High-Affinity Capture of Proteins by Diamond Nanoparticles for Mass Spectrometric Analysis

X. L. Kong, L. C. L. Huang, C.-M. Hsu, W.-H. Chen, C.-C. Han, and H.-C. Chang*

Institute of Atomic and Molecular Sciences, Academia Sinica, P.O. Box 23-166, Taipei, Taiwan 106

Carboxylated/oxidized diamond nanoparticles (nominal size 100 nm) exhibit exceptionally high affinity for proteins through both hydrophilic and hydrophobic forces. The affinity is so high that proteins in dilute solution can be easily captured by diamonds, simply separated by centrifugation, and directly analyzed by matrix-assisted laser desorption/ionization (MALDI) time-of-flight (TOF) mass spectrometry (MS). No preseparation of the adsorbed molecules from diamonds is required for the mass spectrometric analysis. Compared to conventional MALDI-TOF-MS, an enhancement in detection sensitivity by more than 2 orders of magnitude is achieved for dilute solution containing cytochrome *c*, myoglobin, and albumin because of preconcentration of the probed molecules. The lowest concentration detectable is 100 pM for a 1-mL solution. Aside from the enhanced sensitivity, the overall performance of this technique does not show any sign of deterioration for highly contaminated protein solutions, and furthermore, no significant peak broadening and band shift were observed in the mass spectra. The promise of this new method for clinical proteomics research is demonstrated with an application to human blood serum.

Matrix-assisted laser desorption/ionization (MALDI)[1] time-of-flight (TOF) mass spectrometry (MS) is a mainstream tool in current high-throughput mass analysis of biopolymers.[2] The MALDI technique, however, suffers from the shortcoming that it lacks sample specificity and its performance deteriorates markedly for samples containing multiple components and excessive amounts of salts or surfactants.[3] Surface-enhanced laser desorption/ionization (SELDI) is one of the techniques[4−10] developed to circumvent these problems. In this method,[4] micrometer-sized (typically 80−300 μm in diameter) agarose beads made for affinity chromatography columns were used to capture proteins of interest in crude sample solutions. The microbeads were then recovered, washed, placed on the LDI probe tip, and analyzed with regular MALDI-TOF-MS. Unfortunately, direct analysis of the surface-bound proteins is often accompanied with undesired decrease in mass resolution as well as mass accuracy ascribed to the interference from the beads in ion formation and extraction. One solution to this problem is to directly immobilize proteins onto the surface of the LDI probe without use of the microbeads.[7] The approach again suffers from the shortcoming that the number of binding sites is quite limited, $\sim 1 \times 10^{13}$ molecules/cm^2 or $\sim$160 fmol/mm^2 for a single layer of proteins on the probe surface. The obstacle was later removed by immobilization of the proteins to high molecular weight dextrans precoated covalently on the LDI probe.[8] An approximate 500 times more sample could be loaded, although the dextran immobilization process is rather time-consuming.

We have previously shown[11] that diamond is an exceptional platform for protein adsorption and immobilization. The optical transparency, chemical inertness, and biological compatibility of the material endow diamond nanoparticles with novel and promising biotechnological applications. Preliminary tests with cytochrome *c* physisorbed to carboxylated/oxidized diamond particles of 5 and 100 nm in size indicate that the specially prepared diamond surfaces exhibit remarkably high affinity for proteins containing amino acid residues with basic side chains. This unique feature along with the fact that diamond is optically transparent up to the UV region motivated us to explore the possibility of using diamond nanoparticles for SELDI-TOF-MS. The advantage of using nanoparticles over microbeads is manifold. First, nanoparticles have a much larger surface area-to-mass ratio, nearly 3 orders of magnitude higher than that of microbeads; second, the extent to which nanoparticles interfere with the laser desorption/ionization process is diminished because of the smallness of the particles; third, nanoparticles can be embedded more firmly in the LDI matrix crystals than microbeads, thereby reducing material loss during sample preparation and analysis. There have been several applications of metallic, semiconducting as well as polymeric nanoparticles for mass spectrometric analysis of biopoly-

* To whom correspondence should be addressed. E-mail: hcchang@po.iams.sinica.edu.tw.

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mers in the past[12-17] only that they were used mainly as energy-absorbing materials and served different purposes.

Diamond nanoparticles with a nominal size of 100 nm are employed in this work to capture proteins in highly diluted and contaminated solutions. The surfaces of these particles are prepared by strong oxidative acid pretreatment and have been characterized to be predominantly carboxylated and oxidized.[1,18] Additionally, these particles can suspend in aqueous solutions with long-term stability, indicating that their surfaces are grossly hydrophilic in nature. While the hydrophilic diamond nanoparticles exhibit high affinity for proteins, the adsorbed molecules can be easily eluted with matrix solution for MALDI-TOF mass analysis. It is a straightforward assay that neither covalent immobilization of target proteins nor preseparation of the captured proteins from diamonds is required. The assay is particularly useful for analysis of proteins in very dilute solution owing to reduction of the probed sample volume through high-affinity capture and preconcentration of the analyte by the nanodiamonds.

