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A high sensitive and contaminant tolerant matrix for facile detection of membrane proteins by matrix-assisted laser desorption/ionization mass spectrometry

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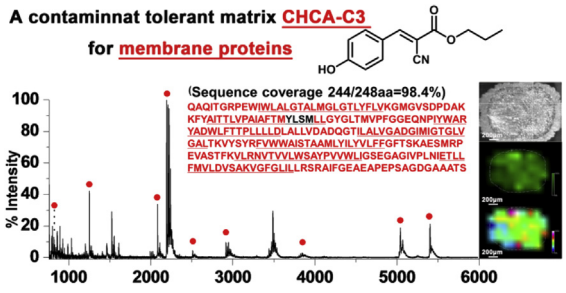
HIGHLIGHTS

- CHCA-C3 matrix improves the LOD of hydrophobic peptides 10- to 100-fold compared with traditional CHCA matrix.
- High quality spectra and uniform sample distribution can be achieved in the presence of high concentrations of contaminants.
- The mechanism of ionization-enhancing and self-desalting effect were investigated.
- CHCA-C3 matrix improves the identification in TMDs of MPs and sequence coverage (~100%).
- A combined method improves the LOD of integral MPs three orders of magnitude compared with traditional matrices.

GRAPHICAL ABSTRACT



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ABSTRACT

Despite the significance of membrane proteins (MPs) in biological system is indisputable, their specific natures make them notoriously difficult to be analyzed. Particularly, the widely used Matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) prefers analyses of hydrophilic cytosolic proteins and has a limited ionization efficiency towards hydrophobic MPs. Herein, a hydrophobic compound (E)-propyl α-Cyano-4-Hydroxyl Cinnamylate (CHCA-C3), a propyl-esterified derivative of α-cyano-4-hydroxycinnamic acid (CHCA), was applied as a contaminant tolerant matrix for high sensitivity MALDI-MS analyses of MPs. With CHCA-C3, the detection limits of hydrophobic peptides were 10- to 100-fold better than those using CHCA. Furthermore, high quality of spectra could be achieved in the presence of high concentration of chaotropes, salts and detergents, as well as human urinary and serum environment. Also, CHCA-C3 could generate uniform sample distribution even in the presence of contaminants. This high contaminant-resistance was revealed to be ascribed to the enhanced

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High sensitivity
Contaminant tolerance

hydrophobicity of CHCA-C3 with a lower affinity towards hydrophilic contaminants. The application of CHCA-C3 is further demonstrated by the analysis of trypsin/CNBr digests of bacteriorhodopsin containing seven transmembrane domains (TMDs), which dramatically increased numbers of identified hydrophobic peptides in TMDs and sequence coverage (~100%). Besides, a combined method by using CHCA-C3 with fluoride solvent and a patterned paraffin plate was established for analysis of integral MPs. We achieved a low detection limit of 10 fmol for integral bacteriorhodopsin, which could not be detected using traditional matrices such as 3,5-dimethoxy-4-hydroxycinamic acid, 2,5-dihydroxyacetophenone even at sample concentration of 10 pmol.

1. Introduction

Membrane proteins (MPs) function as important transporters, adhesion-anchors, receptors, recognition and enzymes, which are responsible for signal transduction, regulatory processes, as well as cell-cell and cell-environment communications [1,2]. A number of pandemic human diseases and cancers result from the dysfunction of MPs [3]. Despite accounting for ~1% of the protein with known three-demensional structures in the present Protein Data Bank database, MPs represents more than half of the currently approved drug targets in human medicine [4]. The recent revolutions in developing antibodies against plasma MPs further augment the extensive interest in MPs.

Mass spectrometry (MS) nowadays has become one of the most important and essential tools for exploration of the membrane proteome [5,6]. Nevertheless, we know the structure and function of MPs so far lags far behind the soluble proteins, and the characterization of MPs is still notoriously difficult and under-represented [7–9]. There are three major issues for the successful identification of MPs: (I) extremely low expression levels in comparison to cytosolic proteins; (II) highly hydrophobic nature, especially for integral MPs that have amphipathic regions; (III) high resistance to proteolysis and rare charged residues like arginine and lysine in transmembrane domains (TMDs), leading to generation of large and hydrophobic peptides which are difficult to detect in MS analysis.

