Homogeneous detection of concanavalin A using pyrene-conjugated maltose assembled graphene based on fluorescence resonance energy transfer

Qiushui Chen, Weili Wei, Jin-Ming Lin*

Analysis Center and Department of Chemistry, Tsinghua University, Beijing 100084, PR China

A R T I C L E   I N F O

Article history:
Received 8 March 2011
Received in revised form 21 April 2011
Accepted 4 May 2011
Available online 11 May 2011

Keywords:
Graphene
Maltose
Concanavalin A
Homogeneous
Fluorescence resonance energy transfer

A B S T R A C T

In this work, we proposed a novel biosensor to homogeneously detect concanavalin A (ConA) using pyrene-conjugated maltose assembled graphene based on fluorescence resonance energy transfer (FRET). Maltose-grafted-aminopyrene (Mal-Apy) was synthesized and characterized by mass spectra, UV–vis and fluorescence spectra. The Mal-Apy was further employed for fluorescence switch and ConA recognition. When Mal-Apy was self-assembled on the surface of graphene by means of π–π stacking interaction, its fluorescence was adequately quenched because the graphene acted as a “nanoquencher” of the pyrene rings due to FRET. As a result, in the presence of ConA, competitive binding of ConA with glucose destroyed the π–π stacking interaction between the pyrene and graphene, thereby causing the fluorescence recovery. This method was demonstrated the selective sensing of ConA, and the linear range is 2.0 × 10⁻⁷ to 1.0 μM with the linear equation y = 1.029x + 0.284 (R = 0.996). The limit of detection for ConA was low to 0.8 nM, and the detection of ConA could be performed in 5 min, indicating that this method could be used for fast, sensitive, and selective sensing of ConA. Such data suggests that the graphene FRET platform is a great potential application for protein–carbohydrate studies, and would be widely applied in drug screening, histomolecular recognition and disease diagnosis.

© 2011 Elsevier B.V. All rights reserved.

1. Introduction

Proteins that specifically interact with certain carbohydrate groups are important in various cellular processes, including cell recognition, immune response, adhesion and inflammation by bacteria and viruses (Jelinek and Kolushova, 2004; Lis and Sharon, 1998; Mammen et al., 1998; Simanek and Korhonen, 1993). Recently, a class of such proteins, the lectins (e.g., concanavalin A (ConA), peanut agglutinin (PNA)), which could bind to mono- and oligosaccharides with high specificity, came into the forefront of biological research (Lis and Sharon, 1998). ConA, a legume lectin from Jack beans, can specifically bind to the glucose moiety of cell membrane glycoprotein, thereby initiating T-cell activation, cell mitogenesis, agglutination and apoptosis (Akhand et al., 1997; Amin et al., 2007; Fayad et al., 2005; Tamura et al., 1995; Zhao et al., 2002). Many approaches have been developed to detect ConA because it is an important target for studying the carbohydrate-protein interactions (Banerjee and Chen, 2007; Disney et al., 2004; El-Boubbou et al., 2007; Huang et al., 2009; Medintz et al., 2003; Sato et al., 2008). Recent efforts have led to the development of carbohydrate chips by either covalent or noncovalent immobilization strategies (Fukui et al., 2002; Park et al., 2004; Wang et al., 2002). Fukui et al. (2002) and Wang et al. (2002) have successfully fabricated a glycan chip by immobilizing various carbohydrates on nitrocellulose or nitrocellulose-coated glass slides, which might be used for high-throughput analysis of carbohydrate-protein interactions. However, the process was complex and time-consuming. In addition, Banerjee and Chen (2007) grafted maltose onto the surfaces of Fe₃O₄ magnetic particles; based on the specificity binding with glucose moieties, ConA were collected by magnetic iron; the consequent optical properties reduction associated with the aggregation of carbohydrate-grafted magnetic particles led to the development of assays for selective sensing of ConA. Carbohydrate-polymer conjugates were also developed to detect ConA and Escherichia coli (E. coli) by polyvalent interactions between ligands and proteins (Disney et al., 2004). Even though much effort has been devoted to developing new sensors for ConA, most of previous approaches need further washing, high speed centrifugalization or separation before sample analysis (Banerjee and Chen, 2007; El-Boubbou et al., 2007; Gildersleeve et al., 2008; Guo et al., 2007; Huang et al., 2009). Therefore, developing a fast and simple method for homogeneous detection of ConA is of great significance.