EXPERIMENTAL SECTION

Chemicals and Materials. Synthetic abrasive diamond powders of sizes in the range of 100 nm were obtained from Kay Industrial Diamond. Bovine heart cytochrome *c* (BCC), bovine serum albumin (BSA), horse heart cytochrome *c* (HCC), horse heart myoglobin (Mb), 4-hydroxy-α-cyanocinnamic acid (4HCCA), and trifluoroacetic acid (TFA) were all from Sigma, and they were used without further purification. Components for phosphate buffer salines were from Acros Organics, deionized water was from a Milli Q plus system (Millipore), and all blood serum samples were from healthy individuals (male) without obvious signs of disease.

Diamond Surface Preparation. Diamond powders were carboxylated and oxidized in strong acids following the procedures of Ushizawa et al.[18] The sample was first purified in a 9:1 (v/v) mixture of concentrated H_2SO_4 and HNO_3 at room temperature for 1 day, subsequently in 0.1 M NaOH aqueous solution at 90 °C for 2 h, and finally in 0.1 M HCl aqueous solution at 90 °C for 2 h. The resulting carboxylated/oxidized diamond nanoparticles were thoroughly washed with deionized water and separated by centrifugation with a Kubota 3700 centrifuge at 12 000 rpm. Two stock suspensions, each containing 1 and 0.1 mg of diamond nanoparticles/mL, were prepared with deionized water for later use.

Protein Adsorption Isotherm. Adsorption isotherms were obtained for three proteins (HCC, Mb, BSA) attached to the carboxylated/oxidized diamonds in 20 mM phosphate buffers at pH 3−12. The amount of proteins adsorbed (mg/g) was deter-

mined from the change in protein concentration before and after addition of the diamond powders into the solution. To ensure equilibration of the adsorption, the protein solution and the diamond suspension were thoroughly mixed together with a shaker (ELMI RM-2L) for 2 h, after which the mixture was centrifuged and the supernatant collected. A UV−visible spectrophotometer (Hitachi U-3200) served to determine the protein concentration based on the Soret absorption band at 409 nm for both HCC and Mb and the tryptophan absorption band at 280 nm for BSA.[19-21] All the spectra were acquired with a quartz cell (Hellma) of 1-mm path length at an instrumental resolution of 2 nm.

MALDI Sample Preparation. The matrix solution consisted of 4HCCA in 0.001:1:2 (v/v) TFA−acetonitrile−water with a concentration of 10 mg/mL. To illustrate the utility of diamond nanoparticles for sample volume reduction, protein solutions of different concentrations (0.1−100 nM) were prepared with deionized water. An aliquot (500 μL) of the protein solution was first mixed with the diamond suspension (0.2−10 μL) in a centrifuge tube for 2 min, followed by separation of the particles with a microcentrifuge (Hettich, MIKRO20) at 12 000 rpm for 5 min. After removal of the supernatant, the matrix solution (1.5−10 μL) was added, rinsing the inner wall of the centrifuge tube, and simultaneously mixed with the precipitate. In this experiment, the volumes of both diamond suspension and matrix solution were carefully chosen according to the protein concentration, solution pH, and adsorption isotherm to ensure complete utilization of these two reagents. An aliquot (1 μL) of the mixed solution was deposited on a stainless steel probe (2 mm in diameter) and air-dried at room temperature.

Following the same procedures, diamond nanoparticles were employed to capture proteins in 20 mM phosphate buffers at well controlled pH. To illustrate the capability of protein purification by diamond nanoparticles in highly contaminated solution, a phosphate buffer with 10-fold higher concentration (200 mM) was used. In this study, to remove contaminants such as phosphates, the protein-covered nanodiamonds were additionally rinsed with deionized water (0.5 mL in each rinse unless specified otherwise) before mixing with the matrix solution for final MALDI-TOF-MS measurements.

