Talanta 117 (2013) 119–126

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Microchip bioreactors based on trypsin-immobilized graphene oxide-poly(urea-formaldehyde) composite coating for efficient peptide mapping

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ARTICLE INFO

Article history:
Received 13 June 2013
Received in revised form
19 August 2013
Accepted 28 August 2013
Available online 3 September 2013

Keywords:
Microchip
Bioreactor
Graphene oxide
Proteolysis
Trypsin
Mass spectrometry

ABSTRACT

Trypsin was covalently immobilized to graphene oxide (GO)-poly(urea-formaldehyde) (PUF) composite coated on the channel wall of poly(methyl methacrylate) microchips to fabricate microfluidic bioreactors for highly efficient proteolysis. A mixture solution containing urea-formaldehyde prepolymer and GO nanosheets was allowed to flow through the channels. The modification layer on the channel wall could further polycondense to form GO-PUF composite coating in the presence of ammonium chloride. The primary amino groups of trypsin could react with the carboxyl groups of the GO sheets in the coating with the aid of carboxyl activating agents to realize covalent immobilization. The feasibility and performance of the novel GO-based microchip bioreactors were demonstrated by the digestion of bovine serum albumin, lysozyme, ovalbumin, and myoglobin. The digestion time was significantly reduced to less than 5 s. The obtained digests were identified by MALDI-TOF MS with satisfactory sequence coverages that were comparable to those obtained by using 12-h in-solution digestion. The present proteolysis strategy is simple and efficient, offering great promise for high-throughput protein identification.

1. Introduction

Proteomics is considered to be the next step after genomics in the study of biological systems. One of the most important tasks of proteomics is to develop high throughput approaches to separating and identifying a large number of proteins from a wide variety of biological sources [1,2]. In proteome research, protein digestion is an important procedure prior to subsequent mass spectrometry (MS)-based peptide mapping [3]. The typical time of the commonly used in-solution proteolysis is in the range of several hours to half a day [4,5]. It is incompatible with the high-throughput identification of proteins. Moreover, the autolysis of proteases generates some peptide fragments that may interfere with the identification of the target proteins. To solve the problems of in-solution proteolysis, proteases were immobilized on various particles, fibers, and the inner surface of microchannels to minimize their autolysis and to increase the amount of proteases during heterogeneous proteolysis [6–8].

Since the pioneering work of Manz and Harrison, microfluidic chips have received more and more attentions owing to their minimal sample/reagent consumption, high performance, portability and high degree of integration [9,10]. Microfluidic devices are powerful platforms for handling small-volume samples (nL to μL) in microchannels to perform enzymatic reactions [11], immunoassay [12], etc. Microfluidic chips can dramatically change the speed and scale of biomedical analysis and should find a wide range of applications in protein identification [13].

Proteases have been immobilized in the channels of microchips by sol–gel encapsulation [14], covalent linking [15], and adsorption [16] to fabricate microfluidic bioreactors for the rapid digestion and identification of proteins in combination with MS techniques. Because the enzymes were immobilized in microchannels, they became much more stable and highly resistant to environmental changes, providing molecular-level interactions between the immobilized proteases and the flowing protein samples. In addition, the autolysis of proteases and the amount of the interfering peptides in the digests were minimized [8].

Since Novoselov and Geim successfully isolated graphene in 2004, it has attracted tremendous scientific and technological attention because of its unique nanostructure and properties [17,18]. Graphene is an important allotrope of carbon with a two-dimensional nanostructure of sp^2-bonded carbon atoms that are arranged in a chicken wire or honeycomb pattern [19,20]. It indicates great promise for a variety of applications such as electronics, sensors, drug delivery, batteries, fuel cells, solar cells,

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0039-9140/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.talanta.2013.08.052

supercapacitors, hydrogen storage and functionalized materials because of its excellent electrical and thermal conductivity, strong mechanical strength, and high surface area [21–29].

As a chemical precursor of graphene, graphene oxide (GO) is basically a single atomic layer of carbon covered with epoxy, hydroxyl, carbonyl and carboxyl groups [30]. It can be facilely prepared by chemical oxidation of graphite and subsequent sonication exfoliation. GO has been employed to prepare nanocomposites [31], chemically modified graphenes [32], antibacterial paper [33], and conjugate with proteins [34]. Because hydrophilic GO can be well dispersed in aqueous solution and should find a wide range of applications in the fabrication of microchip bioreactors for highly efficient proteolysis. Proteases can be immobilized in microchips via GO-based materials assembled in the channels. Recently, we immobilized trypsin in the layer-by-layer coating of GO and chitosan on glass fibers by adsorption to fabricate in channel fiber bioreactors for efficient protein digestion [35]. Because GO bears a great amount of carboxyl groups that can be employed to immobilize trypsin via amide bonds, it indicates great promise for the fabrication of microfluidic bioreactors.