Recently, nanomaterials have provided an alternative approach for bioanalysis platform, such as carbon nanotube (Yang et al., 2008), graphene oxide (Lu et al., 2009), gold (Huang et al., 2009; Sato et al., 2008) and magnetic particles (Banerjee and Chen, 2007; El-Boubbou et al., 2007). Graphene, which is a single-atom-thick...
carbon material and shows remarkable electronic and thermal properties (Geim and Novoselov, 2007; Novoselov et al., 2004; Zhang et al., 2005), has attracted great attention in bioanalysis applications. Graphene oxide is used for bioanalysis due to its strong π-stacking interaction with DNA (Lu et al., 2010; Lu et al., 2009), aptamers (Chang et al., 2010; He et al., 2010; Wen et al., 2010), and molecular beacons (Lu et al., 2010) based on fluorescence variations. It is reported that graphene oxide can quench the fluorescence of dyes through fluorescence resonant energy transfer (FRET) (Dong et al., 2010; Loh et al., 2010; Swathi and Sebastian, 2008, 2009). However, graphene could provide higher fluorescence quenching efficiency than graphene oxide (Kim et al., 2010). The quenching efficiency of pristine graphene is estimated to be as large as 10^3 (Xie et al., 2009). Thus, it is possible to realize sensitive fluorescent sensing using graphene as a good quencher.

In this work, a maltose-grafted-aminopyrene (Mal-Apy) and graphene assembly system was developed for homogeneous detection of ConA by combining with the concept of FRET. First, 1-aminopyrene (1-AP) was successfully conjugated with maltose for fluorescence switch and ConA recognition. As shown in Fig. 1, Mal-Apy was self-assembled with graphene by means of π-stacking interactions between pyrene rings and graphene. Then, the fluorescence was adequately quenched because the graphene acted as a "nanoquencher" of the pyrene rings. Consequently, in the presence of ConA, competitive binding of ConA with glucose destroyed the π–π interaction between the pyrene rings and graphene, thereby causing the fluorescence recovery. This novel fluorescence transduction system was also demonstrated the selective sensing of ConA, and the linear range is 2.0 × 10⁻² to 1.0 μM with the linear equation y = 1.029x + 0.284 (R = 0.996). The kinetic analysis of the graphene FRET platform showed that this method can be used for a fast analysis of ConA. This strategy included three key features. Compared to previous reports, this study provided a novel method to realize the homogeneous detection of ConA. The π-stacking of 1-AP onto graphene could be employed as a fluorescence switch for the selective sensing of ConA. Moreover, Mal-Apy could improve the water-solubility and stability of graphene due to the π–π stacking (Gao et al., 2010; Su et al., 2009; Xu et al., 2008), which would provide graphene wider application prospects in the bioanalysis field.

2. Experimental

2.1. Materials and reagents

ConA and bovine serum albumin (BSA) were obtained from Sigma–Aldrich Co. (MO, USA). Graphene suspension was purchased from Chengdu Organic Chemicals Co. Ltd, Chinese Academy of Science (Chengdu, China). 1-AP (purity >98% wt%) was purchased from Acros Organic (Geel, Belgium). Maltose (purity > 97% wt%) was purchased from Sinopharm Chemical Reagent Co. (Shanghai, China). NaBH₃CN was purchased from Sigma–Aldrich Co. (MO, USA). Sodium phosphate buffer (PBS buffer, 10 mM, pH 7.0) was prepared at ambient temperature containing MnCl₂ (0.1 mM) and CaCl₂ (0.1 mM). All chemicals were of analytical grade and used without further purification. Deionized water was used in all the experiments.

2.2. Apparatus

Tapping mode atomic force microscopy (AFM) images were recorded using a Nanoscope III (DigitalInstrument) scanning probe microscope. The AFM samples (5 μL, 0.01 mg/mL) were prepared on mica glass, followed by drying at room temperature. Mass spectrum analysis was carried out by the ESI-Q-TOF-MS (Bruker Daltonics, Billerica, MA) under positive ion mode. The UV–vis spectra were recorded on a Hitachi U-3900 UV–vis spectrometer (Tokyo, Japan).

2.3. Preparation and purification of Mal-Apy

The preparation of Mal-Apy was achieved by coupling 1-AP with maltose via reductive amination (Lamari et al., 2003) as illustrated in Fig. S1. 1-AP (150 μmol) was suspended in 12 mL methanol/water (1:1, v/v) containing 20 mM phosphate solution and 100 μmol maltose. 300 μmol NaBH₃CN was added to the solution 1 h later. The coupling reaction was performed at 80 °C by stirring the solution for 48 h. The crude was purified by chromatography (silica, CH₃OH:CH₂Cl₂ = 9:1), and then vacuum dried for 24 h at 50 °C, 0.076 MPa. The final yield of Mal-Apy was 39.6% (21.5 mg, 39.6 μM).

