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An effective and in-situ method based tresyl-functionalized porous polymer material for enrichment and digestion of membrane proteins and its application in extraction tips

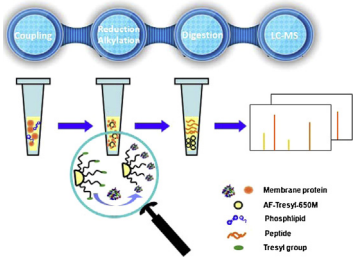
Jiaxi Wang, Mingxia Gao *, Guoquan Yan, Xiangmin Zhang *

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

HIGHLIGHTS

- The tresyl-functionalized porous polymer material has been developed for enrichment membrane proteins.
- The material can capture and digest the membrane proteins extracted from rat liver in 4% SDS or 60% methanol solution.
- The material was successfully applied in extraction tips to capture and digest the membrane proteins.
- This method is suitable for large-scale characterization of membrane proteins.

GRAPHICAL ABSTRACT



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ABSTRACT

Membrane proteins are one of promising targets for drug discovery because of the unique properties in physiological processes. Due to their low abundance and extremely hydrophobic nature, the analysis of membrane proteins is still a great challenge. In this work, an effective and in-situ method were developed to enrich and digest membrane proteins by adopting tresyl-functionalized porous polymer material. With tresyl groups, the material can effectively immobilize membrane proteins via covalent bonding on the surface. The material became a facile carrier to enrich membrane proteins from the rat liver in detergents and organic solvents owing to its outstanding binding capacity and excellent biocompatibility. Moreover, it was further applied in extraction tips to capture and in-situ digest the pretreatment membrane proteins in two different solutions. A total of 600 membrane proteins (51% of total protein groups) and 359 transmembrane proteins were identified by nano-LC-ESI-MS/MS in 4% sodium dodecyl sulfate (SDS), and similar results were achieved in the 60% methanol solution. All these results demonstrated that the new approach is of great promise for large-scale characterization of membrane proteins.

1. Introduction

Membrane proteins (MPs) perform a vital role to the survival of organism in biological and physiological processes, such as molecular transport, cell communication and signal transduction. The dysfunction of MPs is linked to deleterious human disease

* Corresponding authors. Tel.: +86 21 65643983; fax: +86 21 65641740.
E-mail addresses: mxgao@fudan.edu.cn (M. Gao), xmzhang@fudan.edu.cn (X. Zhang).

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including cancer, diabetes, cardiac disorders and so on [1]. These evidence strongly suggest MPs as an interesting molecular targets for drug discovery. Hence, studying the biological activities of MPs can facilitate us with deep insight into their roles in a cell or tissue, thereby to find a novel therapeutic way to treat the disease [2,3]. However, the identification of MPs is still puzzled by their low abundance in biomass, large molecular weight and poor solubility in aqueous solution [4,5].

Accordingly, numerous researches have been explored to enhance the solubility of MPs, usually by the way of adding detergents [6–8] and organic solvent [9–11] into sample solutions. Detergents and organic solvents can be used to improve the solubility of membrane proteins, but they always severely suppress enzymatic activity, interfere with mass spectrometry measurement, particularly subsequent liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) analysis, and generate poor MS data. In other words, the reduction or removal of reagents and interfering contaminants is of vital importance prior to digestion and MS analysis [12]. Moreover, to improve the efficiency of enriching proteins in the solution, a variety of research approaches have been developed. A lot of molecule interactions have been investigated as supports for sample loading on materials, such as van der Waals forces, electrostatic interactions and coulombic forces [13]. However, relying only on the combination of the forces mentioned above will make the progress suffer from cumbersome steps, labor-intensive and incomplete immobilization because of the relatively weaker interaction. Sorts of stronger interactions based on π–π stacking interactions, hydrophobic interactions, and covalent bonding have been extensively used in order to strengthen the forces between proteins and materials [14–20]. Many analytical approaches and strategies have been developed to facilitate membrane protein characterization. Liang and co-workers reported a new tube-gel absorption method and a gradient gel electrophoresis (GGE) system to investigate the MPs [21,22]. Peng et al. [23] recommended a similar method for purification, SDS-PAGE analysis and digestion of MPs and identification by LC-MS/MS. Despite these methods achieved some progress, there still are some problems such as significant protein loss, incomplete digestion, poor reproducibility and operation cumbersome to be solved.

