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Isolation of Cytochrome C for Proteomics with Lindqvist-type Polyiodate Modified Metal Organic Framework

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ABSTRACT

Separation and purification of Cytochrome C (Cyt-C) is important for proteomic. High efficient and selective pretreatment method for Cyt-C in real samples are always needed. Herein, polyniobate ($K_7H[Nb_6O_{19}]\cdot 13H_2O$, Nb_6O_{19}) is modified on a metal-organic framework MIL-125(Ti) through intermolecular hydrogen bonds and an aqueous-stable composite Nb_6O_{19}/MIL-125(Ti) is successfully prepared to pretreat complex protein sample. Protein adsorption studies have shown that Nb_6O_{19}/MIL-125(Ti) can promote the selective adsorption of Cyt-C due to the synergistic effect of electrostatic and hydrogen-bond interactions. At pH=10.0 (Britton-Robinson buffer), the adsorption efficiency of 300 μL 100 $\mu g\cdot mL^{-1}$ Cyt-C onto 1.0 mg Nb_6O_{19}/MIL-125(Ti) can reach 99.5%. The adsorption behavior of Cyt-C fits well with the Langmuir adsorption model, corresponding to a maximum theoretical adsorption capacity of 168.35 $mg\cdot g^{-1}$. Using 3 $mol\cdot L^{-1}$ NaCl as the eluent, a high elution efficiency of 92.19% is obtained. In addition, the results of the sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis confirm that Nb_6O_{19}/MIL-125(Ti) efficiently adsorbed Cyt-C from scrofa heart extraction. LC-MS/MS spectrometry results show that the purification of Cyt-C reduces the abundance from the 12th to the 154th place after Nb_6O_{19}/MIL-125(Ti) treatment. Moreover, low abundant proteins, e.g., Superoxide dismutase 1, IF rod domain-containing protein and Ubiquitin-60S ribosomal protein L40 were considerably enriched. These outcomes confirm the practicability of Nb_6O_{19}/MIL-125 (Ti) as a Cyt-C extractant has potential application value in scrofa heart proteomics.

1. Introduction

Cytochrome C (Cyt-C) is a kind of electron transfer for cellular respiration, in which ATP is produced to sustain cells alive [1]. Abnormal release of Cyt-C from mitochondria to cytoplasm cause cancer, liver/nerve injuries and other diseases [2]. Therefore, the quantitative analysis of Cyt-C is an important subject of proteomics. Before analysis of Cyt-C in real samples, separation/purification and enrichment of Cyt-C are always essential for two reasons. On the one hand, the content of Cyt-C is lower than the detection limit of usually used methods. On the other hand, complex matrix in bio-samples interfere the analysis of Cyt-C seriously. Besides, after removal of Cyt-C, other low-abundance proteins in bio-samples that can hardly been detected in the presence of Cyt-C can be identified easily. Hence, exploring new method or new materials for isolation of Cyt-C are of great importance for proteomics [3]. Cimen et al. The PHEMAH cryogel was complexed with the Cu^{2+} ions directly via MAH for the adsorption of Cyt-C from aqueous solutions and rat liver homogenate [4]. Zhang et al. Epitope molecularly imprinted polymers (EMIPs) were synthesized using a novel cyclodextrin-based ionic liquid as functional monomer for specific recognition of Cyt-C in aqueous solution via the combination of epitope and surface imprinting approach [5]. However, these methods are costly and time-consuming, and the lower adsorption capacity of Cyt-C is obtained.

Solid-phase extraction using nanomaterials or nano-composites provides efficient approaches for isolation of proteins. Bharath

Abbreviations: BR, Britton-Robison; Cyt-C, cytochrome c; BSA, bovine serum albumin; Mb, myoglobin; POMs, polyoxometalates; PONbs, Polyoxoniobates; MOFs, Metal-organic frameworks; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

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