2.4. Fluorescence tests

The fluorescence spectrum was measured by F-7000 Hitachi spectrometer (Tokyo, Japan). In our experiment, 0.15 mg/mL graphene was added into 1.0 μM Mal-Apy (in 10 mM PBS buffer, pH 7.0, with 0.1 mM MnCl₂ and 0.1 mM CaCl₂). For further characterization, ConA solutions with different concentrations (0, 0.002, 0.005, 0.01, 0.02, 0.05, 0.1, 0.2, 0.5, 1.0, 2.0, 5.0 μM) were added into above solution and incubated for 30 min before measurement. In specificity studies, the graphene system was incubated with different concentrations of ConA and BSA solutions in PBS for the same time, respectively. The fluorescence was monitored at 447.0 nm with the excitation of 340.0 nm at room temperature.

3. Results and discussion

3.1. Characterization of graphene and Mal-Apy

The graphene used in this experiment was characterized by AFM on mica. As shown in Fig. 2, the cross-sectional view of the AFM image showed that the average thickness of graphene sheet was about 1.12 ± 0.04 nm, which was somewhat larger than single layer graphene oxide (0.78 nm). Such thickness was reasonable for the wrinkles structure of graphene, which was also observed in previous reports (Chang et al., 2010; Dong et al., 2010; Xu et al., 2008). The optical characterizations of graphene were also conducted by monitoring the UV–vis and fluorescence spectra (Fig. S2).
The UV–vis absorbance spectra of pure graphene indicated that the graphene had a strong absorption band at 229.0 nm, which correspond to C=C π→ π* transitions (Sun et al., 2008). As shown in Fig. S2, there was no fluorescence emission of graphene at 447.0 nm with excitation of 340.0 nm. This could indicate that graphene had no contribution to the detection signal of ConA in further experiments.

The Mal-Apy was prepared by forming a Schiff base between maltose and −NH₂ of 1-AP. The synthetic route was showed in Fig. S1. UV–vis and fluorescence and mass spectroscopy were employed to characterize this product. Fig. 3a shows that the UV–vis absorbance peaks of Mal-Apy existed at 239.5, 278.0 and 347.5 nm, and the UV–vis absorbance peaks of 1-AP existed in 354.0 nm, 281.0 nm and 242.0 nm. Therefore, the UV–vis absorbance peaks of Mal-Apy showed slight blue shift compared to 1-AP. The blue shift should be ascribed to the derivation of 1-AP with maltose. Further, the characteristic UV–vis absorption spectra also indicated that there were intact pyrene rings in Mal-Apy. And the intact pyrene rings were the prerequisite to interact with graphene through π-stacking. The maximum emission wavelength of Mal-Apy was 447.0 nm compared with the 440.0 nm of 1-AP, showed a slight red shift (Fig. 3b).

The result also implied the successful derivation and retaining of pyrene rings. ESI-mass spectra were also carried to clearly prove the structure of Mal-Apy. Fig. 4a shows the first order mass spectrum of Mal-Apy. The characteristic fragment m/z 566.15 was assigned to [Mal-Apy+Na]+. The MS/MS further showed the structure of Mal-Apy (Fig. 4b). The fragment ion m/z 404.14 was created by reduction of a glucosyl group (m/z 162.01), and this result provided the evidence that there was glucosyl group in Mal-Apy. This is very important because the glucosyl group played a crucial role in the specific interaction with ConA.

3.2. FRET on the graphene platform

There were two reasons that 1-AP was chosen for the present study. First, 1-AP has fluorescence properties that could be used for labeling maltose. The other important reason is pyrene rings owned the plane configuration which could stack on the graphene and could be used as fluorescence molecule switch because of the π-stacking with graphene. The fluorescence quenching of Mal-Apy based on the FRET between pyrene rings and graphene was evaluated by fluorescence spectra. Fig. 5 shows the fluorescence quenching of Mal-Apy at various concentrations of graphene. The fluorescence intensity decreased rapidly when the graphene was added into the 1.0 μM Mal-Apy solutions. Moreover, the fluorescence intensity decreased with increasing concentration of graphene. About 87.9% fluorescence was quenched when the graphene concentration was 0.15 mg/mL. The fluorescence quenching efficiency by graphene had good reproducibility. In three parallel experiments, the remaining fluorescence of Mal-Apy in the presence of 0.15 mg/mL graphene were 14.7%, 12.1% and 12.2% with only ca. 1.2% standard deviation, indicating a good reproducibility of graphene quenching efficiency. The high efficiency quenching was considered as the direct consequence of noncovalent binding of pyrene rings on the graphene surface and the energy transfer from pyrene rings to graphene (Chang et al., 2010; Yang et al., 2008). The planar configuration of graphene
and pyrene rings guarantees the close proximity of Mal-Apy with graphene and efficient fluorescence quenching due to π-stacking between graphene and pyrene rings. In addition, Müllen (Su et al., 2009) and Shi (Xu et al., 2008) reported that graphene could be stabilized in the aqueous solution by large aromatic molecules based on the π–π interaction. This water-solubility fluorescence probe, containing water-solubility biofunctionalized group and large aromatic group, will be of significance in fundamental and applied science for the non-soluble graphene.

