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Analytica Chimica Acta 561 (2006) 32–39
ANALYTICA CHIMICA ACTA
www.elsevier.com/locate/aca

Chemically modified carbon nanotubes as material enhanced laser desorption ionisation (MELDI) material in protein profiling

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Received 29 September 2005; received in revised form 27 December 2005; accepted 5 January 2006

Abstract

Biomarkers play a potential role in the early detection and diagnosis of a disease. Our aim is to derivatize carbon nanotubes for exploration of the differences in human body fluids e.g. serum, through matrix assisted laser desorption ionisation/time of flight mass spectrometry (MALDI/TOF-MS) that can be related to disease and subsequently to be employed in the biomarker discovery process. This application we termed as the material enhanced laser desorption ionisation (MELDI). The versatility of this technology is meant to increase the amount of information from biological samples on the protein level, which will have a major impact to serve the cause of diagnostic markers.

Serum peptides and proteins are immobilized on derivatized carbon nanotubes, which function as binding material. Protein-loaded suspension is placed on a stainless steel target or buckypaper on aluminum target for direct analysis with MALDI-MS. The elution method to wash the bound proteins from carbon nanotubes was employed to compare with the direct analysis procedure. Elution is carried out by MALDI matrix solution to get them out of the entangled nanotubes, which are difficult to desorb by laser due to the complex nanotube structures. The advantage of these optimized methods compared to the conventional screening methods is the improved sensitivity, selectivity and the short analysis time without prior albumin and immunoglobulin depletion. The comparison of similarly modified diamond and carbon nanotubes exhibit differences in their nature to bind the proteins out of serum due to the differences in their physical characteristics. Infrared (IR) spectroscopy provided hint for the presence of tertiary amine peak at the crucial chemical step of iminodiacetic acid addition to acid chloride functionality on carbon nanotubes. Atomic absorption spectroscopy (AAS) was utilized to quantitatively measure the copper capacity of these derivatized carbon nanotubes which is a direct measure of capacity of this material to bind the number of proteins from serum.

Keywords: Carbon nanotubes; MALDI; Protein profiling; Elution; IMAC; Biomarker

1. Introduction

The discovery of "multi-walled carbon nanotubes (MWNT)" by Iijima [1] and of "single-walled carbon nanotubes (SWNT)" independently by Bethune et al. [2] has given a new biologically important material in addition to the newly developed materials e.g. cellulose [3], silica [4] and poly(glycidyl methacrylate/divinylbenzene) beads [5], to be used in proteomic research. The unique physical and chemical properties with a diameter in the range of nanometers have caused great research interest in this nanofield. Nanoscale field effect transistor devices with carbon nanotubes as the conducting channel are used to detect protein–protein binding [6]. In recent studies, functionalised carbon nanotubes are reported to be used as the MALDI matrix [7–10] for the study of micromolecules as well as macromolecules. This whole scenario of the effective biocompatibility of carbon nanotubes has emerged an idea to use this material as a material enhanced laser desorption ionisation (MELDI) support material. The main aim of this study is to provide a rapid and sensitive protein profiling material and method, leading towards the identification of disease markers from a variety of biological samples.

The classical approach for discovering disease-associated proteins continues to be the two-dimensional polyacrylamide

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doi:10.1016/j.aca.2006.01.012

gel electrophoresis (2D-PAGE) [11,12]. Although 2D-PAGE is unchallenged in its ability to resolve thousands of proteins, it is laborious, requires large quantities of protein, lacks critical reproducibility standards from one laboratory to another and is not easily converted into a diagnostic test. Recent advances in mass spectrometry (MS) and multidimensional liquid chromatography (MDLC) combined with tandem mass spectrometry (MS/MS) [13,14], already offer an alternative to or an interface with two-dimensional electrophoresis for the simultaneous detection and identification of multiple protein species, like matrix-assisted laser desorption ionisation/time of flight mass spectrometry (MALDI/TOF-MS) [15–19]. There have been many examples of the use of MALDI for the determination of disease biomarkers, with the primary focus being diagnostics for all forms of cancer [20–22]. Compared to other MS-applications, the MELDI-technology is much handier and timesaving regarding the sample loading, measurements and allows high throughput analysis with robotic systems. Therefore, the development and improvement of recent techniques based on carbon nanotubes materials for protein profiling and biomarker discovery is underscored.

