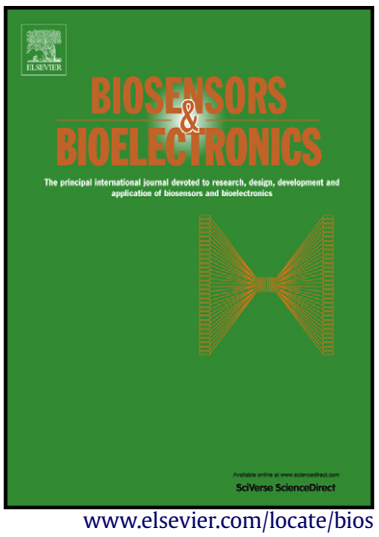

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Structure Effect on Graphene Modified Enzyme Electrode Glucose Sensors

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Abstract

We explored and investigated the structure effect on reduced graphene oxide (rGO) sheets modified enzyme electrode with glucose oxidase (GOD) by structure characterizations and electrochemical measurements. The rGO sheets with different defect density, layers, and oxygen concentration were chosen to modify enzyme electrode, and all modified enzyme electrode obtained excellent electrocatalytic activities and performances towards glucose. The abundant defects in rGO induce easy absorption of GOD. At low oxygen concentration, rGO sheets help to induce the direct electron transfer (DET) on electrode; and at higher oxygen concentration, reduction of H_2O_2 occurred instead of DET on the surface of electrode. When rGO modified enzyme electrode under the working model of H_2O_2 reduction, enlarge the oxygen functional group could lead the more absorption of GOD, resulting in the improvement of affinity and sensitivity to the biosensors. The modified enzyme electrode can provide faster response, higher sensitivity, and better affinity by optimizing and controlling the structure of graphene and its derivative.

Keywords: graphene; glucose; biosensor; enzyme electrode

1. Introduction

Since 1962 Clark and Lyons(Clark and Lyons 1962) proposed the concept of glucose enzyme electrodes, people has developed three generation glucose biosensors, the first generation based on monitoring of reduction of hydrogen peroxide, the second generation inserted electron transfer mediator, and the third generation detected direct electron transfer (DET).(Wang 2001) (Shan et al. 2009) For fifty years research and exploration, glucose biosensors based on glucose oxidase (GOD) electrode, has been widely studied and investigated in diabetes clinical detection, biological analysis, environmental monitoring, and food processing industries.(Wang 2001) Nowadays, with the development of nanoscience and nanotechnology,(Du et al. 2011) the study on modified enzyme electrode biosensors by nanomaterials and nanostructures received broad attention due to their excellent electric properties, high surface area, and ease of functionalization.(Ma et al. 2012; Wang et al. 2011b) The modification of enzyme electrode through nanomaterials and nanostructures, such as silicon nanowires,(Elfström et al. 2008) ZnO nanostructures,(Lei et al. 2010; Lei et al. 2011) and carbon nanotubes,(Bai et al. 2012) become a highlight point in nanoscience and bioelectronics.

Graphene, a two dimensional (2D) honey bomb lattice of carbon atoms discovered in 2004,(Novoselov et al. 2004) has captured remarkable attentions due to its excellent physical and chemical properties, such as a large surface area (theoretically, 2630 m^2/g for single-layer graphene), excellent electrical conductivity, high thermal conductivity, strong mechanical strength and good biocompatibility.(Guo and Dong 2011b) Among all graphene's considerable properties, high surface area, easily functionalized, excellent electron transfer, and good biocompatibility, make it would be a suitable application to enzyme electrode biosensors(Guo and Dong 2011a).