Three independent measurements were conducted for mass analysis of human blood serum, including (1) MALDI-TOF-MS without any pretreatment, (2) MALDI-TOF-MS with the ZipTip pretreatment, and (3) MALDI-TOF-MS with the diamond nano-particle pretreatment of the sample. In experiment (1), 1 μL of blood serum was mixed with 50 μL of the 4HCCA solution, after which 2 μL of the serum−matrix mixture was deposited on the stainless steel probe and air-dried. In experiment 2, a C18 pipet tip (Millipore, ZipTip) was first activated following the standard protocol of the manufacturer. The blood serum (50 μL) was then passed through the ZipTip repeatedly 5 times. After rinsing 3 times with 0.1% TFA and 5% methanol in water, the molecules attached to the resin were eluted with a 0.001:1:1 (v/v) TFA−acetonitrile−

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water mixture (10 μL). Half of the elution was deposited on the probe, on which it was mixed with the 4HCCA solution (2 μL) and air-died. In experiment 3, 10 μL of blood serum was diluted 100 times with deionized water and then mixed with 10 μL of the diamond nanoparticle suspension (1 mg/mL). After equilibration for 2 min, the combined solution was centrifuged for 5 min and the supernatant was removed. The precipitate was then washed once with deionized water (1000 μL), collected by centrifugation (3 min), and finally mixed with the 4HCCA solution (5 μL) as described above. An aliquot (1 μL) of the mixture was deposited on the probe for MALDI-TOF-MS measurements.

MALDI-TOF-MS. Mass spectra were acquired using a delayed ion extraction linear time-of-flight mass spectrometer built in-house with a flight length of 2.2 m and an acceleration voltage of 20 kV.[22] A Nd:YAG laser (Continuum Minilite) operating at 355 nm evaporated and ionized the protein molecules with a typical energy of 20 μJ/pulse. By properly adjusting the distance between the focusing lens and the sample target, the laser intensity was controlled to a level just above the ion generation threshold. The ionized molecules were detected by a triple microchannel plate assembly (Galileo) biased at -2.8 kV. Mass spectra were acquired with 50$-$200 laser shots with a LeCroy 9314 digital oscilloscope.

Scanning Electron Microscopy. Mixtures of diamond nano-particles and the 4HCCA matrix were examined using a scanning electron microscope (Hitachi S-2400). The samples were prepared on a glass slide (following the same procedures for preparation of the MALDI sample), secured to a mount fixture with double-sided tapes, and coated with a thin layer of gold ($\sim$23 nm) by ion sputtering prior to microscopic inspection.

RESULTS AND DISCUSSION

Protein Adsorption. In a previous work,[11] we found that carboxylated/oxidized diamonds are remarkably good adsorbents for proteins and polypeptides. The tenacious adsorption comes from the interplay of ionic interaction, hydrogen bonding, van der Waals interaction, and hydrophobic interaction between protein and surface.[20] Figure 1 displays the adsorption properties of diamond for BSA, MB, and HCC at solution pH's corresponding to their respective isoelectric points (pI = 4.7, 6.9, and 10.5).[23] The protein$-$surface interaction is so strong that the adsorption isotherms of all three proteins show a very sharp increase and quickly saturate at low concentrations. The amounts of the proteins adsorbed vary sensitively with solution pH but all maximize at pH $\approx$ pI (cf. Figure 2). A large fraction of the protein molecules remains attached to the surface even at pH > pI. Particularly noteworthy is that of BSA, which has a pI = 4.7 but shows a high degree of adsorption to the carboxylated diamond surfaces at pH 7. At this pH, the net charge of free BSA in water is about -18 (ref 24) and the ionic interaction between the negatively charged protein and the anionic diamond surface is expected to be repulsive. The feature demonstrates the important contribution of hydrogen bonding and hydrophobic interaction to the adsorption process.[21]

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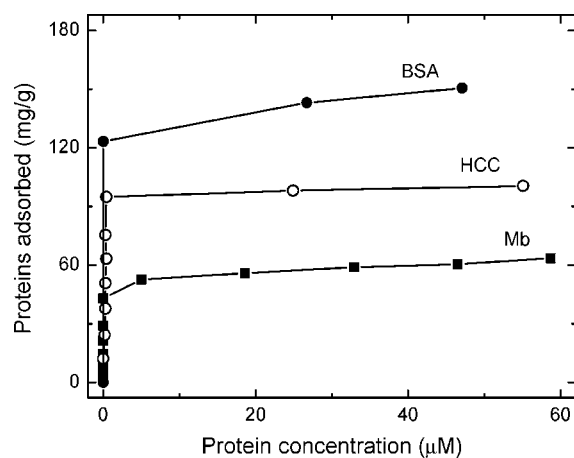(24) Peters, T., Jr. *Adv. Protein Chem.* **1985**, *37*, 161$-$245.