To resolve the above-mentioned issues, a number of pretreatment techniques have been developed to facilitate the MS-based analyses of MPs, including using different types of additives [10–15], using purification techniques such as strong cation exchange chromatography [16] and affinity purification [17], as well as using various cleavage protocols such as CNBr and proteinase K [18], etc.

Currently, Matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) is one of the most commonly used ionization techniques and has been proven to be extremely useful for peptide mapping, structure characterization, tissue imaging and identification of protein posttranslational modifications [19,20]. However, MALDI-MS is usually problematic for analysis of MPs [21,22]. One the one hand, low ionization efficiency caused by inherently high hydrophobicity of MPs is one of the major problems for successful MALDI-MS analysis. On the other hand, the use of additives, such as chaotropes, salts and detergents, in sample solution for maintaining the activity of MPs or for facilitating their dissolution or separation are another widely criticized problem [7–9]. The presence of additives would result in poor analyte-matrix co-crystals and hamper ionization of analyte of interest, thereby causing significant decrease of signal sensitivity and reproducibility [23–25]. Thus, multiple pretreatment steps are usually required to remove these compounds, which subsequently lead to more labor, inevitable sample loss, and potential contaminant [24,25].

To address these issues, one very promising approach is to develop new matrices with improved MALDI performance [26–29]. However, a very limited number of novel matrices have been resorted to improve the signal intensity or sensitivity of under-represented MPs. For example, a cleavable combination of detergents and MALDI matrix was prepared by Norris et al., serving as detergents and matrix (upon acid hydrolysis) for improved analysis of hydrophobic proteins [30]. In 2012, Fukuyama et al. pointed out that using hydrophobic alkylated dihydroxybenzoic acid (ADHB) as matrix additive could significantly improve the analysis sensitivity of hydrophobic peptides due to the hydrophobic interactions between the alkyl chain of ADHB and peptides, however, an additional matrix and the need of finding a “hot spot” were required for hydrophobic peptides analysis [31]. Based on this finding, they further investigated alkylated trihydroxyacetophenone (ATHAP) as a hydrophobic matrix for enhanced analysis of hydrophobic peptides (10-fold sensitivity enhancement) and MPs [32,33]. Meanwhile, Ghafly et al. also found out that the signal intensities of hydrophobic peptides was strongly correlated to the hydrophobicity of GUMBOS ionic liquid matrix [34]. Therefore, all these pioneering works indicate that the increased hydrophobic affinity between matrix and hydrophobic proteins/peptides may result in improved detection of MPs.

In our previous study, an esterified CHCA derivative, (E)-propyl α-cyano-4-hydroxyl cinnamylate (CHCA-C3), was reported as a contaminant tolerant MALDI matrix for improved profiling of intact protein molecules [35]. In view of the hydrophobic nature of CHCA-C3, we thus launched a systematically study on using CHCA-C3 as a matrix for analysis of MPs. Compared with the conventional matrix CHCA, the limits of detection (LODs) for hydrophobic peptides using CHCA-C3 matrix were improved by one to two orders of magnitude. High quality of spectra and homogeneous sample distribution were achieved even in the presence of high concentrations of various contaminants or complex surroundings (e.g., urea, NaH_2PO_4, human urine). To our knowledge, a matrix possessed both high sensitivity and contaminant-resistant character has not been reported before in MPs analysis. Moreover, the possible mechanisms for the ionization-enhancing effect and contaminant-resistance of CHCA-C3 were also investigated. Then, the application of CHCA-C3 was further demonstrated by the successful analysis of both digested and intact bacteriorhodopsin (BR, containing seven TMDs).

2. Materials and methods

2.1. Chemicals and materials

The details are shown in the supporting information.