The study of graphene and its derivative applied to enzyme electrode biosensors derived from 2009. Niu et al. (Shan et al. 2009) firstly demonstrated graphene as an enhanced material for direct electrochemistry of GOD to construct glucose biosensors. Lin et al. (Wang et al. 2010) demonstrated that N-doped graphene displayed a higher electrocatalytic activity for the reduction of hydrogen peroxide and fast direct electron transfer kinetics for GOD. Dai et al. (Liu et al. 2010) fabricated glucose biosensors through the covalent attachment between carboxyl acid groups on graphene oxide (GO) sheets and the amine of GOD, and Qiu et al. (Qiu et al. 2011) utilize synergy effect of GO and chitosan to fabricate glucose biosensor. In a word, graphene and its derivative modified enzyme electrode showed broad linearity, good sensitivity, stability and reproducibility. However, the effects of graphene and its derivative structures on the properties of modified enzyme electrode reported vague.

In this paper, we studied the relationship between reduced graphene oxide (rGO) structure and electrochemical properties of rGO sheets modified enzyme electrode with GOD. Three rGO sheets with different layers, defect density, and oxygen content were characterized by field emission scanning electron microscopy (FESEM), Raman spectra, and X-ray photoelectron spectra (XPS). Low oxygen concentration of rGO sheets help to induce the direct electron transfer (DET) on electrode; and higher oxygen concentration no longer generate DET but reduce H_2O_2 on the surface of electrode. Increasing the oxygen functional group could lead the improvement of affinity and sensitivity to the biosensors. The structure effect of rGO sheets modified enzyme electrode with GOD on the mechanism and kinetics of enzyme catalysed reaction was discussed in details. All rGO sheets modified enzyme electrode with GOD performed good electrocatalytic oxidation and got decent sensitivity to glucose detection, implying potential application to the construction of variety glucose sensors.

2. Material and methods

2.1 Regents

The rGO sheets with different structures were purchased from Tianjin Pula Nanotech Ltd. GOD and Nafion were purchased from Sigma-Aldrich. Glucose and H_2O_2 were obtained from Sinopharm Chemical Reagent Co., Ltd.

2.2 Preparation of rGO modified GOD electrodes

Before modification, the glass carbon electrode (GCE, 3mm in diameter) was polished with sand paper followed by 1.2, 0.8, and 0.05 μm alumina slurry, respectively. After successive sonication in ethanol and deionized water, the electrode was rinsed with deionized water and dry at 60°C.

The prepared electrode was modified by a simple casting method as follow. First, rGO sheets with different structures (rGO01, rGO02 and rGO03) were dispersed in ethanol and ultrasonic agitation for 30 min, and then rGO ethanol dispersion (3 μL, 1mg/ml) dropped onto the surface of GCE and dried in the air. Second, a 5 μL GOD solution (109 U/mg, 10.0 mg/mL in phosphate buffer solution (PBS, 0.01 M, pH=7.4)) was dropped onto the surface of rGO/ GCE and dried in the dark. Finally, a 5μL 5 wt. % Nafion solution was further coated on the GOD/rGO/GCE and dried for 24 h at 4℃ to form a film.

The same method of rGO sheets modified Pt electrodes (PtE, 3mm in diameter) were produced as above.

2.3 Apparatus

The morphologies and structures of rGO sheets were characterized by employing FESEM (Zeiss, SUPRA-55), Raman spectrometer (Jobin-Yvon, JY-HR800, 514nm), XPS (Axis Ultra^{DLD}), and FT-IR Spectrometer (Perkin Elmer, Spectrum One). The cyclic voltammetric and amperometric response measurements of Nafion/GOD/rGO/GCE were investigated by advanced electrochemical interface (Solartron Analytical, SI 1287).

3. Results and Discussion

3.1 Morphology and structure Characterization

FESEM images of rGO sheets (rGO01, 02, and 03) with different structures are shown in Fig. 1 (a), (b) and (c), respectively. All rGO sheets are in micrometre size. In addition, apparent corrugated are observed in rGO01 and rGO02, and plane surface is obtained in rGO03.

