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ORIGINAL ARTICLE

Derivatized graphitic nanofibres (GNF) as a new support material for mass spectrometric analysis of peptides and proteins

Andreas Greiderer · Matthias Rainer · Muhammad Najam-ul-Haq · Rainer M. Vallant · Christian W. Huck · Günther K. Bonn

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Abstract Graphitic nanofibres (GNFs), 100–200 nm in diameter and 5–20 μm in length have been modified in order to yield different affinities (Cu^{2+} and Fe^{3+} loaded immobilized metal affinity chromatography (IMAC) as well as cation and anion exchange materials) for the extraction of a range of biomolecules by their inherited hydrophobicity and the hydrophilic chemical functionalities, obtained by derivatization. Modified GNFs have for the first time been employed as carrier materials for protein profiling in material-enhanced laser desorption/ionization (MELDI) for the enrichment and screening of biofluids. For that purpose, the derivatized GNF materials have comprehensively been characterized regarding surface area, structural changes during derivatization, IMAC, as well as ion exchange and protein-loading capacity and recovery. GNF derivatives revealed high protein-binding capacity (2,000 μg ml^{-1} for insulin) and ideal sensitivities, resulting in a detection limit of 50 fmol μl^{-1} (for insulin), which is crucial for the detection of low abundant species in biological samples. Compared to other MELDI carrier materials, sensitivity was enhanced on GNF derivatives, which might be ascribed to the fact that GNFs support desorption and ionization mechanisms and by absorbing laser energy in addition to matrix.

Keywords Nanomaterials · Graphitic nanofibres (GNF) · Derivatization · Mass spectrometry (MS) · Matrix-assisted laser desorption/ionization (MALDI) · Material-enhanced laser desorption/ionization (MELDI) · Protein profiling

A. Greiderer · M. Rainer · M. Najam-ul-Haq · R. M. Vallant · C. W. Huck (✉) · G. K. Bonn
Institute of Analytical Chemistry and Radiochemistry, Leopold-Franzens University, Innrain 52a, 6020 Innsbruck, Austria
e-mail: christian.w.huck@uibk.ac.at

Introduction

Proteomics, including biomarker discovery as one of its most important tasks and spheres of activity, is nowadays regarded as the key to obtaining more insight into fundamental biological processes and relationships in order to facilitate the development of new drugs and disease diagnosis (Xiao et al. 2001; Li et al. 2002).

The successful establishing of proteome research as an independent scientific domain was closely associated with the introduction and improvement of mass spectrometry with efficient ionization techniques. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI–TOF–MS) (Hillenkamp et al. 1991; Aebersold and Mann 2003; Reyzer and Caprioli 2005) and electrospray ionization mass spectrometry (ESI–MS) (Whitehouse et al. 1985) are both routinely employed, as they are characterized as "soft ionization" methods, allowing mass spectrometric investigation of high molecular weight and thermally labile bioanalytes, like proteins and peptides with high efficiency, high sensitivity and low fragmentation. MALDI–TOF–MS, in particular, has been established as a powerful technique for protein investigations from various types of biological samples, like tissues (Avasarala et al. 2005) or serum (Petricoin et al. 2002; Adam et al. 2002), as the upper mass detection limit has been shifted to over 300 kDa. These protein investigations, which are also referred as "protein profiling" or "protein mass fingerprint" techniques, employ a selective preconcentration and enrichment step prior to MS analysis in order to reduce the sample

complexity and allow the analysis of low-abundant species in the sample (Merrell et al. 2004).

However, protein profiling has been reported to suffer from methological and bioinformatic artefacts, which severely question the validity and reproducibility of the obtained results regarding biomarker evaluations (Diamandis 2004). Due to this reason, the characterization and investigation of new support materials for selective enrichment of serum peptides and proteins, combined with their mass spectrometric analysis is regarded to be essential to improve selectivity as well as sensitivity in biomolecule analysis. This approach, which has been introduced as material-enhanced laser desorption/ionization MELDI–MS (Feuerstein et al. 2005, 2006; Rainer et al. 2006, 2007), does not only focus on the binding properties of the materials with respect to their affinity, but considers the carrier morphology and structure in addition, which has been shown to provide advantages regarding signal intensities as well as reproducibility. In this context, nanomaterials are regarded to have promising structural characteristics for improved application in protein profiling and subsequent analyte identification, as has recently been demonstrated for C_{60} fullerene derivatives (Vallant et al. 2007) as well as other related MELDI nanomaterials, like derivatized carbon nanotubes (CNT) (Najam-ul-Haq et al. 2006a, b) and nano crystalline diamond (NCD) (Najam-ul-Haq et al. 2006a, b). The design and development of nano-structured materials, being surface-derivatized with carrier proteins (like that of serum albumin), for specific accumulation of low-molecular weight compounds have been shown to positively contribute to biomarker discovery (Geho et al. 2004), particularly since low-molecular weight molecules, present in biofluids, are supposed to be related to cancer and other disease patterns, being thus of potential clinical utility for therapeutic intervention (Zhang et al. 2004).