Figure 1. Adsorption isotherms of horse heart cytochrome *c* ($\bigcirc$), horse heart myoglobin ($\blacksquare$), and bovine serum albumin ($\bullet$) on 100-nm diamonds at pH 10.5, 6.9, and 4.7, respectively. The typical error involved in the measurements is $\pm$10%.
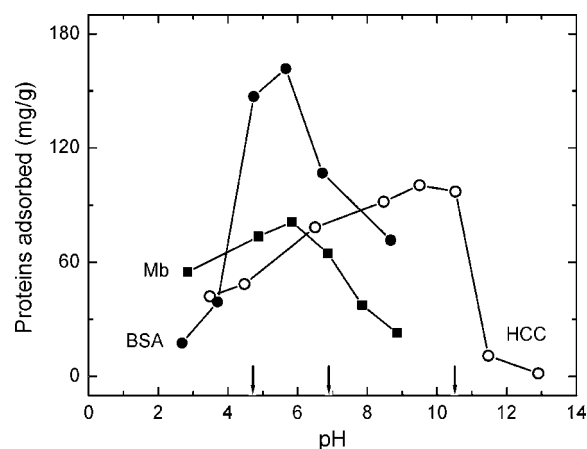

Figure 2. Amounts of horse heart cytochrome *c* ($\bigcirc$), horse heart myoglobin ($\blacksquare$), and bovine serum albumin ($\bullet$) adsorbed on 100-nm diamonds as a function of solution pH at saturation. Isoelectric points of HCC, Mb, and BSA are indicated at 10.5, 6.9, and 4.7, respectively. The typical error involved in the measurements is $\pm$10%.

The amount of HCC adsorbed onto the 100-nm diamonds saturates at 99 mg/g or, equivalently, 8.0 μmol/g at pH 10.5 (Figure 1). Given a specific surface area of $\sim$60 m^2/g for the nanoparticles,[11] this yields an adsorption density of $\Gamma = 8.0 \times 10^{12}$ molecules/cm^2. Cytochrome *c* has a molecular dimension of 2.5 $\times$ 2.5 $\times$ 3.7 nm,[25] and hence an orientation-dependent monolayer density in the range of $(1.0-1.6) \times 10^{13}$ molecules/cm^2 is expected. Compared to the calculated densities, the experimental value $\Gamma = 8.0 \times 10^{12}$ molecules/cm^2 suggests that more than half of a monolayer forms on the diamond surface at the isoelectric point for HCC. A similar conclusion is reached for Mb, which has the highest measurable adsorption density of $\Gamma = 4.8 \times 10^{12}$ molecules/cm^2 at pH 5.8. The value just falls short of the monolayer density range $(6.3-11) \times 10^{12}$ molecules/cm^2 calculated from its crystallographic dimension of 2.5 $\times$ 3.5 $\times$ 4.5 nm. Applying the same rationale to BSA, which is a prolatelike ellipsoid ($\sim$14 $\times$ $\sim$4 nm),[24] suggests that the protein may also form a closely packed monolayer on the surface of the diamond

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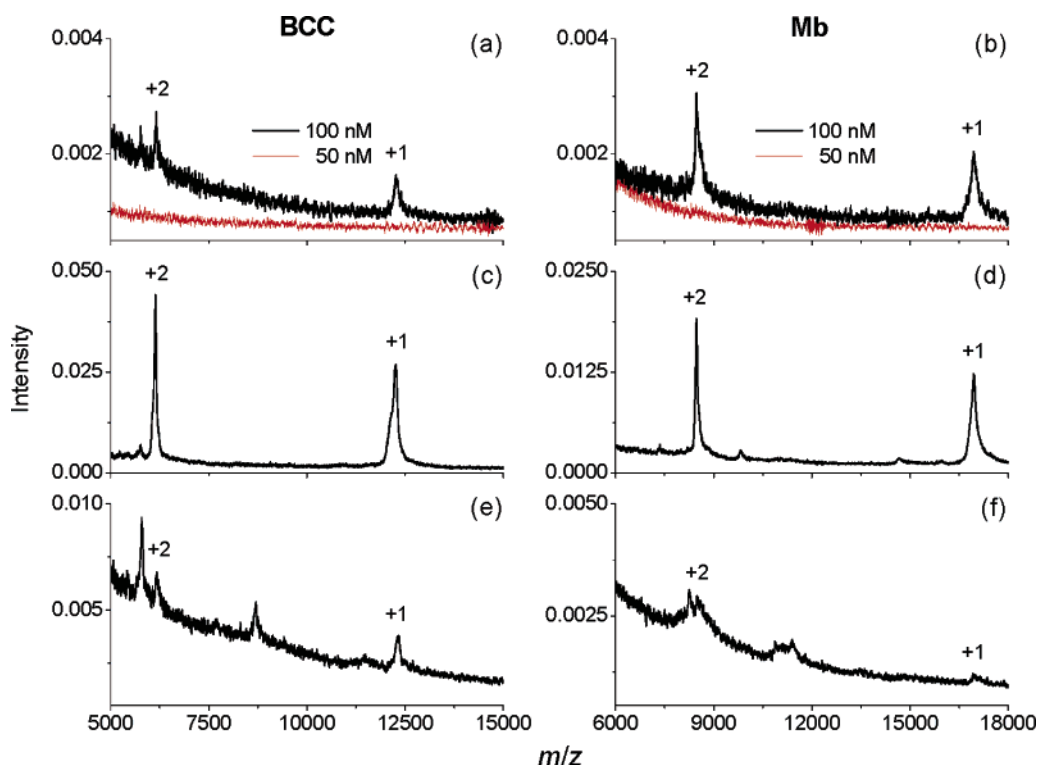
Figure 3. MALDI-TOF mass spectra of bovine cytochrome *c* (BCC) and horse heart myoglobin (Mb), obtained without any pretreatment (a, b) and with the diamond nanoparticle pretreatment for protein solutions of 50 and 0.2 nM for (c, d) and (e, f), respectively.