The structure of defects and layers characterization of rGO sheets are performed by Raman spectra in Fig. 1 (d). Well-defined D band (~1330 cm^{-1}), the first-order scattering from a zone-boundary phonon, was relatively intense in all rGO sheets, implying the defective crystal structure.(Ferrari et al. 2006) G bands (~1580 cm^{-1}), assignable to the E_{2g} phonon of sp2 carbon atoms, were obtained in all rGO sheets as well. The relative ratio of D and G band intensity (I_D/I_G) reflect the defect density in rGO sheets.(Lim et al. 2010) Here, rGO01, rGO02 and rGO03 with I_D/I_G at 1.12, 0.99and 0.98, indicated the relatively high defect density in rGO01 and lower defect density in rGO02 and rGO03. 2D bands (~2670 cm^{-1}), the second order of zone-boundary phonons, were enhanced in sample rGO01 and relative smooth in rGO02 and rGO03. Due to the relative intensity ratio of G and 2D (I_G/I_{2D}) reflects the layers information of graphene, the higher I_G/I_{2D}, the more layers, (Reina et al. 2009) the less layers of rGO01 than rGO02 and rGO03 were indicated.

The oxygen concentration and functional groups were characterized by XPS shown in Fig 1(e). The peaks of O1s were enhanced due to the increasing of oxygen contents from rGO01 to rGO03. The atomic concentration of C, O, and N of rGO sheets was calculated and demonstrated in Table 1. A small amount Nitride exited in the rGO sheets due to the reduction reaction. FT-IR spectra (Fig S1) also support the large number of oxygen function group exist in rGO samples. C=O stretching vibration peaks locate at ~1650 cm^{-1}, and carboxyl group/hydroxyl peaks locate at 3000~3600 cm^{-1}.

With the deepen oxidation of rGO sheets, sp2 hybridization transited to sp3-bonding. Prolonged oxidation would result in fracturing of graphene lattice and formation of edge plane defects decorated by hydroxyl and carboxylic groups.(Bowling et al. 1988)

Briefly, the oxygen concentration of the samples is increased from rGO01 to rGO03; the layers are decreased from rGO01 to rGO 03; while rGO01, rGO02 and rGO03 are with equivalent high level of defect density.

Table 1 Atomic Concentration of C, O, and N of samples a, b and c

Sample	C1S (%)	O1S (%)	N1s (%)
01	94.3	5.3	0.4
02	81.6	17.9	0.5
03	70.6	29.8	0.4

3.2 Electrochemical Characterizations

Due to electron transfer kinetics is dependent on not only the density of electronic states but also surface microstructure(McCreery 2008), and increasing in electron transfer rate constant on the carbon basal plane correlate with the appearance of edge plane defects,(Jia et al. 2007) cyclic voltammograms (CV) were carried out on the rGO sheets modified enzyme electrode with GOD to determine the relationship between rGO structure and current. In Fig 2, CV curves of enzyme electrode with and without rGO sheets were performed in 0.1 M PBS and scan rate at 50 mV. Compared to the enzyme electrode without rGO, the currents of rGO modified enzyme electrode are enhanced and redox waved obviously. Moreover, the cyclic current of rGO modified enzyme electrode is getting broader from rGO01 to rGO03. It is implied that the electrochemical activity is improved by rGO and redox reaction related to GOD occurred. Fig S2 can help to support the above assumption. When rGO sheets modified GCEs without GOD detect in presence of 3mM glucose, no obviously redox peaks are observed in CV curves and the cyclic current enhanced gradually from rGO01 to rGO03. It is verified that the redox waves is related to GOD not oxygenated species from rGO. For rGO01 modified enzyme electrode, the cathodic and anodic peak potentials (Epc and Epa) are -0.469V and -0.431V, the peak-to-peak separation (ΔEp) is 38mV, and the current intensity ratio of cathodic to anodic (I_{pc}/I_{pa})was ~1, suggesting quasi-reversible redox process fast electron communication achieved. The formal potential (E^0), calculated by average value of Epc and Epa, is at -0.450 V (Fig 2 black curve). Moreover, the CV measurement of rGO01 modified enzyme electrode under various scan rates from 10 to 100 mV in 3mM glucose solution are presented in Fig 3