Different nano-materials such as colloidal gold (Kirk and Bohn 2004), gold nanoparticles (Teng et al. 2004) and magnetic nanoparticles (Ho et al. 2004) have already been successfully developed for MALDI–MS sample preparation and offer new opportunities for proteomic-based biomarker discovery. Nanowires and nanocantilever arrays provide promising vectors for targeted delivery of anticancer drugs and imaging contrast agents and allow the detection of precancerous and malignant lesions from biological fluids (Ferrari 2005). Graphitic nanofibres (GNF), obtained by catalytic chemical vapor deposition (C-CVD), generally possess a diameter of 100–200 nm, a length of 5–20 μm and typically exhibit cylindrical and conical structures, being ascribed by stacked curved graphite layers that form cones or cups (Melechko et al. 2005). This exceptional structural constitution causes GNF carbon materials to possess extraordinary electrical, mechanical and thermal properties, which might advantageously influence the adsorption of organic compounds onto the carrier material (Cuervo et al. 2008) as well as the desorption/ionization process in MALDI–TOF–MS and improve the energy-transfer efficiency (Guo et al. 2006).

In this work, we report on the synthesis of different graphitic nanofibre derivatives and their application to specific binding of peptides and proteins from human serum samples, followed by the direct analysis of bound serum constituents with MALDI–TOF–MS for mass pattern analysis. For that purpose, the GNF derivatives were comprehensively characterized regarding their physical, morphological, structural properties as well as protein binding and elution properties. Several examples prove the successful application of the derivatives as MELDI carrier material for protein profiling.

Experimental

Chemicals and Reagents

Acetic acid (100%), acetone (≥99.5%), acetonitrile (G chromasol V HPLC ≥ 99.9%), 6-aminocaproic acid (>99.0%), argon (99.9%), calcium chloride dehydrated purum (≥97.0%), carbon tetrabromide (≥97.0%), copper(II)-sulfate (p.a. ≥99%), dichloromethane (≥99.0%), ethanol (≥99.0%), ethylenediaminetetraacetic acid disodium salt dihydrate (99.0%), graphite nanofibres (95%), hexane (≥99.0%), hydrochloric acid (37% fuming), iminodiacetic acid (≥99%), iron(III) chloride (≥99.0%), malonyl dichloride (≥97.0%), methanol (G Chromosol V ≥ 99.9%), nitric acid (≥65.0%), potassium dihydrogenphosphate (≥99.0%), di-potassium hydrogenphoshate (≥99.0%), ortho-phosphoric acid (≥85.0%), sodium acetate (≥99.0%), sodium carbonate (≥99.9%), sodium chloride (p.a. ≥99.5%), sodium hydroxide (≥99.0%), sodium sulfate anhydrous (≥99%), sulfuric acid (98%), tetrahydrofuran (≥99.5%), thionyl chloride (≥99+ %), toluene (≥99.0%), triethylamine p.a. (≥99.5%), tris(hydroxymehyl) amino mehtane hydrochloride buffer substance pH 8.0, trifluoroacetic acid (≥99.5%), sinapinic acid (SA, ≥99.0% pure) and α-Cyano-4-hydroxycinnamic acid (HCCA, matrix substance for MALDI–MS, ≥99.0%) were obtained from Fluka (Buchs, Switzerland). Serum samples were provided by the Department of Urology at Medical University of Innsbruck, Austria. All chemicals were used as received. Water was purified using a NANOpure Infinity unit (Barnstead, Boston, MA, USA).