nanoparticle. At pH 5.7, the measured adsorption density is 2.4×10^{12} molecules/cm^2, which is significantly higher than the expected value of 1.8×10^{12} molecules/cm^2 if the BSA molecules are all adsorbed with their long axes aligned along the surface.

Mass Analysis of Proteins in Highly Diluted or Contaminated Solutions. Panels a and b of Figure 3 show, respectively, the mass spectra acquired for BCC and Mb with MALDI-TOF-MS without any pretreatment of the samples. The peaks corresponding to singly and doubly charged ions can be readily identified for both proteins in 100 nM solutions (0.5-μL sample solution mixed with 0.5-μL matrix solution on the probe in these two cases). However, no signals were identifiable when the protein concentrations were lowered to 50 nM, indicating that the detection limit of our instrument is somewhere between 25 and 50 fmol for proteins with $m \approx 15$ kDa. With the aid of diamond nanoparticles to preconcentrate the proteins, the signals become easily observable at the concentration of 50 nM (Figure 3c and d), and the detection limit can be readily lowered to 0.2 nM for both proteins (Figure 3e and f). In the latter experiments, 1.5 μL of the matrix solution was used to elute the protein molecules from the diamond nanoparticles to form a slurry and only 1 μL of the slurry was deposited on the probe. Hence, the total amount of the protein molecules deposited on the LDI probe is 67 fmol, given a protein solution of 500 μL and an initial concentration of 0.2 nM. This amount of the protein is close to the detection limit (25−50 fmol) of our instrument within a factor of 2. Importantly, it suggests that the presence of the 100-nm diamond particles does not much deteriorate the performance of the MALDI-TOF mass analysis. Similar to BCC and Mb, BSA was also detected with high sensitivity for multiply charged ions in the associated m/z region, although the intensity distributions significantly differ between

the mass spectra acquired with and without the diamond nanoparticle pretreatment (data not shown).

It is known that the presence of contaminants such as salts and surfactants often precludes direct analysis of biological samples by MALDI-TOF-MS.[3] Indeed, a test of the technique with solution containing phosphate buffers failed to produce satisfactory results. This is shown in Figure 4a, where no signal that can be associated with BCC is found for the protein (100 nM) in 200 mM phosphate buffer at pH 6.5. To recover the signal, a suspension containing 10 μg of diamond nanoparticles was added to the solution and the resulting slurry was analyzed. Figure 4b displays the mass spectrum, in which the protein signal at m/z 12 233 can be clearly identified. Further enhancement of the signal-to-noise ratio could be made by rinsing the diamond nanoparticles once with deionized water prior to mixing with the matrix solution (Figure 4c). Not surprisingly, as a result of the high affinity of the protein to diamond, the high-quality mass spectrum remained even after rinsing the BCC-bound nanoparticles with deionized water 4 times (Figure 4d). Similar results were obtained for both Mb and BSA (data not shown).

