CONSTRUCTION OF GRAPHENE-BASED ENZYME SYSTEM FOR RAPID PHOTO-ASSISTED PROTEOLYSIS

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ABSTRACT

Rapid and efficient protein digestion by sequence-specific proteases is a prerequisite and critical step in proteomics. A novel photo-assisted nanoreactor was designed and constructed for rapid protein digestion. Porous graphene-silica composite material was prepared, and trypsin molecules were further immobilized in the nanopores to construct the functional nanoreactor for protein digestion. By taking advantage of the unique response of graphene to the near infrared light, this nanoreactor can realized rapid protein digestion under near infrared laser radiation, which is much faster than the widely applied overnight in-solution digestion using free trypsin molecules. It's expected that this work would contribute to the rapid and high throughput protein digestion in proteomics.

KEYWORDS

Nanoreactor, Graphene, Silica, Protein, Digestion

INTRODUCTION

As a comprehensive study of protein expression and function from a global perspective, proteomics plays an increasingly important role in biomarker discovery, disease diagnosis and prevention.[1-3] Proteolysis by sequence-specific proteases such as trypsin is a necessary and critical step in proteomics. Conventional in-solution and in-gel digestions by trypsin under certain restricted conditions often suffer from disadvantages such as low stability of trypsin due to changes of environmental conditions, difficulties to recovery and reuse of trypsin, prolonged digestion time, and limitations for digestion of low concentration proteins.[4] With the rapid development of nanotechnology, nanomaterials can provide an attractive solution due to their high surface area to volume ratio and multiple and designable functions.[5] Various mesoporous materials with nanostructures have been explored to carry enzymes by physical adsorption, which can enhance enzyme stability, increase enzyme to substrate ratio, improve its efficiency, and facilitate separation and recovery for reuse,[6] thereby improving performance than those using free enzymes alone.[7, 8]

On the other hand, heating and microwave irradiation are widely studied as the power source for the protein digestion of enzyme-immobilized system. Although near-IR shows high potential to accelerate the digestion process, research in this filed is still in its infancy due to the lack of near-IR powered nanoreactor. As a type of important functional material, graphene and its derivatives graphene oxide (GO) have continued to draw considerable interests in both theoretical studies and practical applications in the past two decades.[9-11] By virtue of their ultrahigh surface area, excellent chemical and thermal stability, and remarkable electrical and mechanical properties, graphene has tremendous potential for applications in various fields.[12-14] Especially, graphene serves as a scaffold or substrate to form composites with other functional materials such as metals, oxides, and polymers, which has been extensively explored in biomedicine.[15] More importantly, graphene is characterized as near infrared light absorber, and it can convert light into local heating under near infrared laser radiation, which provides a new avenue for construction of smart light responsive nanostructures. Herein, we constructed a graphene-based nanoreactor for rapid near-IR assisted proteolysis.

DSIGN AND CONSTRUCTION OF NANOREACTOR

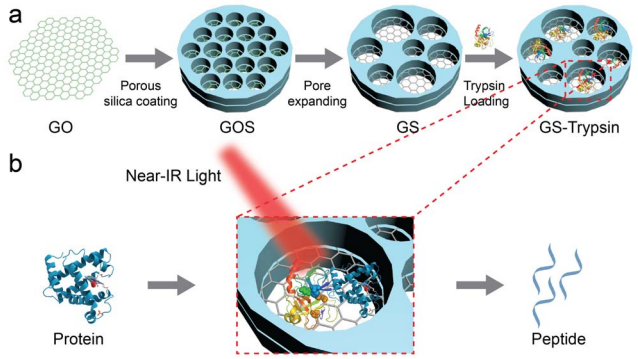

Figure 1: Schematic illustration of the construction of graphene-based enzyme system (a) and rapid photo-assisted protein digestion (b)

Figure 1a illustrates the design and construction strategy of the protein digestion reactor. Graphene oxide (GO) was used as the substrate, and a porous silica layer was assembled on both sides of graphene oxide (GOS) using the cetyltrimethylammonium bromide (CTAB) as the structure-directing agent. The nanopores were expanded and followed by reduction of graphene oxide to graphene silica composite (GS) to facilitate the loading of enzyme molecules and Near-IR absorption. The GS nanostructures were first activated via a bio mimic strategy using dopamine solution and then easily constructed by loading the trypsin

molecules, followed by purification to remove the unattached trypsin molecules.