Derivatization of graphitic nanofibres (GNF)

Acid chloride formation

Fibres (0.5 g) were oxidized with a 50-ml of H_2SO_4/HNO_3 mixture (1:1 v/v) for 120 min at 120°C to result in carboxylic acid functionalities at sp^2 C–H edges of the graphitic layers (Tijmen et al. 2002). After filtration of the residue, washing with water and drying under vacuum, the oxidized GNFs were reacted with thionylchloride (15 ml) at 85°C for 12 h under argon. Finally, the excess of thionylchloride was removed by vacuum distillation.

Derivatization with iminodiacetic

GNFs with acid chloride functionalities (0.4 g) were suspended in a solution of 1.5 g iminodiacetic acid (IDA), having been dissolved in 25 ml THF and 50 µl Et_3 N and refluxed over night under argon to create GNF-IDA. After filtration of the product and washing with a 0.2 M Na_2CO_3 solution and water, GNF-IDA was loaded with Cu^{2+} or Fe^{3+} ions followed by stirring in a 100 mM $CuSO_4$ or 100 mM $FeCl_3$ solution for 2 h at room temperature.

Derivatization with 6-aminocaproic-acid

The cation exchanger material was synthesized by treating the acid chloride material (0.4 g) with 1 g of 6-aminocaproic acid, being dissolved in 25 ml dry THF, containing 50 µl Et_3 N at 82°C under argon over night. The residue was washed thoroughly in a sintered-glass filter with deionized water.

Derivatization with triethylamine

To obtain GNF-$N^+Et_3OH^-$ as an anion exchange material, the acid chloride (0.4 mg) was treated with (25 ml) Et_3 N for 24 h under reflux and under argon. The excess Et_3 N was removed by distillation and the residue finally washed with 1 mM NaOH. The reaction scheme of all GNF derivatizations, together with the reaction conditions is shown in Fig. 1.

Characterization of derivatized GNF materials

Morphological and structural studies of derivatized GNFs were performed by scanning electron microscopy (SEM), using a JSM-5310LV Scanning Microscope (JOEL LTD, Tokyo, Japan), by infrared spectroscopy, employing a attenuated totally reflection fourier transform infrared (ATR-FTIR) equipment (Nicolet 5700, Thermo Electron Corporation, MA, USA) in the range of 500–4,500 cm^{-1} and by BET measurements on a 212 Perkin–Elmer Shell sorptiometer.

Continuing characterization studies of the GNF derivatives were accomplished by the determination of the Cu^{2+} content of GNF–IDA–Cu^{2+} by atomic absorption spectroscopy (AAS) on a PU9100X atomic absorption spectrometer (Philips, Einhoven, The Netherlands), employing external calibration with $CuSO_4$ in the range of 0.01–0.06 mM and by quantification of the acid and the base capacity of GNF–C_6H_{12}–COO^- and GNF–NEt_3^+, respectively, by pH-measurements (MP 220 pH-meter, Mettler Toledo, Columbus, OH, USA) after a 2-point calibration (pH 10.0 and 4.0) at 20°C.

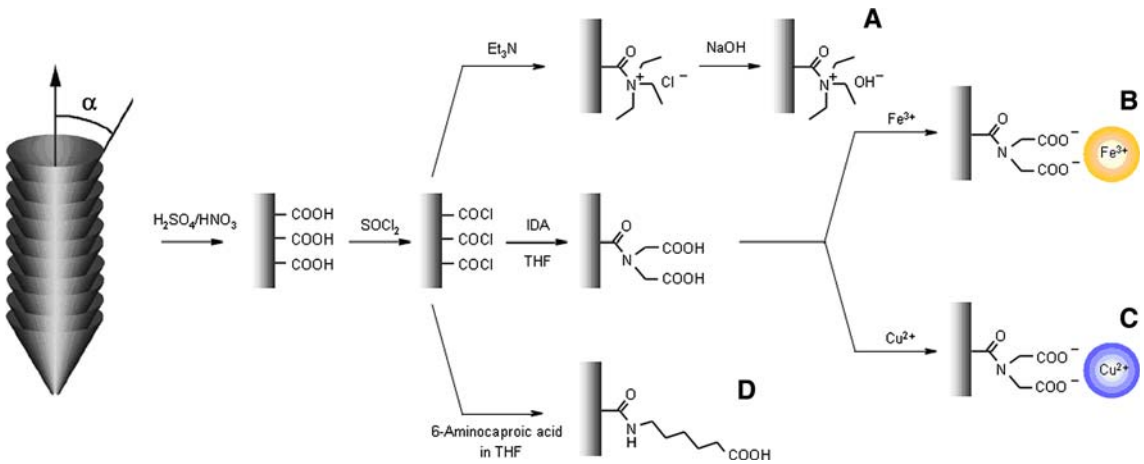

Fig. 1 Reaction scheme for modified GNF-materials for selective protein enrichment. **a** GNF–$N^+Et_3OH^-$; **b** GNF–IDA–Fe^{3+}; **c** GNF–IDA–Cu^{2+} and **d** GNF–C_6H_{12}–COO^-H^+. Oxidation procedure and thionylchloride treatment is same for all these four synthetic procedures