Aside from playing an active role to concentrate proteins in highly diluted and contaminated solutions, diamond nanoparticles also hold great promise for capturing proteins selectively in mixed solutions. This promise is shown in Figure 2, where the attachment of cytochrome *c* to diamond is seen to prevail at pH 9, whereas myoglobin prevails at pH 6. Such adsorption selectivity is indeed confirmed by MADLI-TOF-MS. Figure 5 displays the mass spectra of 1:1 BCC/Mb mixtures in 20 mM phosphate buffers at different pH's. As a result of the preferential adsorption of BCC over Mb, only very weak features associated with the gaseous Mb ions are identifiable at pH 8.7. In accord with the
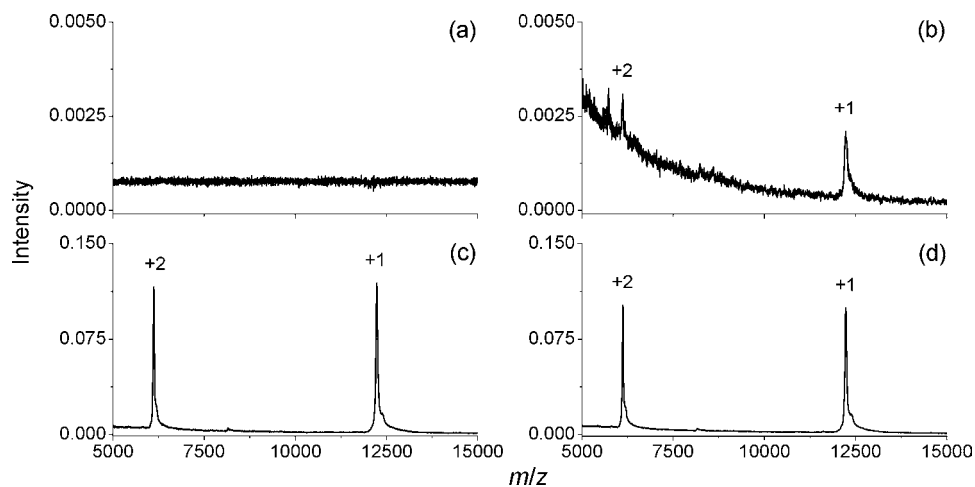
Figure 4. MALDI-TOF mass spectra of 100 nM bovine cytochrome *c* in 200 mM phosphate buffers at pH 6.5, obtained without any pretreatment (a) and with the diamond nanoparticle pretreatment (b). The mass spectra shown in (c) and (d) were obtained with the diamond nanoparticle pretreatment but before submission for MALDI-TOF mass analysis, the protein−diamond mixtures were washed once and four times with deionized water, respectively.
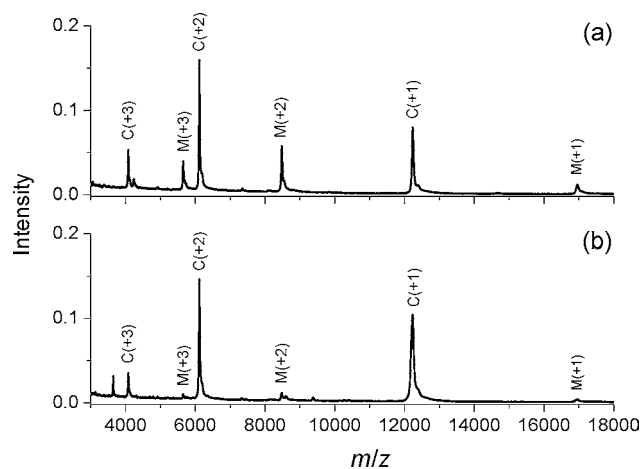

Figure 5. MALDI-TOF mass spectra of bovine cytochrome *c* and horse heart myoglobin (both 50 nM) in 20 mM phosphate buffers at pH 5.0 (a) and 8.7 (b). Both spectra were acquired with the diamond nanoparticle pretreatment. The charge states of cytochrome *c* and myoglobin are denoted by the numbers associated with "C" and "M", respectively.
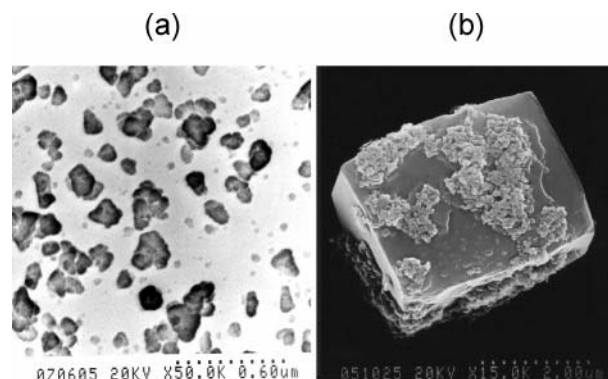

Figure 6. Scanning electron microscopy images of diamond nanoparticles alone (a) and diamond nanoparticles mixed with the 4HCCA matrix before laser irradiation (b). The scale bars in (a) and (b) are 0.6 and 2.0 μm, respectively. Note that the matrix crystal is covered with diamond nanoparticles and their agglomerates.

adsorption isotherm measurements (cf. Figure 2), both proteins cannot be detected at pH 12, at which the repulsive diamond surface is no longer accessible for noncovalent attachment.