Notably, the graphene is characterized by their excellent photothermal property. Besides contributing to the high loading of enzyme, the porous silica can provide with high surface area for protein digestion. Furthermore, under the near-IR radiation (Figure 1b), the graphene can absorb near infrared light, and convert the light into local heat to power the trypsin catalysis, resulting in easier cleavage of peptide bonds. Therefore, the rapid photo-assisted digestion can be realized by the designed graphene-based enzyme system.

EXPERIMENTAL RESULTS

Figure 2a show the scanning electron microscopy (SEM) images of the prepared graphene oxide. Apparently, the graphene oxide has a very smooth surface and it is hard to observe the layer structure due to their two dimension structure and extremely low thickness. However, after coating with the silica shell, as shown in figure 2b the thickness of the two dimension materials increase apparently, while the surface of the nanomaterials is still relatively smooth. Interestingly, the graphene-silica composites after pore expanding treatment showed coarse surface, and many nanopores could be observed, and they were randomly distributed (Figure 2c). Transmission electron microscopy (TEM) images (Figure 2d) further reveal that the presence of many small nanopores in the nanosheets, and the nanomaterials with interconnected nanopores show unique network structure.

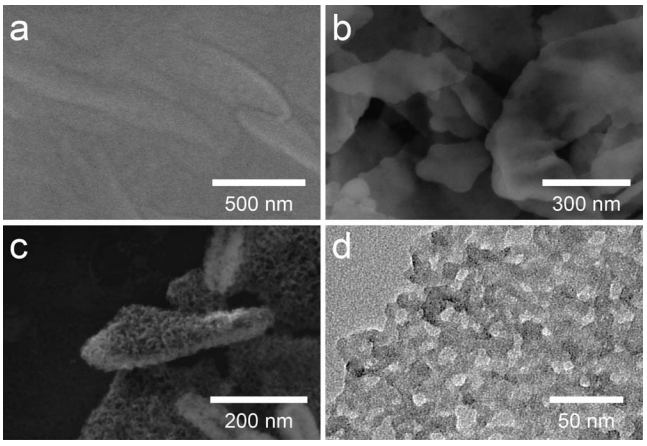

Figure 2: SEM (a-c) and TEM (d) images of graphene oxide (a), GOS (b), and GS (c and d)

The porous structure of graphene-silica composites were characterized by N_2-sorption measurement (Figure 3). The adsorption/desorption isotherms of the graphene-silica composites have type IV curves with an H2 hysteresis loop at relatively high P/P_0 according to the IUPAC classification,[16] suggesting the composites possess the porous structure. The average Brunauer-Emmett-Teller (BET) specific surface area and pore size of the graphene-silica affinity composites are calculated to be 303.6 $m^2 g^{-1}$ and 9.8 nm, respectively. Figure 3b displays the pore-size distribution derived from the adsorption branch using the Barrett-Joyner-Halenda (BJH) method. Nanopores with multiple sizes can be clearly observed, which is consist with the TEM results. It is noteworthy that the porous structure of the graphene silica composites are beneficial to enhance their performance in loading trypsin molecules and practical protein digestion owing to their high surface areas and numbers of active sites. Especially, these composites with unique architecture consist of multiple pore sizes, which would improve mass transport during catalytic reaction.[17, 18]

