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Selective enrichment and desalting of hydrophilic peptides using graphene oxide

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ABSTRACT

The wide variety and low abundance of peptides in tissue brought great difficulties to the separation and identification of peptides, which is not in favor of the development of peptidomics. RP-HPLC, which could purify small molecules based on their hydrophobicity, has been widely used in the separation and enrichment of peptide due to its fast, good reproducibility and high resolution. However, RP-HPLC requires the instrument and expensive C18 column and its sample capacity is also limited. Recently, graphene oxide has been applied to the adsorption of amino acids. However, the enrichment efficiency and selectivity of graphene oxide for peptides remain unclear. In this study, the adsorption efficiency and selectivity of graphene oxide and RP-C18 matrix were compared on trypsinized α-actin and also on tissue extracts from pituitary gland and hippocampus. For α-actin, there exhibit similar elution peaks for total trypsinized products and those adsorpted by GO and C18 matrix. But peptides adsorbed by GO showed the higher hydrophilic peaks than which adsorbed by C18 matrix. The resulted RP-HPLC profile showed that most of peptides enriched by graphene oxide were eluted at low concentration of organic solvent, while peptides adsorbed by RP-C18 matrix were mostly eluted at relatively high concentration. Moreover, mass spectrometry analysis suggested that, in pituitary sample, there were 495 peptides enriched by graphene oxide, 447 peptides enriched by RP-C18 matrix while in hippocampus sample 333 and 243 peptides respectively. The GRAVY value analysis suggested that the graphene oxide has a stronger adsorption for highly hydrophilic peptides compared to the RP-C18 matrix. Furthermore, the combination of these two methods could notably increase the number of identification peptides but also the number of predicted protein precursors. Our study provided a new thought to the role of graphene oxide during the enrichment of peptides from tissue which should be useful for peptidomics study.

1. Introduction

Bioactive peptide plays an important role in the growth, development, immune regulation, metabolism, and other physiological processes. Peptidome refers to all components of the endogenous polypeptide from organs, tissues, cells and humors of our body. Peptidomics is an emerging discipline of studying the structures and functions of polypeptides as well as the interrelationship between them [1–3]. Peptidomics is not only a supplement of genomics but also filled the gap between metabonomics and proteomics [4].

Given the important functions of peptides and the significant role peptidomics has taken on, peptidomics has become a hot topic of scientific research, while the first step is the extraction, separation and identification of peptides. To protect the peptides from the enzymatic degradation in the process of extraction, high temperature treatment or rapid freezing was used frequently [5–8]. In addition, organic solvent or strong acid have been also used since these treatments might prevent the degradation of proteins and peptides effectively and also improve the solubility of peptides [9]. In this study, we used the hyperthermic to inactivate the enzyme, followed by both organic solvent and acid treatment to improve the extraction efficiency for the reason that the solubility of polypeptides is different.

Except for extraction, separation and enrichment of peptides could determine the efficiency of peptide identification. In recent years, many procedures including ultrafiltration, capillary elec-

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trophoresis and high performance liquid chromatography (HPLC) have been used for seperation and enrichment. Among them, reversed phase high performance liquid chromatography (RP-HPLC), which based on different hydrophobicity, has been widely used for its fast analysis, high resolution and good repeatability [10]. However, due to the complexity of polypeptides, only a single purification method might not achieve the desired results. Therefore, the combination of different methods has shown certain advantages [8,11,12].

Graphene oxide as a novel two-dimensional material of a flake-like shape of carbon atoms, is a transparent and good conductor [13–15]. It has been used as matrix in matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF/MS) to enrich and ionize the sample of both polar compounds and non-polar compounds such as amino acids, polyamines, nucleosides, steroids [15,16]. Besides, the special structure of a single layer and sheet form makes graphene oxide a large surface area, and it can interact with peptide which could be used to detect the interaction between peptides and proteins and also used as extraction agent to purify amino acids of Glu, His, and Trp during Solid-Phase Extraction [15,17–20]. In addition, it also used as high capacity capacitor for its high conductivity of electric and heat and also applied to biosensor [21–28]. On account of its specific features of adsorption, we used graphene oxide to enrich polypeptides in this study in addition to RP-C18 matrix and then tested their adsorption and pReferences

Hippocampus and pituitary can secrete amount of active peptides and therefore used as materials in this study. Here, graphene oxide and RP-C18 matrix were used to enrich peptides from brain tissue, and followed by RP-HPLC analysis and MS/MS identification, showing that they had good adsorption of strong or weak hydrophilic polypeptide respectively with easy operation and large amount of enrichment. So the combination of two methods could effectively improve enrichment effect, thereby increasing the identification rate of polypeptide, which was an effective means for peptide enrichment from samples of large size and complexity.

