Peptide Enrichment

Enrichment of Low-Abundance Peptides and Proteins on Zeolite Nanocrystals for Direct MALDI-TOF MS Analysis**

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Proteomics, one of the most important research areas in the post genomics era, is arousing considerable attention because of its pertinence to functional genomics.[1] Its premise is the high-throughput separation and identification of proteins and peptides. Peptide mapping by MALDI-TOF MS has become a major tool for protein identification in proteome analysis.[2] However, many proteins are quite scarce owing to the difficulties of sample isolation. This is especially true for proteins that are expressed in low abundance, such as disease-associated proteins.[3] Moreover, a further decrease in sample yield may occur during the preparation of a peptide digest prior to MALDI-TOF MS analysis which therefore requires that peptide preparations undergo an enrichment step beforehand. However, conventional methods for concentrating peptides by simple evaporation result in sample loss through protein adsorption onto the surface of the container. An additional problem with evaporation is the simultaneous concentration of buffer components (e.g. salts) and other contaminants. Alternative methods for peptide enrichment by reversed-phase resin are complex and are typically suitable only for hydrophobic peptides.[4] Therefore a universal and simple peptide enrichment method is in high demand.

Zeolite has attracted considerable attention as a novel chromatographic carrier in the adsorption and purification of proteins.[5] Zeolite particles at the nanometer scale (nanozeolites) show a number of distinct characteristics: large external surface area, high dispersibility in both aqueous and organic solutions, and a variety of tunable surface properties, such as adjustable surface charge and hydrophilicity/hydrophobicity.[6] These properties make them promising candidates for the concentration of trace biomolecules in large solution volumes through various protein–zeolite-surface interactions.

Very recently, our group has successfully separated the selenoprotein-P (Se-P) proteins in mouse plasma through their chelation with Co^{2+}-exchanged nanozeolite/diatomite composites.[7] Herein, we report a remarkable enrichment of low-abundance peptides on zeolite nanocrystals based on their strong adsorption ability[8] and high dispersibility. Meanwhile, nanozeolites exhibited universality toward the peptides tested, and resisted the co-concentration of salts during the enrichment process. More importantly, the peptides adsorbed on the nanozeolites can be directly analyzed by MALDI-TOF MS. Hence, the risk of sample loss from any elution steps is avoided and the concentration procedure is simplified.

All nanozeolite particles were hydrothermally synthesized according to literature procedures.[9] Scanning electron microscopy and dynamic light scattering (DLS) analysis show that the size of zeolite particles is less than 100 nm (Table 1), which endows them with both high dispersibility in peptide solutions and large active surface areas for peptide adsorption. The X-ray diffraction (XRD) patterns confirm their corresponding crystal structures. To study the enrichment effect of nanozeolites on peptide analysis, the nanozeolites were dispersed in a dilute aqueous solution containing three standard peptides (0.04–1 $pg\,\mu L^{-1}$ for each peptide) (peptide I: KHHDKHGH, M_w 995.07, isoelectric point (pI) 8.62; peptide II: CYIQNCPRG, M_w 1050.22, pI 8.06; and peptide III: DRVYIHPFHL, M_w 1296.49, pI 6.92). After centrifugation and redispersion, the suspension of peptide-adsorbed nanozeolites (peptides/nanozeolites) was mixed with a MALDI matrix (α-cyano-4-hydroxycinnamic acid). The mixture was then spotted onto a MALDI plate for MS analysis. For comparison, peptide solutions of 20 and 50 $pg\,\mu L^{-1}$ were prepared without enrichment and also analyzed by MALDI-TOF MS.[4]

Figure 1 displays MALDI-TOF mass spectra of the three peptides with or without nanozeolite-mediated enrichment. The peptides at concentrations lower than 20 $pg\,\mu L^{-1}$ were scarcely detected by MS without the nanozeolite-enrichment step (Figure 1b). However, after enrichment, peaks of all peptides became clearly apparent (Figure 1c), even at a peptide concentration as low as 1 $pg\,\mu L^{-1}$. The intensities and signal-to-noise ratios (S/N) of peptides I and II were even higher than those of the peptides at 50 $pg\,\mu L^{-1}$ that were

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Table 1: Particle sizes and Si/Al ratios of the synthesized zeolite nanocrystals, and the MS data and S/N of peptides/nanozeolite samples after enrichment in the absence or presence of $CaCl_2$.