The determination of the protein-loading capacity of GNF-IDA-Cu^{2+} with insulin was performed as previously described (Rainer et al. 2006). The adsorbed amount of substance was quantified by HPLC, using a Bischoff HPLC system (Leonberg, Germany), composed of a HPLC compact pump, a CSI 6150 degasser and a LAMBDA 1010 UV/VIS photodiode array detector Bischoff and a spherical silica gel Hypersil column (100 × 4.6 mm I.D., ODS, 3 µm) from Sigma–Aldrich (St Louis, MO, USA). The data obtained were interpreted with McDAcq 32 Control Center, V. 2.4.634 software from Bischoff Chromatography.

Sensitivity study

Standard solutions containing insulin, ubiquitin, lysozyme and myoglobin at different concentrations were prepared to test the sensitivity of the MELDI approach on derivatized GNF. After equilibration with 200 µl 0.1% TFA, 1 mg of GNF–IDA–Cu^{2+} was incubated with 200 µl of the respective protein mixture solution (50, 100, 200, 400, 600, 800, 1000 fmol μl^{-1} each protein) for 60 min at room temperature, followed by washing thrice with 200 µl 0.1% TFA and once with 200 µl straight water. One microlitre of the protein loaded GNF–IDA–Cu^{2+} slurry was spotted on a stainless steel target (Bruker Daltonics, Bremen, Germany) and an equal amount of α-cyano-4-hydroxycinnamic acid (HCCA) [saturated solution in ACN/water (1:1 v/v), containing 0.1% TFA] as matrix was added before MALDI–TOF–MS evaluation.

Serum sample preparation and analysis

For incubation on GNF–IDA–Cu^{2+}, GNF–IDA–Fe^{3+} as well as GNF–C_6H_{12}–COO^-, 80 µl of raw serum were first diluted with 120 µl of a 0.05% TFA solution. In case of the anion exchange material, dilution of the raw serum was performed with equal volume of tris-buffer (pH = 8.0). 2 mg of derivatized GNF material was weighed into a 0.5 ml micro-centrifuge tube, activated with 200 µl of a sodium acetate buffer (pH = 4.0) and equilibrated two times with 200 µl 0.05% TFA in the case of the IMAC materials and GNF–C_6H_{12}–COO^-. GNF–NEt_3^+, on the other hand, was equilibrated with the same volume of tris-buffer (pH = 8.0). Two hundred microlitres of the diluted serum sample was added to the equilibrated material and the mixture incubated on a platform shaker (Thermomixer comfort, Eppendorf, Hamburg, Germany) at 1,500 rpm for 90 min at 25°C. To remove unbound proteins and peptides, GNFs were washed three times with 200 µl 0.05% TFA in case of the IMAC materials and GNF–C_6H_{12}–COO^- and with 200 µl pH 8 tris-buffer in case of GNF–NEt_3^+, followed by an additional washing step with deionized water. Finally, 1 µl of the incubated and washed GNFs was spotted onto a MTP 384 ground steel target and 1 µl of the matrix substance solution was added. Throughout the whole study, α-cyano-4-hydroxycinnamic acid (HCCA) was employed as matrix substance, as preliminary test series revealed the supremacy of HCCA over sinapinic acid (SA) in terms of signal intensity as well as spectrum quality and reproducibility. The spectral quality and sweet spots were improved when mixing the loaded GNFs and matrix before letting them dry on the MALDI-target. Proteins and peptides, being loaded on GNF materials were directly analysed by MALDI–TOF–MS (Ultraflex MALDI–TOF–TOF, Bruker Daltonics, Bremen, Germany), employing the linear mode and a detector energy of 1,677 V. Data were collected by averaging 400 laser shots in the mass regions from 2 to 12 kDa. Flex Control V 2.0 (Bruker Daltonics) was used for parameters control during recording; Flex Analysis V. 2.0 (Bruker Daltonics) was used for data evaluation and interpretation.