(a). I_{pc} and I_{pa} linear increased with the scan rates and achieved excellent correlation coefficient (0.997 and 0.996), implying surface control process (Fig 3 (d)). All above characteristics, E^0 at -0.450 V, the quasi-reversible surface-controlled redox process, suggests the DET of GOD on rGO01 modified electrode which can be described as follow: (Shan et al. 2009; Wang et al. 2009; Wu et al. 2010)

GOD (Ox) + glucose → gluconolactone + GOD (Red) (1)

GOD (Red) →GOD (Ox) + ne (2)

For rGO02 modified enzyme electrode, Epc and Epa are -0.269 V and -0.135 V with ΔEp at 134 mV (Fig 2, blue curve). I_{pc} and I_{pa} linear increases with the scan rates from 10 to 100 mV in glucose solution, suggesting surface control process (Fig 3 (b) and (e)). The correlation coefficients were 0.995 and 0.994. In addition, similar redox peaks observed at rGO02 modified GCE without GOD in 2mM H_2O_2 (Fig S3 blue curve), which suggests this quasi-reversible surface control process can be ascribed to formation of hydrogen peroxide as follow:

GOD (Ox) + glucose → gluconolactone + GOD (Red) (3)

GOD (Red) + O_2 → GOD (Ox) + H_2O_2 (4)

$H_2O_2 \rightarrow 2H^+ + O_2 + 2e$ (5)

For rGO03 modified enzyme electrode, Epc and Epa are 0.126 V and 0.184 V with 58 mV separation. I_{pc} and I_{pa} are relative weak and also linear increase with the scan rates from 50 to 120 mV in glucose solution (Fig 3 (c) and (f)). The correlation coefficients were 0.980 and 0.995. And similar redox peaks appeared when rGO03 modified GCE without GOD in 2mM H_2O_2 (Fig S3 red curve). The quasi-reversible redox process and surface control on enzyme electrode can also attribute to the formation of hydrogen peroxide as rGO02 dose.

CV curves of GOD on rGO01, rGO02 and rGO03 modified Pt electrode was conducted in presence of 3mM glucose (Fig S4) to analyze electrochemical property as well. DET feature can be observed from GOD on rGO01 modified PtE (Fig S4 a and d) and formation of hydrogen peroxide could be inferred from GOD on rGO02 (Fig S4 b and e) and rGO03 (Fig S4 c and f) modified PtE.

3.3 Performance of rGO sheets modified glucose biosensors

The real time detection of rGO sheets modified enzyme electrodes were tested by amperometric response demonstrated

in Fig 4 (a), (b) and (c). All three rGO sheets obtain steady-state current responses toward increasing concentration of glucose. The calibration curves to amperometric responses of rGO sheets modified GOD electrodes are shown in Fig 4 (d), (e) and (f). For rGO01 modified glucose biosensor, the linear detect range is from 1 to 60 μM (correlation coefficients = 0.991) and the sensitivity determined by the slop of linear fitting is 3.54 $\mu A/cm^2 \cdot mM$. For rGO02 modified glucose biosensor, the detect range is from 2 to 13 mM (correlation coefficients = 0.998) with sensitivity at $1.77\times10^{-3}\mu A/cm^2 \cdot mM$. The linear detect range of rGO03 modified glucose biosensor is 1 to 10 mM with sensitivity at $1.08\times10^{-2}\mu A/cm^2 \cdot mM$. In comparison, rGO sheets with low oxygen content provided lower detect limit, wider detect range, and higher sensitivity, which could be applied to glucose detection in scruple environment; while high oxygen content rGO sheets modified enzyme electrode is suitable for detection of human blood. In addition, high defect density, fewer layers and oxygen functional group can also affect the sensitivity of rGO modified glucose biosensors. There is no specific relationship between oxygen functional group and detect range.