Laser Desorption/Ionization Mechanism. An interesting question raised is whether the protein molecules remain attached to or are already detached from the diamond nanoparticle surfaces prior to laser irradiation.[26] Figure 6 shows the SEM images of the diamond nanoparticles alone and the particles after mixing with the 4HCCA matrix. For the latter, the nanoparticles are seen to cluster together and predominantly accumulate on the surfaces of the energy-absorbing material when the matrix crystallized (Figure 6b). Similar sample−matrix separation behaviors have been observed for micrometer-sized bacteria,[27,28] although diamond nanoparticles discussed herein are ~10 times smaller.

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With the SEM images, the most likely explanation for our experimental spectra presented so far is that we are actually detecting protein molecules embedded in the matrix, rather than those bound to the diamond surfaces. To test the hypothesis as well as to assess the degree of protein detachment, a control experiment was conducted with a 1:1:1 mixture of BCC, Mb, and BSA. In this experiment, 5 μL of the 4HCCA solution was mixed with the protein-attached diamond nanoparticles, and only 1 μL of the resulting slurry was used for acquisition of the mass spectrum without preseparation of diamond nanoparticles. The remaining 4 μL of slurry was centrifuged, and 1 μL of the supernatant was analyzed by the MALDI-TOF-MS. Figure 7 shows the results of these two measurements. No distinct differences are found between them, supporting the inference that the protein molecules in these two cases (with or without preseparation of diamonds) are most likely desorbed and ionized through a similar mechanism. This leads us to the conclusion that the protein molecules initially attached to the diamond nanoparticles were freed from the surfaces by the matrix solution, after which they cocrystallized with the matrix molecules in air. The conclusion is in line with the result of the adsorption measurement (cf. Figure 2) that the amounts of HCC, Mb, and BSA bound to the
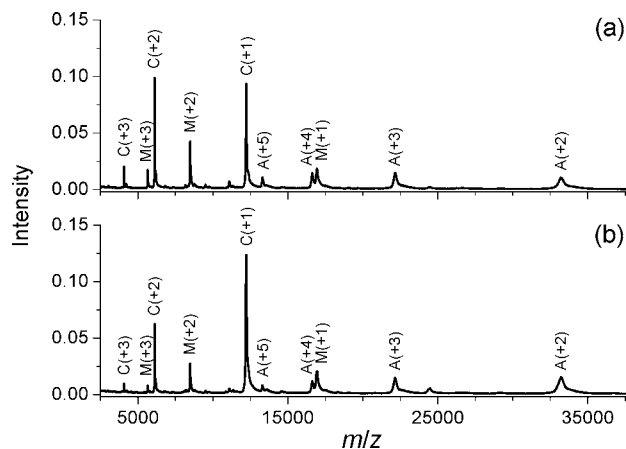
Figure 7. MALDI-TOF mass spectra of a protein mixture composed of bovine cytochrome *c*, horse heart myoglobin, and bovine serum albumin (all 50 nM in deionized water) acquired with the diamond nanoparticle pretreatment. The spectra were acquired with (a) and without (b) preseparation of the nanoparticles before MALDI-TOF mass analysis. The charge states of cytochrome *c*, myoglobin, and albumin are denoted by the numbers associated with "C", "M", and "A", respectively.

carboxylated/oxidized diamonds decrease substantially at pH <3 because of the repulsion built up between the proteins and the surfaces. Such an acid-induced desorption is expected to proceed readily in the organic acid matrixes containing 0.1% TFA and may be facilitated by competitive adsorption of the matrix molecules to the diamond surfaces as well. Thanks to the optical transparency, chemical stability, and the smallness of the diamond nanoparticles, no apparent peak broadening and band shift are detected in the mass spectra as shown in Figure 7b.

Applications to Blood Serum Analysis. Clinical proteomics is a process by which researchers can come to understand the function of proteins in physiological disease states by probing biomarkers. The analysis of human blood serum by MALDI or SELDI has been demonstrated to be a powerful technique in searching for these markers.[29-31] We explore in this section the potential application of diamond nanoparticles to analyze blood serum for clinical proteomics research. The human blood sample was obtained from healthy individuals, clotted, and subsequently separated by centrifugation. The isolated serum was divided into 50-μL portions and immediately stored in a -20 °C refrigerator until use.