Results and discussion

Characterization of derivatized GNFs

Oxidation of GNFs

IR spectroscopy revealed the success of the derivatization reactions of GNFs. Changes in the infrared spectra have been obtained after treatment of GNFs with H_2SO_4/HNO_3 in the region of 1,716 cm^{-1}, which belongs to C=O stretching vibration (oxidation process). Moreover, an enhancement of C–C-absorption around 1,200 cm^{-1} has been observed after reaction, which can be attributed to the degradation of aromatic ring structures at exposed sp^2 C–H edges by oxidation with strong acids and generating new C–C single bonds (Kirk and Bohn 2004). Further investigation of the GNF morphology with SEM confirmed the successful oxidation process of GNFs, as a treatment of the unmodified GNF material with the oxidation mixture (H_2SO_4/HNO_3 mixture) caused the nanofibres to swell in accordance to literature (Tijmen et al. 2002). This behaviour is clearly demonstrated in Fig. 2, whereas Fig. 2a shows a typical charge of unmodified GNF and Fig. 2b illustrates the SEM of the nanofibres after H_2SO_4/HNO_3 treatment.

Quantification of Cu^{2+} and acid/basic sites on GNFs

The successful reaction of oxidized GNF to GNF–IDA–Cu^{2+} (IMAC material) by the chemical attachment of iminodiacetic acid was confirmed by quantification of the copper capacity, using atomic absorption spectrometry

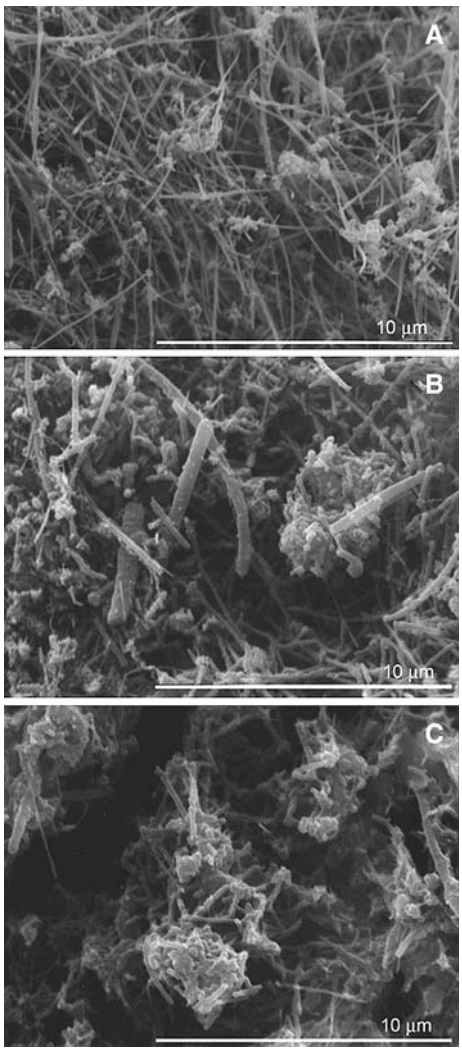

Fig. 2 SEM pictures showing unmodified GNF (**a**), oxidized GNF (**b**) and GNF–IDA–Cu^{2+} (**c**). GNF are swollen after treatment with H_2SO_4/HNO_3 mixture

(AAS). The copper capacity was calculated by external standard calibration and was determined to be 0.33 µmol Cu^{2+} per mg GNFs (0.022 mg Cu^{2+} per mg GNFs). High binding capacities are thus expected with regard to the pre-concentration of serum protein samples, as proteins exhibit high affinity to immobilized Cu^{2+} centers, preferentially building histidine-Cu^{2+} complexes (Abbaspour and Kamyabi 2004). The derivatisation of GNF to the GNF ion exchange materials (GNF–NH–C_6H_{12}–COO^- and GNF–NR_3^+) was controlled by quantification of the acid or base uptake (Li et al. 2005). Calculation of acid sites on GNF–CON–C_6H_{12}–COO^- resulted in 0.31 µmol H^+ per mg GNFs, which is in the same order of magnitude than the Cu^{2+} capacity of GNF–IDA–Cu^{2+}. The number of basic counter ions on GNF–NEt_3^+ material by base uptake was determined to be 0.04 µmol OH^- per mg GNFs. This considerable lower amount of anion exchange groups can be explained by the steric demand of triethylamine groups which need significantly more surface area than primary or secondary amines.