3.4 Mechanism and kinetics of enzyme catalysed reaction

To explore the kinetics of enzyme catalysed reaction on rGO sheets modified enzyme electrodes, the apparent Michaelis-Menten constant (K_m), calculated from the linear form of Lineweaver–Burk equation, was introduced.(Lei et al. 2012; Li et al. 2012)

$$1/j = (k=K_m/j_{max})\cdot(1/C)+1/j_{max}$$

Where j is the current density, j_{max} is the saturation value of current density, and C is the concentration of glucose. Here, K_m reflected the bioactivity and affinity of GOD.(Wang et al. 2011a) The corresponding Lineweaver–Burk plots (1/j vs 1/C) of rGO sheets modified glucose biosensors are presented in Fig 5. K_m and j_{max} are 0.682 mM and 0.483 $\mu A/cm^2$, 3.473 mM and 0.045$\mu A/cm^2$, and 1.36 mM and 0.337$\mu A/cm^2$ for rGO01, rGO02 and rGO03 modified glucose biosensors, respectively.

rGO01, with low oxygen content, few layers and large number of defects, modified enzyme electrode showed the best affinity of GOD suggesting high electrical communication between glucose and the redox centres of the GOD, and lead to DET on the enzyme electrode. As well known, the redox centre of GOD, flavine adenine dinucleotide (FAD) which undergoes a two-electron coupled with two-proton redox reaction, is deeply embedded in a protective protein shell of enzyme molecule.(Cai and Chen 2004) So it is extremely difficult to achieve the DET on the surface of electrodes.

While, graphene could promote the electron transfer(Shan et al. 2009) and facilitate the DET process between the GOD and electrode due to its extraordinary electron transport property and high special surface area. It is believed that graphene sheets, as carbon nanotube dose, become positioned in tunnelling distance of the cofactors of GOD with little consequence to denaturation, and the conformational change of GOD in the microenvironment enable the accessibility of active site for GOD to the electrode.(Guiseppi-Elie et al. 2002; Liu et al. 2005) The structure of rGO01 sheet is close to graphene, so that it induced the DET on the glucose enzyme electrode.

With the oxygen content increasing in rGO sheets, sp2 hybridization transited to sp3-bonding, and the electron was immobilized to oxygen atom resulting in DET cannot be obtained anymore. So rGO02 and rGO03 modified enzyme electrode responded to glucose by the formation of detect of electrolysis H_2O_2. Due to GOD immobilized into rGO sheets via peptide bonds between the amine groups of GOD and the carboxylic acid at edge of rGO,(Liu et al. 2010) the more oxygen functional group in rGO sheets the more immobilization of GOD onto rGO sheets. The improved sensitivity and K_m of rGO03 modified enzyme electrode can be ascribed to the higher absorption of GOD.

4. Conclusion

In summary, the structure effect on rGO sheets modified enzyme electrode with GOD biosensors were explored and investigated. All rGO sheets with different structures modified glucose biosensor obtained excellent electrocatalytic activity and performance towards glucose. The abundant defects and oxygen function group in rGO induce easy absorption of GOD. At low oxygen concentration, rGO sheets help to induce the direct electron transfer (DET) on electrode; and at higher oxygen concentration, reduction of H_2O_2 occurred inside of DET on the surface of electrode. When rGO modified enzyme electrode under the working model of H_2O_2 reduction, enlarge the oxygen functional group could lead the more absorption of GOD, resulting in the improvement of affinity and sensitivity to the biosensors. Realization of the structure effect on mechanism and kinetics of rGO modified biosensors can be the benefit to the application to the electrochemical biosensors of fast response, high sensitivity, and excellent affinity, and would provide super platform to nano biosensors and bioelectronics.