2. Ethics statement

This study was carried out in strict accordance with the guidelines established by the Committee on the Use and Care of Animals at the Hunan Province, P. R. China. The protocol was approved by the Ethics Committee of the University of Hunan normal university (Permit Number: 010008). All efforts were made to minimize suffering. All the experiments were approved by and were conducted in the Committee.

3. Materials and methods

3.1. Materials

Dithiothreitol and trifluoroacetic acid were purchased from Sigma-Aldrich (St. Louis, MO, USA). Urea was obtained from SERVA (Heidelberg, Germany). RP-C18 matrix was purchased from YMC (Tokyo, Japan), α-actin with 95% purity from rabbit skeletal muscle was from Cytoskeleton Inc (Denver, CO, USA). Graphene oxide was a kind gift from Dr. Jianghui Jiang in Hunan University (Changsha, Hunan, China). Water required from experimental solution preparation and mass spectrometry was got from a Millipore Milli-Q Plus Purification System (Bedford, MA, USA). All other reagents were domestic products of the highest grade available. C57BL/6 male mice (SPF grade, age of 8 weeks) were purchased from Hunan Slack King of experimental animals (Changsha, China).

3.2. Digestion of α-actin by trypsin

α-Actin from rabbit skeletal muscle was digested by as described before [29]. Briefly, the α-actin powder was re-suspended in sterile H_2O, boiled for 5 min for denature, and reduced by dithiothreitol, then alkylated by iodoracetamide. 10 vol of 25 mM NH_4CO_3 was added and incubated with trypsin in 37 °C for 16 h the obtained peptide mixture were lyophilized and stored at −80 °C until use.

3.3. Extraction of the polypeptides

Ten adult C57BL/6 male mice were decapitated without pretreatment, pituitary glands and cerebrums were stripped out and thrown directly into physiological saline for rinse, and then the hippocampus was dissected from the brain. It is worth noting that the above experimental operations were performed on ice. Pituitary and hippocampus from five random mice were mixed and followed by parallel processing.

Four sets of samples suspending in moderate Milli-Q water were boiled in water bath for 5 min and placed to room temperature, and then grinded three times for every 3–5 min until the clots were completely homogeneous. The mixture was centrifuged at 8000g for 10 min at 4 °C. Supernatant was decanted at 4 °C and the pellets were mixed with 750 μL of either 0.25% acetic acid or 8 M urea which was freshly prepared. Tissue was homogenized in the extraction solution using sonication in ice bath followed by centrifuged at 14,000g for 30 min at 4 °C. Both the water fractions and the urea or acetic acid fractions were mixed for concentration and stored until use at −80 °C.

3.4. Peptides enrichment by graphene oxide and C18 matrix

Graphene oxide was synthesized from graphite powder according to previous reports [30,31]. The lateral size for GO used here is as following: diameter, 500 nm–5 μm; thickness, 0.8–1.2 nm; the single layer ratio is about 99%, and the purity is great than 99%. The major functional groups in GO are C—C, C—H and C═O, which are account for 44.7% and 34.7% respectively. Other functional groups such as C—O—C and COOH are account for 11.4% and 9.3% respectively. Peptides were enriched by graphene oxide reported by Dong et al. [15] using a modified procedure, as described below. Graphene oxide weighting 2 mg was rinsed successively with acetonitrile (ACN) and water and suspended in 0.3 mL of 50% methanol. After sonication for 5 min, 10 μL of the suspension was pipetted immediately into 100 μL of sample solution. The intermixture was then sonicated at ice bath for 10 min. After centrifugation at 10,000g for 10 min at 4 °C, the pellet of graphene oxide on which the peptides were attached was resuspended in 30% and 40% acetonitrile. After sonication for 20 min at ice bath, the analyte was centrifuged at 14,000g for 10 min at 4 °C. The supernatant was lyophilized and stored at −20 °C until use. At the same time, 2 mg RP-C18 matrix were rinsed with 0.3 mL acetonitrile and water, then 10 μL of C18 matrix was pipetted into 100 μL of sample solution, and the following steps were same with above description.

3.5. HPLC analysis and ultrafiltration

The obtained peptides were solubilized with Milli-Q water and separated using RP-HPLC of C18 column (4.6 × 250 mm). Briefly, eluent A was a compound of 99.9% H_2O and 0.1% TFA and B eluent was prepared with 99.9% ACN and 0.1% TFA. Column started to work after excitation by 100% ACN for 30 min and washing by 100% H_2O for 30 min with a high-pressure gradient elution of A and B. The flow rate was 0.7 mL/min when sample was separated on C18 column.