Herein, we developed a distinctive method for cosmically characterization of hydrophobic membrane proteins based on the porous polymer material with tresyl group (AF-Tresyl-650M) which could effectively immobilize membrane proteins via nucleophilic substitution reaction. Tresyl functional group at the end of AF-Tresyl-650M is an effective reactive group to protein amino residues, and the immobilizing reaction would perform more facilely and efficiently under homogeneous conditions without nonspecific adsorption of proteins. In both organic solvents and aqueous solution containing SDS, the material could enrich and purify MPs effectively, which greatly expands the application range of the material. Compared with the periodic mesoporous organosilica material [20], 2.9 and 4.1 times improvement on the identified proteins and membrane proteins number were achieved (1222 vs 420, and 616 vs 149). It is worth mentioning that we developed a facile way of filling the material into the tips' head, which will successfully avoid the constant transfer of the solution and improve the efficiency of MS analysis [24,25]. Our extraction tips is easier and cheaper to fabricate. Also depending on the volume of the sample, the size of tips can be chosen. The unique property of the AF-Tresyl-650M material ensures a mild condition in different phase systems to realize the multiprocess of dissolution, enrichment, digestion and MS analysis but without cross influence (Scheme 1). In brief, we developed a high-efficiency, quick and accurate method to analyze MPs by adopting the AF-Tresyl-650M material. We believe that our approach would be of great benefit to membrane proteomics analysis.

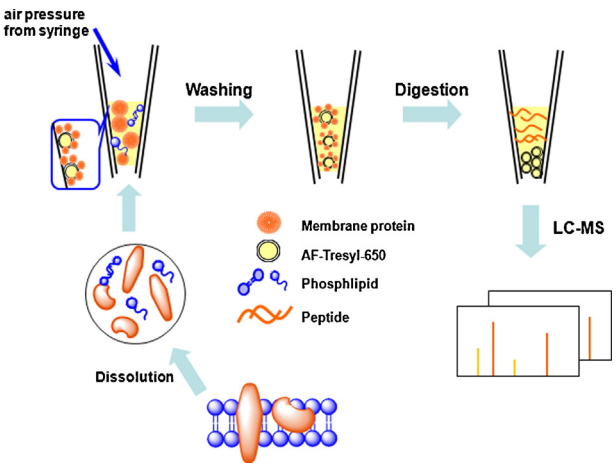
2. Material and methods

2.1. Materials

AF-Tresyl-650M was purchased from Tosoh (Shanghai, China). Acetonitrile and methanol (HPLC grade) were ordered from Fisher Scientific. Trifluoroacetic acid (TFA), Tris–HCl and α-cyano-4-hydroxycinnamic acid (CHCA), dithiothreitol (DTT), iodoacetamide (IAA), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), phenylmethylsulfonyl fluoride (PMSF) and cytochrome-C were purchased from Sigma–Aldrich (St. Louis, MO, USA). Sequencing grade-modified trypsin was purchased from Promega (Madison, WI, USA). Sodium dodecyl sulfate (SDS), NaH_2PO_4, Na_2HPO_4, $CaCl_2$, NaCl and NH_4HCO_3 were purchased from Shanghai Chemical Reagent Co. (Shanghai, China). Milli-Q water prepared by Milli-Q system (Millipore, Bedford, MA, USA) was used in all experiments.

2.2. Application of AF-Tresyl-650M to proteins enrichment in proteomics research

We firstly used a standard protein cytochrome-C (Cyt-C) (Mr = 12,400 Da, pI = 10.7) as a model. The Cyt-C standard protein was dissolved in 4% SDS accompanied by shaking. A certain amount of AF-Tresyl-650M material was mixed with the Cyt-C to optimize the factor. After being incubated at 37 °C for 2 h, the AF-Tresyl-650M material was retained by separation. Then the material was dispersed in 0.1 M Tris–HCl buffer (pH 8.0) containing 0.5 M NaCl, shaken at 37 °C for 1 h to block tresyl groups. Before the proteolysis on the surface of material, the material was washed with deionized water for five times. In order to achieve appropriate condition to digest, the material was rinsed with 25 mM NH_4HCO_3. Next, trypsin in NH_4HCO_3 buffer (25 mM, pH 8.0) was added into the above-mentioned system with a final enzyme/substrate ratio of 1:40 (w/w), and then the mixture was incubated at 37 °C for 2 h. After the digestion, the mixture was centrifuged. Then the supernatant collected was directly detected by MALDI-TOF MS analysis.



Scheme 1. Schematic illustration of the extraction tips strategy applied in efficient analysis of the membrane proteins.