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Novel synthesis of glucose functionalized magnetic graphene hydrophilic nanocomposites via facile thiolation for high-efficient enrichment of glycopeptides

Xiaoyan Feng, Chunhui Deng, Mingxia Gao, Guoquan Yan, Xiangmin Zhang*

Department of Chemistry and Institutes of Biomedical Sciences, Fudan University, Shanghai 200433, China

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ABSTRACT

High-efficient enrichment of glycopeptides prior to mass spectrometry is essential for glycoproteomics analysis. Hydrophilic interaction chromatography (HILIC) approach is a prominent strategy for glycopeptides identification. In this work, glucose functionalized magnetic graphene hydrophilic nanocomposite (MagG/Au/Glu) was synthesized as a novel HILIC material via a facile surface modification strategy. Different from previous click synthesis of saccharides-functionalized materials, glucose was easily thiolated via Traut's reagent and then immobilized on the materials via efficient Au-S coupling, greatly simplifying the synthesis process. Combining the rapid magnetic response, huge surface area from graphene and excellent hydrophilicity from glucose, MagG/Au/Glu nanocomposites afforded convenience of the operation and affinity for glycopeptides. Thus, the nanocomposites exhibited superior performance of high sensitivity, selectivity and reusability in glycopeptide enrichment from tryptic digests of standard glycoprotein HRP. Encouragingly, with the usage of MagG/Au/Glu nanocomposites, a total of 305 glycopeptides assigned to 108 glycoproteins were identified from complex real sample human serum, indicating a great potential for the application of glycoproteomics research.

1. Introduction

Owing to structural diversity and heterogeneity, protein glycosylation carries abundant biological information and mediates a variety of key biological processes, such as signal transduction, immune regulation and cell growth and differentiation [1–4]. More importantly, the occurrence and development of diseases are accompanied by aberrant glycosylation, including immunodeficiency, neurodegenerative diseases and cancers [5,6]. And some glycosylated proteins have proved to be disease clinical markers and therapeutic targets [7]. Thus, a comprehensive study of protein glycosylation is of vital significance. At present, although the advanced mass spectrometry (MS) technology with speediness and accuracy has been widely used in proteomics research [8,9], direct MS analysis of glycosylation still remains challenging. Due to low abundance and poor ionization efficiency, glycopeptides are severely suppressed in MS signal by non-glycopeptides [10,11]. Therefore, it is indispensable to enrich and separate glycopeptides from complex samples prior to MS analysis.

In recent years, several strategies including lectin affinity, hydrazide chemistry, boric acid affinity and hydrophilic interaction chromatography (HILIC), have been developed for the enrichment of glycopeptides [12–16]. Among them, HILIC approach, based on the difference in hydrophilicity between glycopeptides and non-glycopeptides, has been proved to be a promising approach, owing to broad glycan adaptability, non-destructiveness to glycan structure, high repeatability, simple enrichment process and good compatibility with MS identification [17,18]. With excellent hydrophilicity and similar polyhydroxy structure to the glycan, saccharides, such as glucose and maltose, showed specific affinity for glycopeptides [19]. Accordingly, saccharides-functionalized materials, possessing strong interaction with glycopeptides, could be wonderful choices for HILIC approach. Chu et al. [20] designed a glycosyl amino acid HILIC stationary phase via click chemistry and the material possessed good glycopeptide enrichment characteristics. Fe_3O_4-GO@nSiO_2-PAMAM-Au-maltose was also reported for efficient and selective enrichment of glycopeptides [21]. As in the cases above, click strategy is generally adopted for the material synthesis, including immobilization of saccharides on substrate materials and fabrication of key intermediate. However, although click reaction itself has proved to be an efficient strategy, the derivative reaction to generate intermediate alkyne-saccharides usually suffers from tedious operation, time-consuming procedure and harsh conditions [22], which restrict the operability and practicability. Hence, it is

* Corresponding author.
E-mail address: xmzhang@fudan.edu.cn (X. Zhang).

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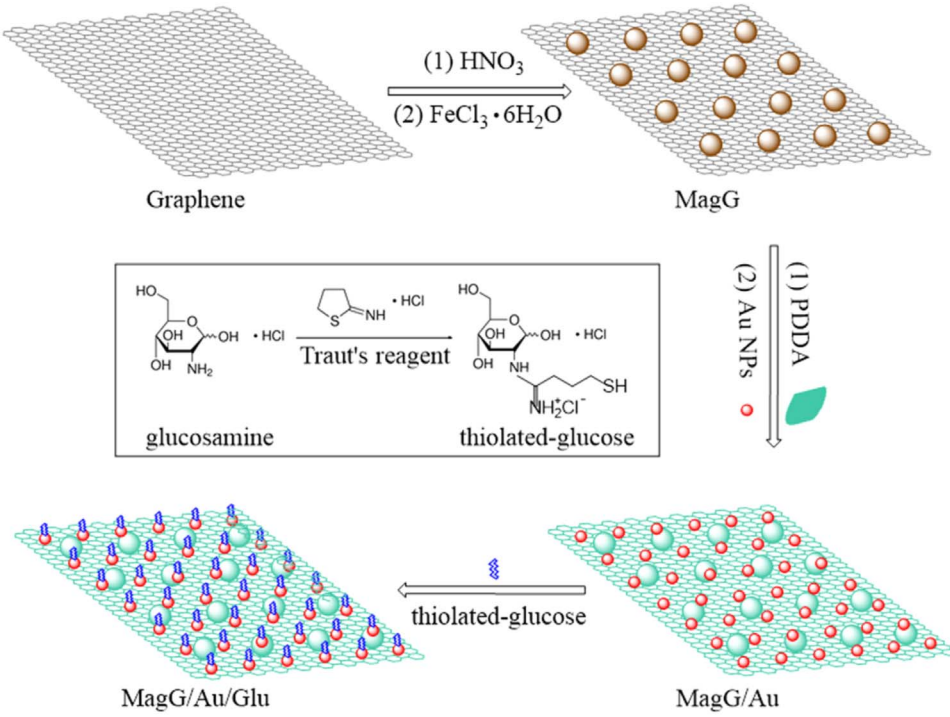

