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Low-level expression of purine bases in BALB/3T3 cells monitored by ultrasensitive graphene-based glass carbon electrode

Guang-Gang Gao [a], Guo-Biao Xu [a], Jin-Lian Li [a], Ji-Wen Cui [a], Dong-Mei Wu [a,*], Hong-Bin Qiu [b,*]

[a] College of Pharmacy, Jiamusi University, Jiamusi 154007, China
[b] School of Public Health, Jiamusi University, Jiamusi 154007, China

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ABSTRACT

Using an ultrasensitive chemically reduced graphene oxide and ionic liquid modified glass carbon electrode (RGI–GCE), separated electrochemical signals of adenine and hypoxanthine in both human breast cancer (MCF-7) and mouse embryonic fibroblast (BALB/3T3) cells were observed. For the first time, low-level expression of purine bases in noncancerous BALB/3T3 cells can be electrochemically monitored. The metabolism of purine bases in carcinogen agent-contaminated BALB/3T3 cells was also investigated through the change of electrochemical signals ascribed to different purine bases, which opens a new electrochemical approach to the exploration of a low-level purine mechanism in noncancerous cells.

Purine bases, including guanine (G),[1] xanthine (X), adenine (A), and hypoxanthine (HX) as catabolic intermediates of nucleotide catabolism, closely relate to many physiological functions of cells and show different metabolic levels for noncancerous and cancer cells [1–3]. The research of metabolic-level changes of purines is extremely important for prevention and therapy of cancer [4,5]. The intracellular purines are traditionally detected by high-performance liquid chromatography (HPLC) that is usually combined with complicated pretreatment, high cost, and long detection time. As a different technique, electrochemical detection has drawn much consideration in cell analysis during recent years. Especially, we found a new in situ method to deal with cell samples by which cell culture pretreatment and detection can be conducted in the same cell culture dish. This in situ method significantly improved the electrochemical response of cells, simplified the operation process, reduced the experiment time, avoided the use of trypsin, and even was applied in the study of the effectiveness of antitumor drugs on tumor suppression [6,7]. Given this, there is no doubt that the electrochemical detection of intracellular purines to unveil the status of cellular carcinogen or gene mutation is a direct and time-saving method.

Since Ci and coworkers reported the electrochemical detection of leukemia cells by glass carbon electrode (GCE), multiwall carbon nanotube (MWNT) modified GCE has also been designed to realize the detection of cellular purines in which only one oxidation signal ascribed to G can be observed [8,9]. In our previous work, two electrochemical signals of human breast cancer (MCF-7) or human prostate cancer (PC-3) cells based on MWNT and ionic liquid (IL) complex modified GCE (MWNT–IL–GCE) were detected and are ascribed to oxidation processes of G/X and A/HX, respectively [10,11]. However, the completely overlapped oxidation signal of G and X (or A and HX), as a result of the similarity of their structures, hinders the accurate evaluation of individual metabolic change of intracellular purine bases. In addition, because of the low-level purine metabolism in noncancerous cells [12] and the low sensitivity of the existing electrode [13], so far the study of electrochemical behavior of noncancerous cells cannot be realized. For accurate evaluation of metabolic levels of purines, highly sensitive and regenerable modified GCE remains a big challenge.

As a two-dimensional carbon nanostructure consisting of a single layer of sp^2 carbon atoms, graphene has attracted great interest because of its unique properties such as high theoretical surface area, excellent electronic conductivity, and great mechanical

* Corresponding authors.
E-mail addresses: dmwu@jmsu.edu.cn (D.-M. Wu), qhbin63@163.com (H.-B. Qiu).

[1] *Abbreviations used:* G, guanine; X, xanthine; A, adenine; HX, hypoxanthine; HPLC, high-performance liquid chromatography; GCE, glass carbon electrode; MWNT, multiwall carbon nanotube; IL, ionic liquid; RG, reduced graphene; RGI–GCE, chemically reduced graphene oxide and ionic liquid modified glass carbon electrode; CV, cyclic voltammetry; MNNG, *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine; GO, graphene oxide; PBS, phosphate-buffered saline; DMSO, dimethyl sulfoxide; DMEM, Dulbecco's modified Eagle's medium; APAP, acetaminophen; DMF, dimethylformamide; SEM, scanning electron microscopy; LSV, linear sweep voltammetry; SD, standard deviation.

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strength [14–17]. Our recent work indicated that chemically reduced graphene (RG) modified GCE has shown promising application for the detection of biological compounds, including purine molecules [18]. On the other hand, IL can enhance the sensitivity of MWNT modified GCE transparently due to its ability to extend the electrochemical window and facilitate electron transfer from target purine molecules to electrode surface [19–21]. Here, we anticipated that modification of GCE by both RG and IL may extensively enhance the sensitivity of GCE for its detection of different purine molecules in cells, making it possible to obtain distinguishable and individual oxidation signals ascribed to different purine molecules. In the area of bioelectrochemistry, parallel to the extending investigation of new electrode materials, it is also important to find suitable model cells, such as MCF-7 cells [22] and leukemia K562 cells [23,24], that can be used as targets to explore its nucleotide metabolism, and then we can realize the prediction of viability, proliferation, pathological features, gene mutation, and even carcinogenesis [25,26]. Among diverse cells, the BALB/3T3 cell, as one of the most investigated mammal cells, shows feasibility of carcinogenesis when it is injected into suitable carcinogenic agents and plays a significant role in the study of canceration in vitro [27,28]. So far, canceration of BALB/3T3 cells induced by a carcinogen agent is mostly determined by biologically substantial transformation of foci with a long detection time.