Acknowledgements

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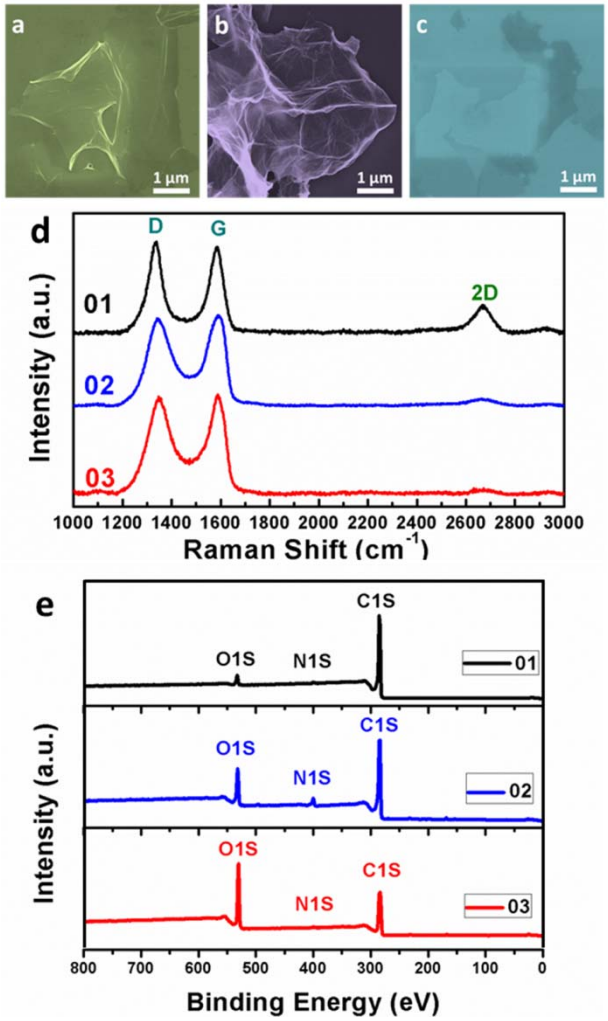

Fig. 1 Morphologies and structures of rGO sheets. FESEM of rGO sheets: (a) rGO01, (b) rGO02 and (c) rGO03; (d) Raman spectra and (e) the whole XPS spectra

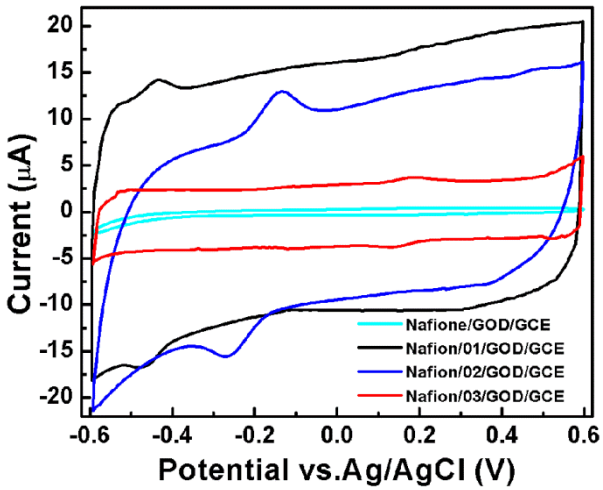

Fig. 2 Cyclic voltammograms of Nafion/GOD/GCE, Nafion/rGO01/GOD/GCE, Nafion/rGO02/GOD/GCE, and Nafion/rGO03/GOD/GCE in PBS (0.01 M, pH=7.4) with the scan rate at 50mV/s.