Specific surface area

The surface areas of 26 $m^2\ g^{-1}$ for GNFs and 18 $m^2\ g^{-1}$ for GNF-IDA-Cu^{2+} were obtained by BET evaluation. As the decreased specific surface area of the GNF IMAC materials in comparison to the unmodified nanofibres is representative for all derivatized GNF materials, this decreased surface area may be ascribed by the introduction of a huge number of functional groups (compare high Cu^{2+} or acid/base capacity of the derivatives) onto the surface of GNFs. Very polar groups like metal ions or carboxyl groups can also cause structural changes of the nanofibres leading to differences in surface area compared to apolar, non-derivatized GNFs.

Insulin loading capacity and recovery rate

Even if it has been proven that the derivatized GNF materials possess a high density of functional surface groups, it is indispensable to determine the material binding and elution properties towards proteins, as the actual analytes of concern. For that purpose, the protein-binding properties of GNF–IDA–Cu^{2+} was evaluated by incubation with insulin solutions (200 µl) of different concentrations, ranging from 250 to 2,500 µg ml^{-1}, for 2 h. Figure 3 demonstrates the trend line for the insulin loading (µg) on GNF-IDA-Cu^{2+} (mg) depending on insulin concentration (µg ml^{-1}). A linear behaviour has been found between adsorbed amount of substance (m_{ads}) and the incubation concentration ($C_{insulin}$) up to approximately 1,500 µg ml^{-1} insulin, whereas a further increase in concentration (up to 2,000 µg ml^{-1}) caused the line to flatten, indicating that the

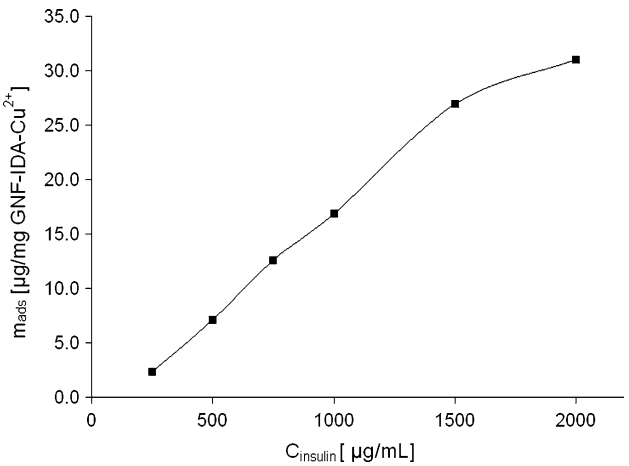

Fig. 3 Insulin loading capacity of GNF–IDA–Cu^{2+} (µg mg^{-1}) determined at different insulin concentration (µg ml^{-1})

maximal loading capacity is approached at 30 μg insulin per mg GNF–IDA–Cu^{2+}.

To determine the recovery rate of bound insulin, comparative HPLC studies were performed before and after incubating GNF–IDA–Cu^{2+} with insulin (1,500 μg ml^{-1}), followed by washing and elution of ligated protein with 600 μl ACN/water (70/30% v/v), containing 0.03% TFA. After the removal of ACN, the remaining eluates were subjected to HPLC analysis. This way, recovery rates were calculated to range from 50 to 55% for insulin as a model protein, being incubated on GNF–IDA–Cu^{2+}. Recovery rates indicate that a considerable amount of insulin is bound unspecifically and is therefore removed by washing.

Detection limit for insulin using GNF–IDA–Cu^{2+} as MELDI support material

Selective binding of proteins and peptides is a major demand on GNF derivatives. So is the sensitivity and limit of detection of the material, as biological samples have typically a high dynamic range, which cause the importance of low abundant species easily to be undermined. Requirements to detect proteins and peptides at a very low level of concentration seem to be fulfilled, as being shown by sensitivity measurements, conducted with a protein mixture at different concentrations (Table 1) with MALDI–TOF–MS. GNF–IDA–Cu^{2+} material was incubated with the protein mixtures and then treated following the protocol for serum samples (see "Serum sample preparation and analysis"). The detection limit for the synthesised GNF-IDA-Cu^{2+} was the reproducibly found to be around 50 fmol μl^{-1} for insulin.