3.3. Fluorescence recovery with ConA

We developed the fluorescence transduction system for ConA detection by the graphene FRET platform. Fig. 6a shows the fluorescence recovery of graphene FRET platform in the presence of different concentrations of ConA. The fluorescence intensity of the graphene FRET platform increased with the increasing concentration of ConA from 0 to 5.0 μM. The linear range is from $2.0 \times 10^{-2}$ to 1.0 μM with linear equation $y = 1.029x + 0.284$, where $y$ is relative fluorescence intensity and $x$ is the concentration of ConA (regression coefficient $R = 0.9996$) (Fig. 6b). In three parallel experiments, the relative standard deviation of relative was 7.9%, indicating a relatively good reproducibility. The limit of detection for ConA was 0.8 nM, based on three times the standard deviation of the control (free of ConA), which is comparable to the best reported results (Guo et al., 2007; Huang et al., 2009). Two possible reasons contributed to this high sensitive. First, the interaction of carbohydrate with ConA on the graphene platform was so strong that ConA could break down the π-stacking interaction between graphene and pyrene rings, which created the sensitive fluorescence recovery for ConA detection (Chang et al., 2010). Further, the high efficient fluorescence quenching and recovery depended on the energy transfer between 1-AP and graphene was critical for the FRET platform. The plane structure of 1-AP matched the one-dimensional graphene was important to the strong interaction and high effective fluorescence quenching. Thus, the direct result of detection ConA by interacting with Mal-Apy on the graphene FRET platform has great potential for fast and simple detection of ConA in complex real samples.

3.4. Kinetic analysis of graphene FRET platform

The kinetic characteristics of the graphene FRET platform were further investigated by monitoring the fluorescence variations as a function of time. Fig. 6c shows that the fluorescence quenching of Mal-Apy (1.0 μM) by graphene (0.15 mg/ml) was very fast. The fluorescence intensity of Mal-Apy was quenched by over 85% in 1 min, indicating the high quenching efficiency of graphene (Kim et al., 2010). However, about 42% of fluorescence recovery was obtained upon addition of ConA (5.0 μM) at the room temperature. The fluorescence recovery reached equilibrium in 5 min, indicating that the π-stacking interaction between graphene and pyrene rings was broken down due to the interaction of Mal-Apy with ConA. This time analysis experiment clearly indicated that the graphene FRET platform could be used for fast and simple detection of ConA.

3.5. Specificity of graphene FRET platform

Maltose binding ConA has high specificity (Munoz et al., 2009; Ooya et al., 2003, 2005). In our experiments, we showed that
the fluorescence-based graphene platform responded to ConA was specific. As shown in Fig. 6d, when tested a series of different concentrations of BSA (0, 0.002, 0.005, 0.01, 0.02, 0.05, 0.1, 0.2, 0.5, 1.0, 2.0, 5.0 μM) in 10 mM PBS buffer, the fluorescence-based graphene platform had lower fluorescence respond to BSA compared to that of fluorescence-based graphene platform. However, significant fluorescence enhancement exhibited when ConA was added. This result was consistent with the reports that glucosyl group has higher affinity toward ConA (Guo et al., 2007; Huang et al., 2009; Sato and Anzai, 2006). In addition, it showed that the pyrene-conjugated carbohydrate assembled graphene platform could be used as a sensitive and selective system for lectin recognition and detection.

4. Conclusions

In conclusion, we have successfully constructed a novel method for homogeneous detection of ConA based on the graphene FRET platform. As a key design of strategy, the bifunctional molecule (Mal-Appy) was successfully synthesized for fluorescence switch and ConA recognition. Because of the high fluorescence quenching efficiency of graphene, this method was also demonstrated to have great potential for fast, sensitive, and selective sensing of other lectins. Although this strategy for protein–carbohydrate studies is encouraging, some efforts should be made to expand the field of applications. More bifunctional molecules should be designed for expanding the protein–carbohydrate studies, and multivalent cooperative interactions between protein and carbohydrate should be further considered for protein–carbohydrate recognition. Anyway, we believe this novel sensor is expected to be an excellent platform for protein–carbohydrate studies and could be applied in drug screening, bimolecular recognition and disease diagnosis.

Acknowledgements

This work was supported by National Natural Science Foundation of China (No. 90813015) and National Basic Research Program of China (2007CB714507).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bios.2011.05.009.

References