Scheme 1. Schematic illustration of the preparation procedure of MagG/Au/Glu nanocomposites.

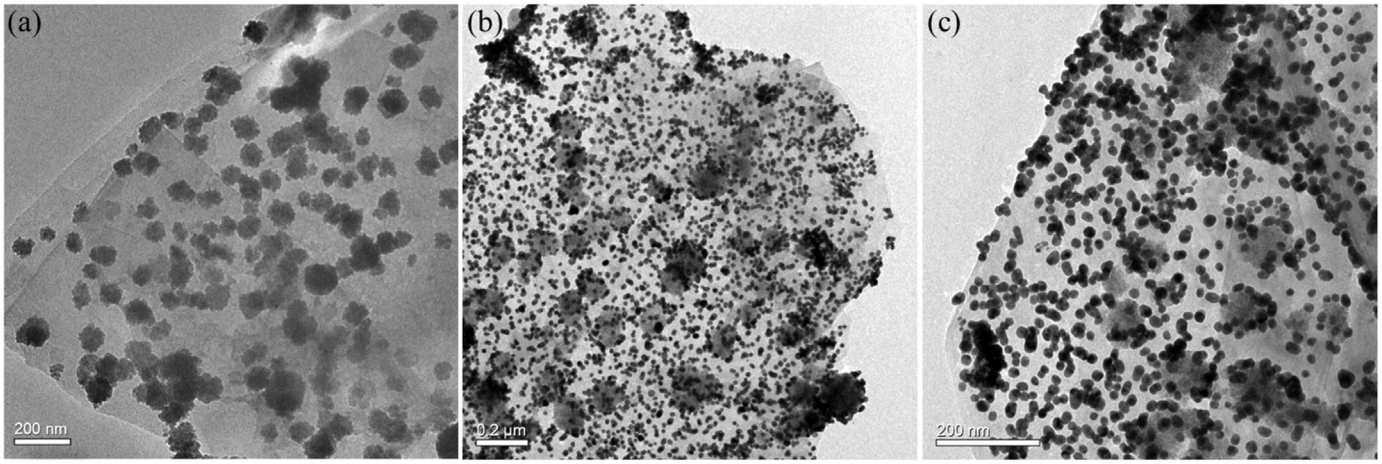

Fig. 1. TEM images of (a) MagG, (b) MagG/Au and (c) MagG/Au/Glu nanocomposites.

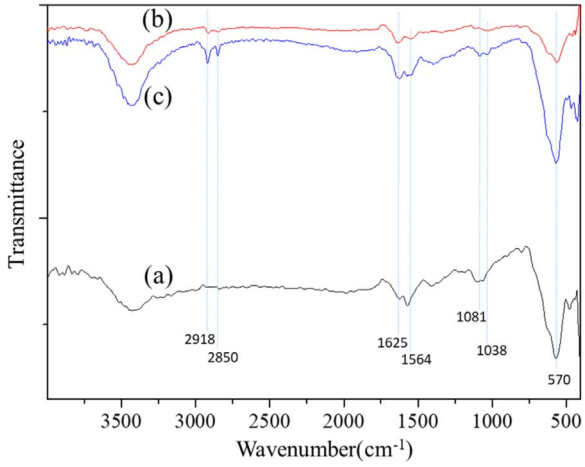

Fig. 2. FT-IR spectra of (a) MagG, (b) MagG/Au and (c) MagG/Au/Glu nanocomposites.

highly desirable to develop a facile saccharides-functionalization route of novel HILIC materials for selective enrichment of glycopeptides.

In virtue of the large surface area, rapid magnetic response and fine biocompatibility, magnetic graphene (MagG) nanocomposite has attracted considerable attention and been widely used in proteomics research. In our previous works, various functionalized MagG nanocomposites were developed and achieved excellent performances in the selective enrichment of post-translational modified peptides, implying that MagG was a favorable substrate material [23–26]. Therefore, synthesis of novel HILIC materials combing MagG nanocomposite with hydrophilic saccharides via facile procedure would remarkably improve the efficiency of glycopeptide identification.

In this work, we presented a novel and facile surface modification strategy to prepare glucose functionalized magnetic graphene hydrophilic nanocomposites (MagG/Au/Glu) and applied them to high-efficient enrichment and isolation of glycopeptides. The modification strategy involved a derivatization step by Traut's reagent which could easily convert the amino groups of glucosamine into thiol groups. And