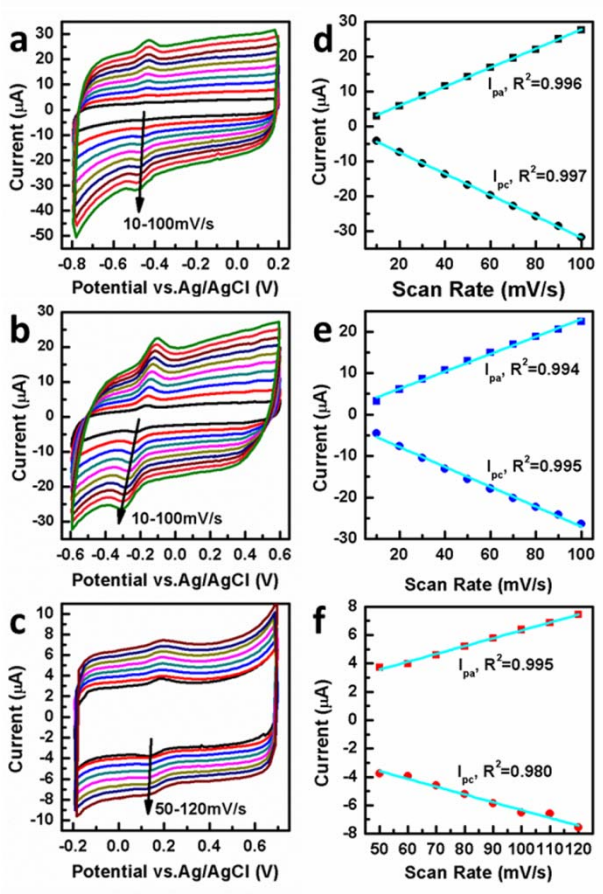

Fig. 3 Cyclic voltammograms of (a) Nafion/rGO01/GOD/GCE, (b) Nafion/rGO02/GOD/GCE, and (c) Nafion/rGO03/GOD/GCE in PBS (0.01 M, pH=7.4) with 3mM glucose solution at various scan rates. (d), (e) and (f) are plots of peaks current (Ip) vs scan rate for Nafion/01/GOD/GCE, Nafion/02/GOD/GCE, and Nafion/03/GOD/GCE, respectively