As a thermosetting polymer, poly(urea-formaldehyde) (PUF) is made from urea and formaldehyde by polycondensation [36]. To prepare PUF, urea and formaldehyde are usually allowed to react in basic mediums to produce water-soluble prepolymer solution. It can further polycondense to form water-insoluble crosslinked PUF network with the aid of curing catalysts such as ammonium chloride [37,38]. As a reactive polymer mixture, urea-formaldehyde prepolymer solution can be employed to prepare GO-based functionalized materials.

In this work, trypsin was covalently immobilized to the GO-PUF composite coating on the channel walls of poly(methyl methacrylate) (PMMA) microchips for efficient proteolysis. The primary amino groups of trypsin were linked to the carboxyl groups of the entrapped GO sheets in the coating via amide bonds to realize covalent immobilization. Moreover, the novel bioreactors were combined with matrix assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) for the efficient digestion and peptide mapping of bovine serum albumin (BSA), lysozyme (LYS), ovalbumin (OVA), and myoglobin (MYO). The fabrication details, characterization, feasibility, and application of the novel microchip bioreactors are reported in the following sections.

2. Experimental

2.1. Reagent and solutions

Ammonium bicarbonate (NH_4HCO_4), urea, formaldehyde, concentrated ammonia (28%), ammonium chloride, acetonitrile (ACN), graphite powder, sodium nitrate, potassium permanganate, and sulfuric acid (98 wt%) were all purchased from SinoPharm (Shanghai, China). BSA from bovine blood, LYS from chicken egg, MYO from horse heart, OVA from chicken egg, trypsin from bovine pancreas, 4-morpholinoethanesulfonic acid (MES), N-hydroxysuccinimide (NHS), trifluoroacetic acid (TFA), and α-cyano-4-hydroxycinnamic acid (CHCA) were supplied by Sigma (St. Louis, MO, USA). All aqueous solutions were prepared in doubly distilled water. Other chemicals were all analytical grade. The stock solutions of BSA, LYS, OVA, and MYO (1 mg/mL each) were prepared in water and were denatured in a 95 °C water bath for 15 min.

2.2. Fabrication of poly(methyl methacrylate) (PMMA) microchip

The PMMA microchips (16 mm × 75 mm × 2.5 mm) used in this work had a simple cross layout. They consisted of a 67 mm-long main channel and a 10 mm-long injection channel. The channels had a trapezoidal cross section with a top width of ~100 μm, a bottom width of ~40 μm, and a depth of ~35 μm. The channel plates of the PMMA microchips were fabricated by in-situ surface polymerization using a silicon template. It was sealed with PMMA cover plates (16 mm × 75 mm × 1 mm) by plasticizer-assisted

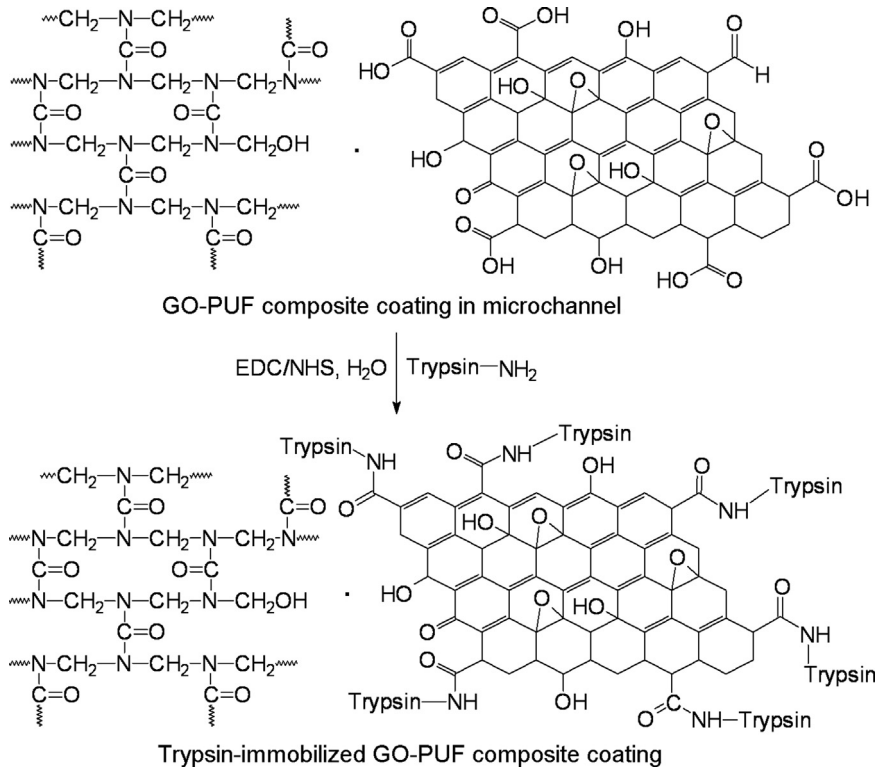

Fig. 1. Reaction routes for the immobilization of trypsin to GO sheets in GO-PUF composite.