The carbon nanotubes (CNT) have been functionalised as an immobilized metal ion affinity chromatography (IMAC) support, to be used as an excellent material for the selective binding of bio-molecules from a complex sample and the further analysis with MALDI/TOF-MS. For this approach iminodiacetic acid (IDA) is attached to CNTs and loaded with copper(II) ions. To investigate the validity and suitability of the derivatized CNTs for protein profiling, human serum samples are used.

The material enhanced laser desorption ionisation (MELDI) technology based on carbon nanotubes, is meant to increase the capacity of proteins bound and subsequently to use in the biomarker studies. It provides very characteristic information based on the specific nature of carbon nanotubes.

2. Materials and methods

2.1. Materials and reagents

Carbon nanotubes were supplied by Nanocyl S.A. Belgium. Iminodiacetic acid (IDA, 98.0%), acetonitrile (for HPLC, ≥99.9%), urea (≥99.5%), 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS, ≥98.0%) and sinapinic acid (SA, ≥99.0% pure) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Trifluoroacetic acid (TFA, ≥99.5% pure) was obtained from Fluka (Buchs, Switzerland). Serum samples were provided by the Department of Urology, at the Medical University of Innsbruck, Austria.

2.2. Derivatization of the carbon nanotubes

The derivatization of carbon nanotubes is a three-step procedure. In the first step the carboxylic groups, which are obtained after purification with oxidizing agents [23,24], are chlorinated with thionyl chloride at 70 °C for 24 h [25]. The excess is removed by vacuum distillation. The next step of amination of the resulting acid chlorides with iminodiacetic acid (IDA) at 70 °C for 90 h in the presence of triethyl amine as a base. The solvent and the excess of IDA are removed by centrifugation and washing with dichloromethane, ethanol and water. In the final step, the carbon nanotubes are loaded with copper(II) ions by putting them into a 100 mM $CuSO_4$ solution for 2 h. During this loading step the iminodiacetic acid forms a bidentate complex with the copper(II) ions. The above-mentioned chemical steps for derivatization of carbon nanotubes are shown in Fig. 1.

2.3. Serum sample preparation

Forty microlitres of serum were shaken with 30 μl of 8 M urea containing 1% 3-[(3-cholamidopropyl)dimethylammonio]-

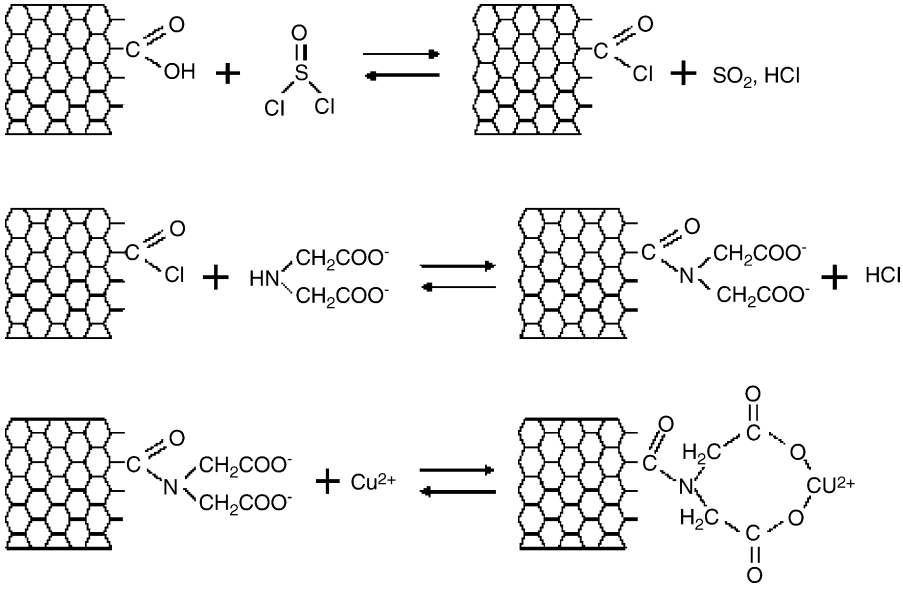

Fig. 1. Chemical steps to derivatize the oxidized carbon nanotubes to get IDA–Cu^{2+} system.