In this study, RG and IL were selected as electrode components, forming an ultrasensitive chemically reduced graphene oxide and ionic liquid modified glass carbon electrode (RGI–GCE) for the detection of purines in MCF-7 and BALB/3T3 cells. For the first time, the oxidation peaks of intracellular A and HX were differentiated and the electrochemical behavior of noncancerous cells was investigated. More interesting, the loss of IL during the detection process that induces the decrease of peak currents can be recharged by operation of cyclic voltammetry (CV) scan in the IL solution, leading to a readily regenerable RGI–GCE. In addition, when BALB/3T3 cells were incubated by introduction of *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (MNNG), concentration change of intracellular purines can be observed transparently by the variation of peak currents, suggestive of an electrochemical method to evaluate the purine metabolic disorder possibly related to cellular carcinogenic status.

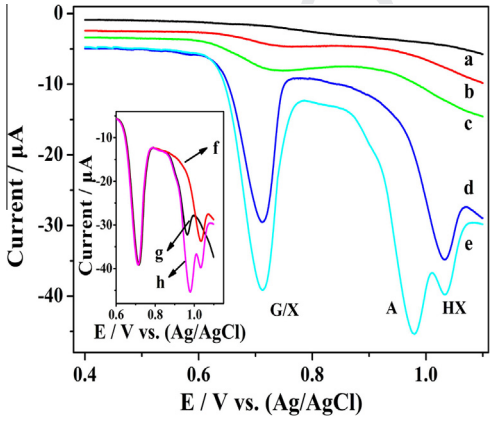

Fig.1. LSV of standard purine mixture of 2.5×10^{-5} mol L^{-1} X, G, HX, and A at different GCEs: (a) bare GCE; (b) RG–GCE; (c) IL–GCE; (d) MWNT–IL–GCE; (e) RGI–GCE. Inset: (f) mixture of G, X, and HX; (g) mixture of G, X, and A; (h) mixture of G, X, A, and HX. Scan rate: 50 mV s^{-1}; temperature: 25 °C.

Materials and methods

Reagents and materials

Graphene oxide (GO) was purchased from Sinopharm Chemical Reagent. 3-Methylimidazolium hexafluorophosphate was purchased from Fluka. Four kinds of purine were obtained from Sigma–Aldrich. Phosphate-buffered saline (PBS) solution containing NaH_2PO_4 and Na_2HPO_4 was deaerated by nitrogen for 15 min before experiments and then kept in an N_2 atmosphere for further experiments. MCF-7 cells were obtained from the Basic Medical Science College at Harbin Medical University (China). BALB/3T3 cells were purchased from Obio Technology (China). All materials related to culture cells, such as dimethyl sulfoxide (DMSO), Dulbecco's modified Eagle's medium (DMEM), culture plate, fetal calf serum, nonessential amino acids, glutamine, and double antibody, were purchased from Life Technologies (USA). MNNG was obtained from Aladdin Industrial (China). Acetaminophen (APAP) solution was prepared using double-distilled water and stored at 4 °C for further use. All other reagents were of analytical purity and were used without further purification.

RG was prepared by the chemical reduction method [29]. Briefly, 2.3 mg of GO was added to 5 ml of water under stirring, followed by the addition of 35 μl of $NH_3{\cdot}H_2O$ and 5 μl of $N_2H_4{\cdot}H_2O$. After sonication for approximately 20 min, GO nanosheets were reduced to graphene nanoplates under refluxing of the mixture for 1 h in an oil bath (95 °C). The final product was then filtered through a nylon membrane (0.22-mm pores) and washed. Then, the RG was dried for 24 h. After that, 1 mg of RG was dispersed into 2 ml of dimethylformamide (DMF) and then was sonicated for 96 h to form flocculent RG DMF solution. IL was dissolved in DMF to form the diluted IL DMF solution with contents as 1 or 3.6% (v/v).

Apparatus

The electrochemical properties of standard purine mixture and cell lysates were recorded on a CHI660D electrochemical workstation (CH Instruments, China) with a conventional three-electrode system comprising a platinum wire as auxiliary electrode, an Ag/AgCl electrode as reference electrode, and a bare or modified GCE as working electrode. Scanning electron microscopy (SEM) was performed with an FEI Quanta 200 scanning electron microscope. The acceleration voltage was set to 10 kV. The sample was stuck on the observation platform and sprayed with gold vapor under high vacuum for approximately 60 s. HPLC detections at 254 nm standard purine mixture and cell lysates were performed on an Agilent 1100 separation module (SpectraLab Scientific) comprising an Agilent XDB-C18 column (4.6 × 250 mm) equipped with a diode array detector (DAD) system at 25 °C. The mobile phase (pH 4.0) was 0.02 mol L^{-1} NaH_2PO_4 solution, and the flowing velocity was set as 1.0 ml min^{-1}. The process of HPLC was as reported in the literature [7]. All electrochemical behaviors of standard purine mixture or cell lysates were studied using linear sweep voltammetry (LSV) in the potential range from 0.0 to 1.1 V with a scan rate of 50 mV s^{-1} at 25 °C.

Fabrication of different electrodes

Bare GCE with a diameter of 3 mm was polished on a polishing cloth with 0.05 μm alumina powder and rinsed with double-distilled water, followed by sonication in acetone, ethanol, and double-distilled water successively. Thereafter, the electrode was allowed to dry at room temperature. MWNT–IL–GCE was prepared according to the literature method [11,30]. RG modified GCE (RG–GCE) was prepared by a simple droplet casting procedure with 2 μl