A representative mass spectrum of the human blood serum acquired with regular MADLI-TOF-MS is shown in Figure 8a. In this experiment, the sample was diluted 50-fold directly with the 4HCCA matrix solution in order to obtain a mass spectrum with decent signal-to-noise ratios. The spectrum shows three strong signals at *m/z* 66 440, 33 220, and 22 150 corresponding to human serum albumin; however, it displays only two distinct features at *m/z* 2000−10 000. Figure 8b presents the mass spectrum of the serum sample purified with the ZipTip method. In contrast to the marked reduction in albumin peak intensity, many new features emerged in the lower *m/z* region owing to desalting and preconcentration of the sample. Interestingly, similar high-quality mass spectra could be obtained after the diamond nanoparticle pretreatment, even though 10-fold less serum was used for the data acquisition (Figure 8c). Compared to the ZipTip result, the acquired mass spectrum is ~5-fold higher in overall peak intensity and is noticeably richer in spectral features over the entire mass range. Furthermore, the albumin peaks are suppressed to a
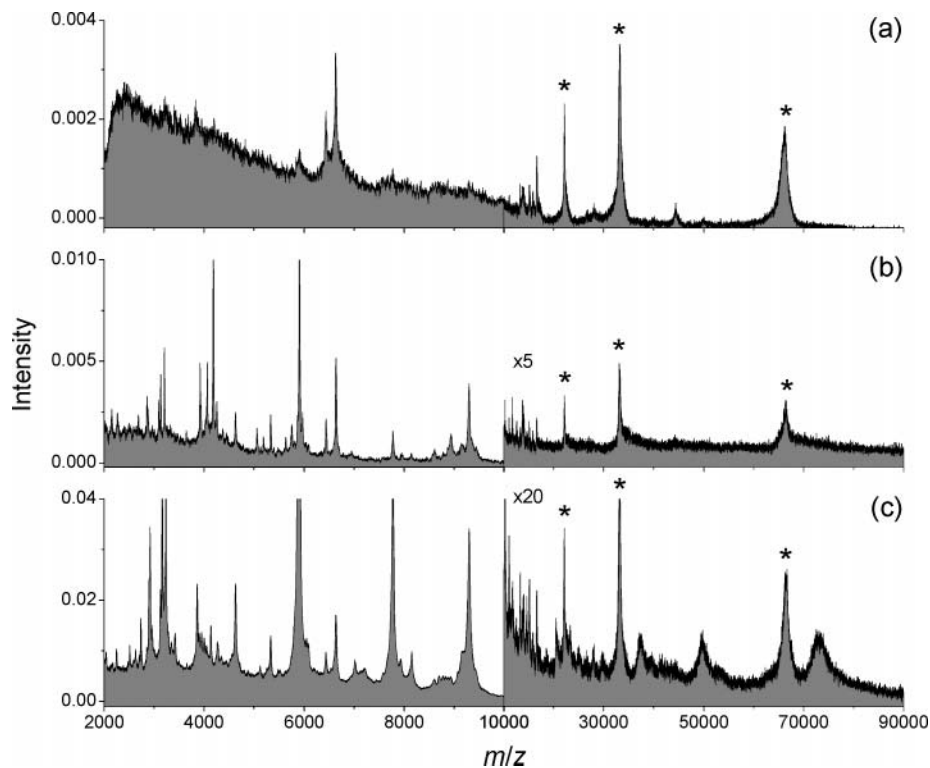

Figure 8. MALDI-TOF mass spectra of human blood serum acquired after 50-fold dilution with the 4HCCA matrix solution (a), after pretreatment with the ZipTip (b), and after 100-fold dilution with deionized water and then treated with diamond nanoparticles (c). The relative quantities of the serum samples consumed in obtaining these spectra are roughly 1:600:50. Note the changes of both *x* and *y* scales at *m/z* 10 000. Albumin peaks are denoted by asterisks (∗).

greater extent with the nanoparticle than the ZipTip pretreatment. Not compromised by the high sensitivity as well as the high selectivity of this method, the whole analysis for each sample can be finished in 10 min.

CONCLUSIONS

We have demonstrated that a combination of sample preconcentration with diamond nanoparticles and laser desorption/ionization with UV matrixes offers a new approach for time-of-flight mass spectrometric analysis of proteins in highly diluted and contaminated solutions. Compared to the existing methods using microbeads, this approach is faster, more effective, and easier to operate, and no concomitant peak broadening and mass shift are observed in the mass spectra. A concentration as low as 100 pM is detectable for high molecular weight proteins such as cytochrome *c*, myoglobin, and albumin in a 1-mL solution. Further decrease of the detection limit by a factor of 10−100 is possible if a prestructured MALDI sample support is used to concentrate the sample to a smaller hydrophilic spot.[32]

A preliminary application of the technique disclosed in this work to human blood serum shows 10-fold higher in sensitivity and noticeably richer spectral features than those obtained with the ZipTip method. Characterized by the high sensitivity and simple operation, the potential of this method to be adopted as a diagnostic tool in clinical proteomics research is evident. Future development of the technology along this line includes modification of the diamond nanoparticle surfaces (e.g., hydrophilic, hydrophobic, and other derivatives)[33] to achieve selective capture of low-abundance proteins in unfrationated biological samples for mass spectrometric assay. It is a novel application of this intriguing material in modern biomedical science and technology.

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