The attained detection limit can be attributed to the miniaturized dimensions (size and shape) of GNF, serving for laser energy transfer to the analyte molecules for desorption and ionization. The thermal properties of GNFs also contribute towards the increase in local temperatures upon laser irradiation. This extra heat is transferred to the analytes to bring them into gaseous phase ions. The pronounced electrical phenomenon of GNF increases the local electric fields to promote the DI process, which is confirmed in less laser energy requirement compared to the other carrier materials like cellulose or poly(glycidyl methacrylate/divinylbenzene) polymers.

Derivatized GNFs for protein profiling

The sp^2 carbon units are geometrically arranged into symmetries, which produce a number of sites for derivatizations with spacers to design various affinities. Protein profiling performed with derivatized GNFs provides high and selective loading capacities. In general, graphitic materials possess perfect electrical and thermal properties for desorption/ionization process in MALDI–TOF–MS. This attributes to the lower laser energy required to perform MALDI–TOF–MS spectra compared to other noncarbon support materials. Due to relatively high surface area of GNFs, they allow high enrichment of analytes, which leads to high sensitivity and reproducibility without prior albumin and immunoglobulin depletion. Figure 4 shows the mass spectra of human serum content in mass range from 2 to 10.8 kDa performed on (a) GNF–IDA–Fe^{3+} (b) GNF–C_6H_{12}–COO^-H^+ (c) GNF–$N^+Et_3OH^-$ and (d) GNF–IDA–Cu^{2+}. Spectra of GNF–IDA–Cu^{2+} reveals highest binding capacities. The intensive peak at ~5,380 Da appears only in GNF–IDA–Cu^{2+} mass spectra; on GNF–IDA–Fe^{3+}, GNF–C_6H_{12}–COO^-H^+ and GNF–$N^+Et_3OH^-$, this abundant protein is completely removed by the washing step. Different peak intensities, especially in the mass range from 7.5 to 10.8 kDa prove the selective enrichment of proteins due to their affinity.

GNFs with various modifications serve as effective carriers to bind analytes out of complex biological samples and furthermore provide the opportunity to directly analyse them with MALDI–TOF–MS. Support materials based on IMAC are often preferred in protein profiling and biomarker research over other affinities, because metals like copper and iron are forming strong and specific chelates with peptides and proteins out of complex serum samples.

Reproducibility

Figure 5 exhibits two spectra recorded from a standard serum sample on different batches of GNF–IDA–Cu^{2+} to

Table 1 Comparison of properties of different MELDI nanomaterials; values of particle size was provided from supplier, copper loading capacities were determined by atomic absorption spectrometry (AAS) and detection limits of insulin were measured on a stainless steel target with MALDI–MS (Bruker, Bremen, Germany)

Carrier material	Particle size (nm)	Loading capacity [mg(Cu^{2+})/mg]	Detection limit for insulin (5.734 kDa)
Fullerences C_{60}-IDA Cu^{2+}	d = 7	0.015	50 fmol/μl, 0.285 μg/ml
C-nano tubes-IDA Cu^{2+}	d = 2l = 1000–10,000	0.06	<100 fmol/μl; 0.573 μg/ml
C-nano fibres-IDA Cu^{2+}	d = 100–200 l = 5,000–20,000	0.022	<50 fmol/μl; 0.285 μg/ml

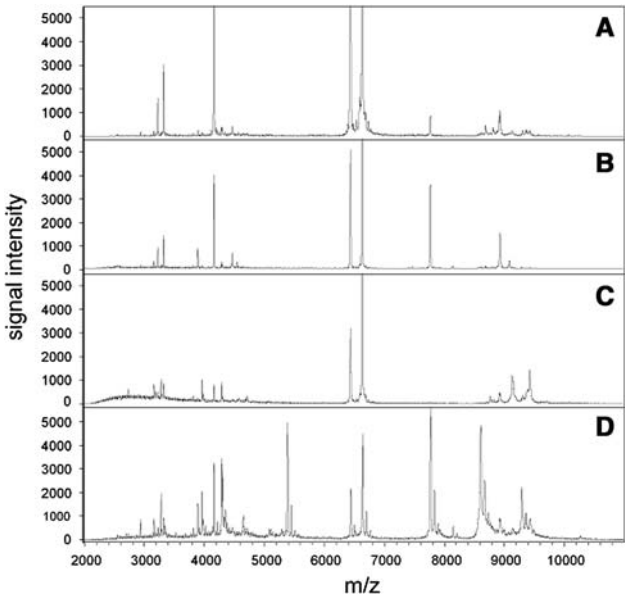

Fig. 4 Mass spectra of human serum in the range from 2 to 10.8 kDa performed on (**a**) GNF–IDA–Fe^{3+} (**b**) GNF–C_6H_{12}–COO^-H^+ (**c**) GNF–$N^+Et_3OH^-$ and (**d**) GNF–IDA–Cu^{2+}; carried out with HCCA as matrix, 400 laser shots recorded per spectra

