatibility and biomolecular recognition, Nano Letters (2) (2002) 285–288.
[13] W. Huang, S. Taylor, K. Fu, Y. Lin, D. Zhang, T.W. Hanks, A.M. Rao, Y.-P. Sun, Attaching proteins to carbon nanotubes via diimide-activated amidation, Nano Letters (2) (2002) 311–314.
[14] K. Fu, W. Huang, Y. Lin, D. Zhang, T.W. Hanks, A.M. Rao, Y.-P. Sun, Functionalization of carbon nanotubes with bovine serum albumin in homogenous solution, Journal of Nanoscience and Nanotechnology (2) (2002) 457–461.
[15] M.L. Guo, J.H. Chen, D.Y. Liu, L.H. Nie, S.Z. Yao, Electrochemical characteristics of the immobilization of calf thymus DNA molecules on multi-walled carbon nanotubes, Bioelectrochemistry 62 (2004) 29–35.
[16] S. Sotiropoulou, V. Gavalas, V. Vamvakaki, N.A. Chaniotakis, Novel carbon materials in biosensor systems, Biosensors & Bioelectronics 18 (2003) 211–215.
[17] J.J. Davis, M.L.H. Green, H.A.O. Hill, Y.C. Leung, P.J. Sadler, J. Sloan, A.V. Xavier, S.C. Tsang, The immobilisation proteins in carbon nanotubes, Inorganica Chimica Acta 272 (1998) 261–266.
[18] J.J. Davis, R.J. Coles, H.A.O. Hill, Protein electrochemistry at carbon nanotubes electrodes, Journal of Electroanalytical Chemistry 440 (1997) 279–282.
[19] D.L. Liu, D.S. Yao, R. Liang, L. Ma, W.Q. Cheng, L.Q. Gu, Detoxification of aflatoxin B_1 by enzymes isolated from *Armillariella tabescens*, Food and Chemical Toxicology 36 (1998) 563–574.
[20] D.S. Yao, S.M. Wen, D.L. Liu, C.F. Xie, Y. Bai, Y.H. Ran, The primary study on the detection of sterigmatocystin by biologic enzyme electrode modified with the multiwall carbon nanotubes, Chinese Journal of Biotechnology 20 (2004) 601–606.
[21] J. Liu, A.G. Rinzler, H.J. Dai, J.H. Hafner, R.K. Bradley, P.J. Boul, A. Lu, T. Iverson, K. Shelimov, C.B. Huffman, F.M. Rodriquez, Y.S. Shon, T.R. Lee, D.T. Colbert, R.E. Smalley, Fullerene pipes, Science 280 (1998) 1253–1256.
[22] J.X. Wang, M.X. Li, Z.J. Shi, N.Q. Li, Z.N. Gu, Electro catalytic oxidation of 3,4-dihydroxyphenylacetic acid at a glassy carbon electrode modified with single-wall carbon nanotubes, Electrochimica Acta 47 (2001) 651–657.
[23] M.L. Guo, J.H. Chen, D.Y. Liu, L.H. Nie, S.Z. Yao, Electrochemical characteristics of the immobilization of calf thymus DNA molecules on multi-walled carbon nanotubes, Bioelectrochemistry 62 (2004) 29–35.

[24] D.L. Liu, D.S. Yao, Y.Q. Liang, T.H. Zhou, Y.P. Song, L. Zhao, L. Ma, Production, purification, and characterization of an intracellular aflatoxin–detoxifizyme from *Armillariella tabescens* (E-20), Food and Chemical Toxicology 39 (2001) 461–466.
[25] G.Y. Wang, Z.H. Wang, S.F. Xiao, Y.M. Wang, G.A. Luo, Electrocatalyic separation for dopamine and epinephrine at multi-wall carbon nanotube modified electrode and simultaneous determination, Chinese Journal of Analytical Chemistry 31 (2003) 1281–1285.
[26] H.P.v. Eqmond, W.E. Paulsch, E. Deijll, P.L. Schuller, Thin layer chromatographic method for analysis and chemical confirmation of sterigmatocystin in cheese, Journal-Association of Official Analytical Chemists 63 (1980) 110–114.
[27] K.A. Scudamore, M.T. Hetmanski, P.A. Clarke, K.A. Barnes, J.R. Startin, Analytical methods for the determination of sterigmatocystin in cheese, bread and corn products using HPLC with atmospheric pressure ionization mass spectrometric detection, Food Additives and Contaminants 13 (1996) 343–358.