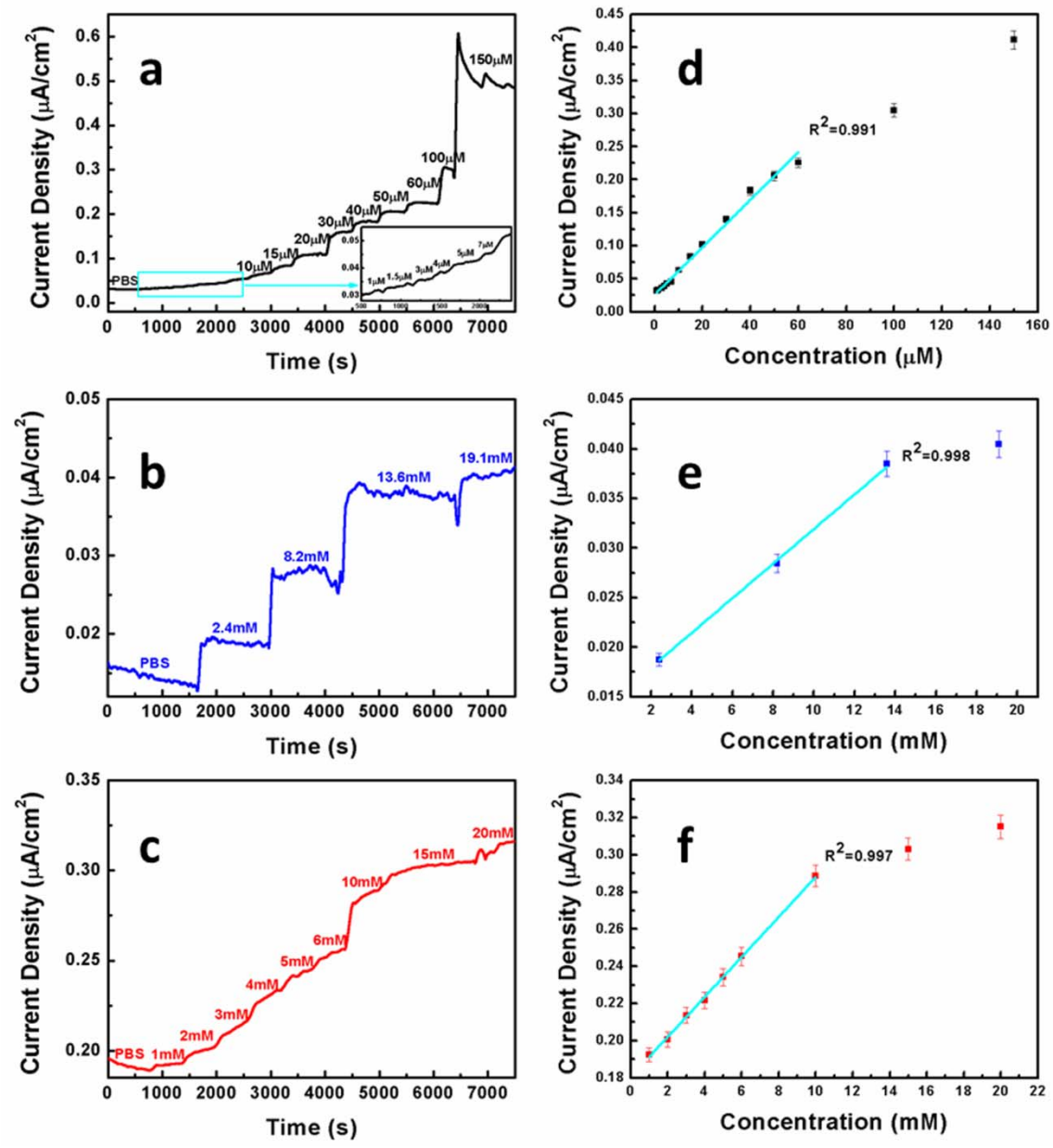

Fig. 4 Amperometric responses of (a) Nafion/01/GOD/GCE, (b) Nafion/02/GOD/GCE, and (c) Nafion/03/GOD/GCE in a series of concentration of glucose. Calibration curves to Amperometric responses of (d) Nafion/01/GOD/GCE, (e) Nafion/02/GOD/GCE, and (f) Nafion/03/GOD/GCE

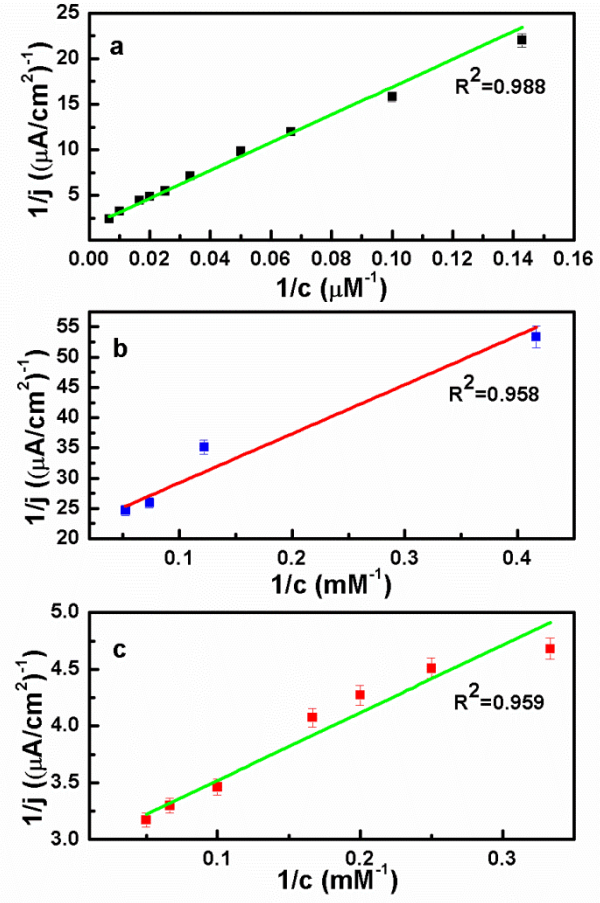

Fig 5 The corresponding Lineweaver–Burk plots of rGO01 (a), rGO02 (b) and rGO03 (c) modified enzyme electrodes

Research Highlights

- This paper studied the structure effect on reduced graphene oxide (rGO) modified glucose enzyme electrode.
- At low oxygen concentration, rGO help to induce the direct electron transfer (DET) on electrode.
- At higher oxygen concentration, reduction of H_2O_2 occurred instead of DET on the surface of electrode.
- Enlarge the oxygen functional group could improve the affinity and sensitivity to the biosensors.