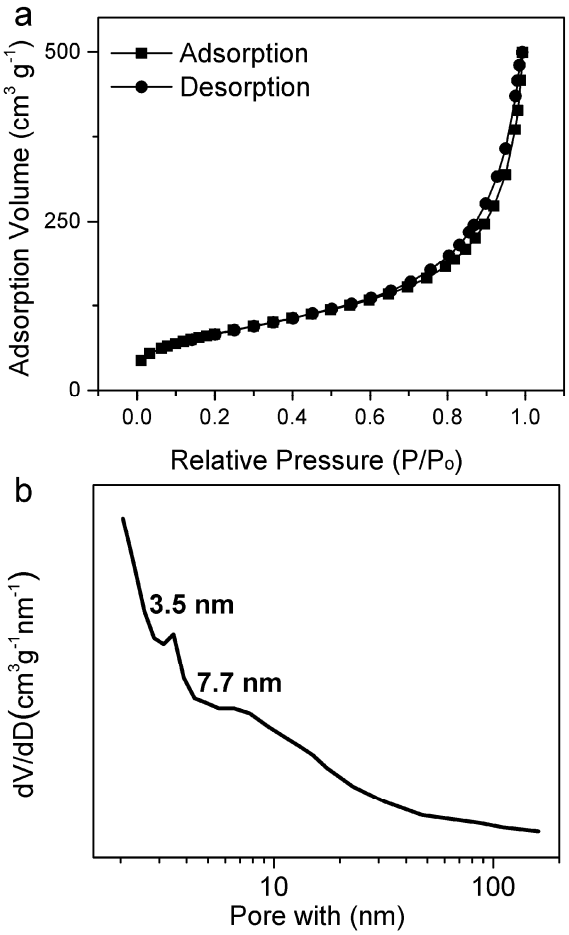

Figure 3: (a) Nitrogen adsorption-desorption isotherm and (b) pore size distribution curve of GS

The trypsin loading was recorded by a UV-vis spectrophotometer. The trypsin was effectively loaded in the graphene-silica composites, due to their relatively large pore size. As shown in Figure 4, the formation of graphene silica composites and successful construction of the protein digestion nanoreactor were confirmed using Fourier transform infrared spectroscopy (FTIR). Apparently, stretching vibrations of O-H at 3434 cm^{-1}, C=O in carbonyl groups at 1741 cm^{-1}, C=C in aromatic rings at 1631 cm^{-1},

and deformation of C-O in epoxide moiety at 1090 cm^{-1} from graphene oxide can be observed. Furthermore, the presence of strong and characteristic Si-O-Si absorption peak around 1100 cm^{-1} demonstrate the formation of silica layer on graphene. Besides the characteristic bands from graphene, the presence of new bands at around 1097 cm^{-1} from SiO_2 and 1663 cm-1, 2928 cm^{-1} and 2965 cm^{-1} from trypsin demonstrate the successful construction of graphene-based enzyme system.

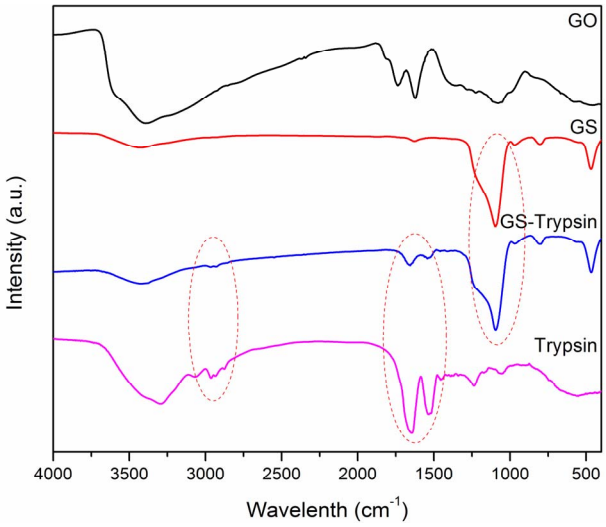

Figure 4: FTIR spectra of the graphene oxide (GO), graphene silica composites (GS), constructed nanoreactor (GS-Trypsin) and trypsin.