Nanozeolite		Si/Al[a]	Size[a] [nm]	Intensity and S/N (in parentheses) of MS signal					
				Peptide I[b]	Peptide II[b]	Peptide III[b]	Peptide I[c]	Peptide II[c]	Peptide III[c]
	β-15	15	95	~0 (~0)	6637 (1540)	116 (27)			
BEA	β-25	25	68	898 (149)	4435 (797)	535 (109)	8959 (845)	6347 (672)	4682 (699)
	β-37	37	81	311 (34)	1876 (448)	157 (163)	2432 (450)	3085 (629)	352 (98)
MFI	S-1	∞	70	707 (128)	1028 (204)	2340 (557)	5920 (638)	207 (118)	2111 (341)

[a] The Si/Al ratio and size of zeolite nanocrystals were determined by energy dispersive X-ray (EDX) analysis and dynamic light scattering (DLS), respectively. [b] The concentration of peptide solutions for enrichment was 1 pg μL^{-1} for each peptide. [c] The concentration of peptide solutions for enrichment was 1 pg μL^{-1} for each peptide, in the presence of 1 M $CaCl_2$.

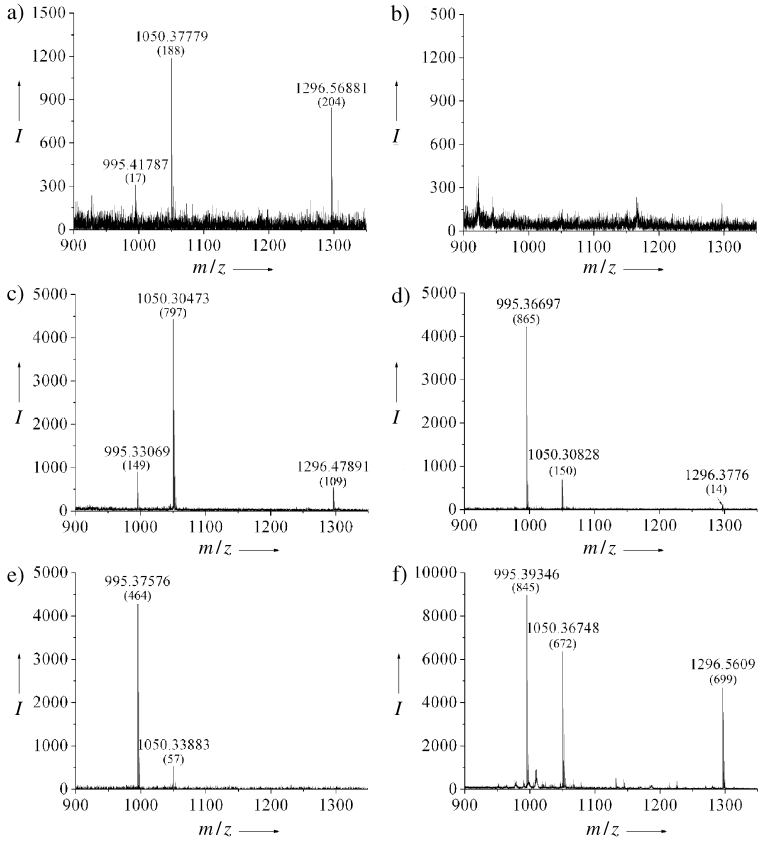

Figure 1. MALDI-TOF mass spectra of the peptide mixtures prepared without enrichment: a) 50 pg μL^{-1} and b) 20 pg μL^{-1}, and the peptides/nanozeolite (β-25) samples after enrichment in peptide mixture solutions of c) 1 pg μL^{-1}, d) 0.4 pg μL^{-1}, e) 0.04 pg μL^{-1}, and f) 1 pg μL^{-1} containing 1 M $CaCl_2$. The data in parentheses are S/N of the corresponding peptides. MALDI-TOF MS experiments were performed on a 4700 Proteomics Analyzer (Applied Biosystems, USA). Samples were desorbed with an Nd-YAG laser (355 nm) operated at a repetition rate of 200 Hz and an acceleration voltage of 20 kV. Measurements were performed in the reflector TOF detection mode.

prepared without the enrichment step (Figure 1a). Furthermore, the presence of nanozeolites neither interfered with the MS signals of the three peptides nor introduced impurities. Notably, when the peptide solution was diluted to 0.4 pg μL^{-1}, the three peptides could still be detected after enrichment (Figure 1d); even when the concentration was decreased to 0.04 pg μL^{-1}, peptides I and II remained detectable (Figure 1e). In this case, a concentration factor of 10^2–10^3 could be estimated.

The application of nanozeolites to enrichment and subsequent MS detection of the peptides was studied by using zeolite beta (β-X, X = Si/Al ratio) and silicalite-1 (S-1) nanocrystals in a 1-pg μL^{-1} three-peptide mixture (Table 1). It was found that, except for the case of zeolite β-15 (the lowest Si/Al ratio), peptides enriched on all nanozeolites exhibited strong MS signals and high S/N, indicating that these nanozeolites are good candidates for peptide enrichment. The discrimination of β-15 against peptide I could be attributed to too strong an adsorption as a result of strong hydrophilic and coulombic interactions[10,5e] which prevents desorption/ionization in MALDI-TOF MS.