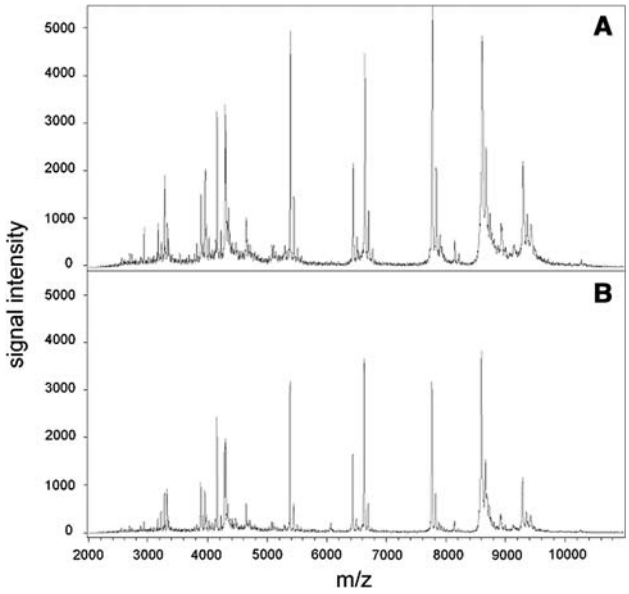

Fig. 5 Two MELDI mass spectra obtained by enrichment of the same serum sample on different batches of GNF–IDA–Cu^{2+} in order to check reproducibility; performed with HCCA as matrix, 400 laser shots recorded per spectra

confirm the required batch reproducibility. Identical levels of reproducibility were obtained on GNF–IDA–Fe^{3+} and anion and cation exchanger-materials (data not shown). The higher reproducibility was attributed to the homogenous loading of peptides and proteins on the derivatized GNFs, because of the efficient interactions of nano surfaces with incubating buffer solutions.

Comparison of different MELDI materials

In the last few years several novel support materials are developed for the MELDI-approach, which includes carbon-based nanomaterials (carbon nanotubes, diamond, C_{60} fullerenes), poly(glycidyl methacrylate/divinylbenzene), cellulose and silica. Table 1 lists the characteristics of carbon-based MELDI carrier material regarding their particle size, loading capacity and detection limits. Graphitic nanofibres are unique in their structural dimensions and provide their entire surface areas as binding sites in comparison to the tubular structures of carbon nanotubes. Graphitic platelets are arranged in various orientations around the fibre axis to provide a number of sites for chemical and physical adsorptions. CNTs, C_{60} fullerenes and GNFs require significantly less laser energy compared to other support material due to their electrical and thermal properties that beneficially influence ionization and desorption process. The reduced laser energy requirement for desorption and ionization avoids the unnecessary fragmentation of the analyte molecules. On the basis of this observation several investigations on matrix-free approaches are currently in progress. Improved detection sensitivity for carbon nanomaterials is also reported because of the improved energy-transfer efficiency in direct LDI (Zhong et al. 2006).

Conclusions

For the first time, modified GNFs are employed as carrier materials for the specific binding of peptides and proteins out of biofluids, followed by their subsequent analysis with MALDI–TOF–MS. The analysis of a series of standards and serum samples by using the MELDI approach clearly demonstrates that derivatized GNFs have appropriate qualities to immobilize a selective range of proteins and peptides through their special affinities and are therefore promising material for mass spectrometric investigations of serum samples. MALDI–TOF–MS sensitivity of <50 fmol μl^{-1} for the reported materials supports the selective binding of low abundant proteins and peptides. Remarkable peptide and protein-loading capacities were quantified through HPLC studies. Performances of functionalized GNFs were improved by optimization of sample preparation and comprehensive physical characterization of GNF material ranging from BET, SEM, IR, HPLC to AAS studies. The interaction of nanosciences with bioscience will serve as a baseline for future studies on the application of graphitic nanofibres for further identification of proteins,

peptides, and other charged species present in human biofluids.

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