The novel protein digestion reactor can realize rapid proteolysis under the near-IR light irradiation, as illustrated in Figure 1b. The effectiveness of the constructed digestion nanoreactor was evaluated by digesting the model protein bovine serum album (BSA). For comparison, we also digested the model protein using the conventional in-solution digestion approach. Normally, it takes 12 hours to digest the protein effectively using the conventional in-solution digestion approach, as shown in Figure 5b. Interestingly, by taking advantage of the constructed nanoreactor, proteins can also be efficiently digested in 1 minute under radiation of near infrared laser. The mass spectrum results (Figure 5a) are comparable to that of using overnight in-solution digestion method. However, it was impossible to effectively digest the proteins in 1 minute using the in-solution digestion method (Figure 5c).

To manifest the universal application of this novel digestion nanoreactor system for various proteins, we further tested it using myoglobin and cytochrome c. As detailed in Table 1, these proteins can also be effectively digested by the constructed nanoreactor with high efficiency. According to obtained mass spectra, the proteins can be identified by coupling with the database search. For comparison, protein digestion of these proteins by the conventional overnight digestion method was also conducted. Similar results were observed, indicating that the rapid protein digestion using the constructed nanoreactor is comparable to that using overnight in-solution strategy. The above results demonstrated the high potentials of the nanoreactor system for rapid photo-assisted protein digestion.

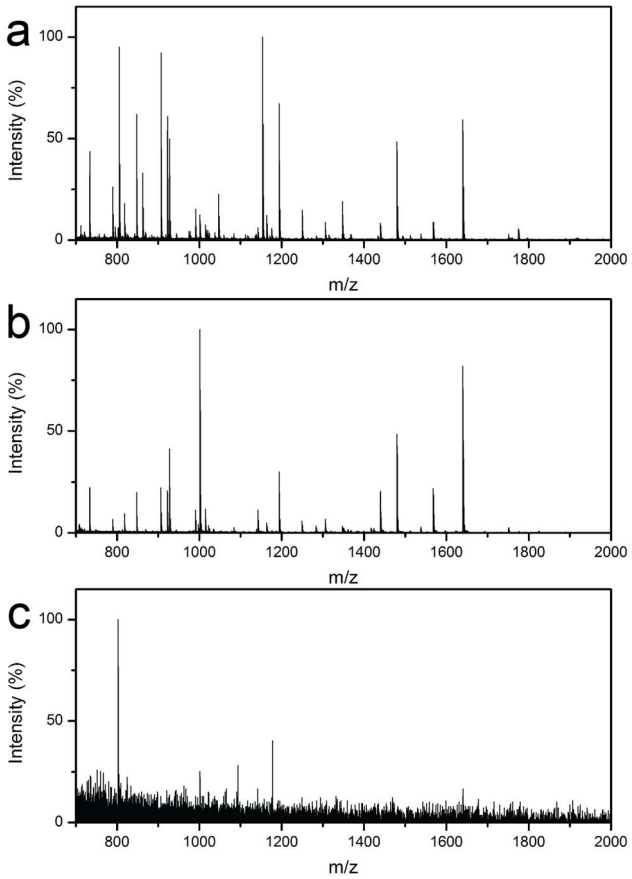

Figure 5: Mass spectra of BSA digested by 1 min photo-assisted digestion (a), 12 h in-solution digestion (b) and 1 min in-solution digestion.

Table 1: Digestion of various proteins.

Digestion method	Protein	Identified peptides	Sequence coverage
Photo-assisted digestion	Myoglobin	16	94%
	Cytochrome c	17	84%
12h in solution digestion	Myoglobin	17	94%
	Cytochrome c	17	84%

CONCLUSION

In conclusion, we have designed and constructed a new enzymatic nanoreactor with the ability to digest protein rapidly under near infrared laser radiation. The graphene based hierarchical nanostructures not only provide cavities to support the bioactive trypsin molecules but also as a light absorber and energy converter to power the digestion nanoreactor. The constructed nanoreactor can realize rapid

protein digestion in 1 minute, which is much more efficient than conventional overnight in-solution digestion using free trypsin molecules. It is expected that this novel nanoreactor would provide new chances for rapid and high throughput proteomics in biomedical applications.

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