During extraction and/or proteolysis procedures, some inorganic salts are normally added to promote dissolution of proteins and peptides, and to maintain an appropriate ionic strength. However, the presence of salts can severely upset the MALDI process and result in poor mass spectra.[11] Therefore, a desalting step necessarily follows conventional protein concentration processes in order to obtain a satisfactory analysis. The influence of salt on the nanozeolite-mediated enrichment process was investigated in peptide solutions containing 1 M $CaCl_2$ (Table 1). In most cases, the three peptides enriched on the nanozeolites in the presence of $CaCl_2$ presented even stronger MS signals with higher S/N (Table 1 and Figure 1f) than those enriched in the absence of $CaCl_2$ (Figure 1c and Table 1). This could be ascribed to the shielding effect of Ca^{2+} ions toward the surface charge of nanozeolites[10] which may facilitate the ionization of adsorbed peptides. The results show that these nanozeolites exhibit an excellent means by which to avoid the problems of high salt levels in protein preparations and to obviate the need for a desalting step.

To evaluate the universality of this approach, β-25 and S-1 nanocrystals were applied to a typical myoglobin tryptic digest. A database search (Protein Prospector)[12] identified about 10 peptides that represent 60–70% coverage of the myoglobin sequence. Figure 2a displays the MS result of a myoglobin (50 pg μL^{-1}) digest enriched by β-25. In comparison with the MS data from the high-myoglobin-concentration (5000 pg μL^{-1}) digest without the enrichment step (Figure 2b), the advantage of zeolite-mediated enrichment is clear: a similar tryptic fragment profile is present, and

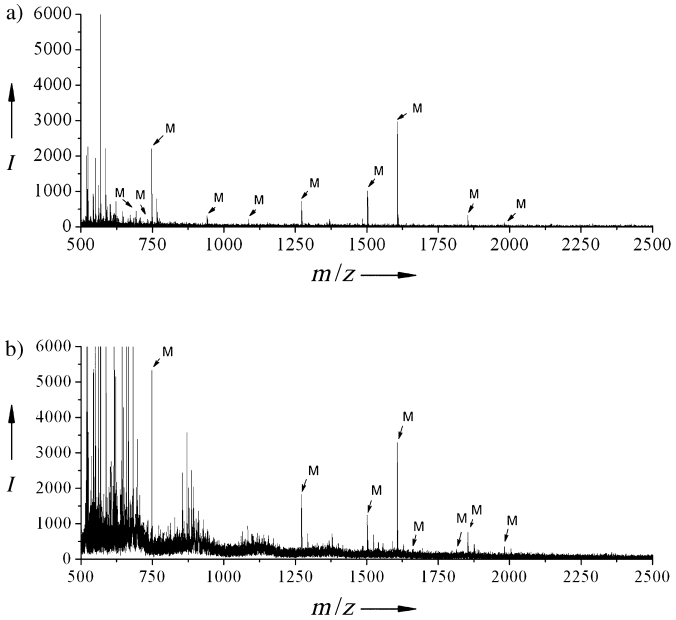

Figure 2. MALDI-TOF mass spectra of myoglobin tryptic digests: a) sample at 50 pg μL^{-1} enriched with β-25 nanocrystals, and b) sample at 5000 pg μL^{-1} without enrichment. The peaks marked with M represent the myoglobin fragments.

the S/N is much higher even for a preparation containing 100-fold less protein (Figure 2a). This indicates that nanozeolites appear not to discriminate against the various peptides.

In summary, nanozeolite particles were applied for the first time in the enrichment and identification of trace peptides and proteins. The direct MALDI-TOF MS analysis protocol eliminates the peptide-elution step and therefore circumvents the risk of sample loss. It was found that nanozeolites with medium and high Si/Al ratios exhibited excellent enrichment and desalting qualities for MALDI-TOF MS analysis of peptides. This technique is expected to open up new horizons for the preconcentration and identification of low-abundance peptides and proteins, as well as a new application of nanozeolites.

Experimental Section

Nanozeolite suspensions (10 μL of 10 mg mL^{-1}) were added to standard peptide or peptide digest solutions (1 mL) at varied concentrations. The resulting mixtures were agitated for 90 min at 37 °C, and centrifuged at 16000 rpm for 20 min. After decanting the supernatant, the residual peptides/nanozeolite pellets were rinsed with water three times and resuspended in acetonitrile (50 %; 2.5 μL) for subsequent MALDI sample preparation. A volume of the above slurry (0.5 μL) was mixed with α-cyano-4-hydroxycinnamic acid (0.25 μL, a saturated solution in 50 % aqueous acetonitrile containing 0.1 % trifluoroacetic acid) on the MALDI plate and applied for MALDI-TOF MS analysis.

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