Macroporous Materials as Novel Catalysts for Efficient and Controllable Proteolysis

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Macroporous Materials as Novel Catalysts for Efficient and Controllable Proteolysis

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A novel nanopore based digestion strategy has been developed by directly adding a macroporous material as catalyst to the conventional in-solution reaction system. Without increasing the enzyme or protein concentrations, this simple digestion approach exhibits high proteolysis efficiency and selectivity due to the in situ fast adsorption of both enzymes and proteins from bulk solution into the macropores of the catalysts, where the target substrates and enzymes are greatly concentrated and confined in the nanospace to realize a quick digestion. Based on the electrostatic interaction matching between the biomolecules and catalysts, selective extraction and digestion of proteins with different isoelectric points can be achieved by adjusting the surface charge of the catalysts. This nanoporous reaction system has been successfully applied to the analysis of a complex biological sample, where 293 proteins are identified, while only 100 proteins are obtained by the standard overnight in-solution digestion. The present nanospace confined digestion strategy will lead to promising advances not only in proteomics but also in other applications where enzymatic reactions are involved.

Over the past decades, mass spectrometry (MS) has become a major method in understanding the protein primary sequence,[1] post-translational modifications,[2] protein−protein interactions,[3] and biochemical functions of proteins[4] in biological systems. Proteolysis is a necessary and determining step in the bottom-up MS-based protein analysis.[5,6] Unlike in the living body where the biological reactions between proteins and cellular enzymes occur in a confined environment and have fast reaction rates, most in vitro enzymatic reactions exhibit slow kinetics, thus the proteins at submicromolar concentrations are difficult to be digested and the widely employed in-solution digestion requires a long reaction time.[5,7] Moreover, many proteins extracted from biological samples are generally complex in nature and express at low abundance,[8] therefore creating enormous difficulty in methodologies.[9] These shortcomings should be overcome in terms of the urgent need of large quantity protein analysis in proteomics.[10,11] Recent improvements in the proteolysis include microreactors and nanoreactors based on microfluidic chips[12−19] and mesoporous materials.[20−22] These methods take advantage of the high concentrations of preimmobilized enzymes or substrates to accelerate the proteolysis kinetics.[12−22] However, the home-designed apparatus is usually difficult to be coupled with the commercial MS instrument.[12−16] As a result, the practical applications of these methods in complex sample analysis are restricted and can not completely replace the traditional in-solution digestion until now.

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Mesoporous materials have been reported as excellent host supports for the immobilization of proteins.[20−29] For the materials with small pore size (e.g., SBA-15), though the proteins or enzymes may be enriched in the mesochannels, their motion would be restricted, leading to the decreased proteolysis efficiency. In order to realize the general enhanced enzymatic reactions, it is desirable to design a host material with large pore size and high pore volume, where both enzymes and proteins can be enriched and confined, while the motion of large biomolecules as well as the enzyme-protein collision will not be restricted. In this regard, macroporous materials with ultrafast mass transfer process are anticipated to be advantageous over the conventional nanoporous materials in accelerating the kinetics of proteolysis.

Herein, on the basis of the regular in-solution digestion technique, a novel enzymatic reaction system has been developed by introducing a macroporous material as novel catalyst for controlled proteolysis without employing any pretreatments. By simply adding a small amount of macroporous ordered siliceous foam (MOSF)[30] (~100 nm in pore diameter) directly in the conventional bulk solution digestion system with enzyme-to-substrate ratio of 1:30 (w/w), the proteolysis efficiency can be greatly improved. As proven, the standard proteins at concentration as low as 2 ng/μL can be digested in 30 min using the present strategy. Moreover, it is further demonstrated that by adjusting the surface charge of catalysts, selective proteolysis has been achieved due to the electrostatic interaction between the catalysts and proteins. Specifically, here we use magnesium oxide to modify the surface of MOSF,[31,32] where an impregnated MOSF (MgO-MOSF) is obtained with relatively basic surface condition. Using the mixture of MOSF and MgO-MOSF as cocatalysts, the proteolysis efficiency is highly enhanced for both positive and negative charged proteins to achieve a broadband digestion. It is expected that the new strategy will lead to promising advances in protein analysis and other bioapplications where enzymatic reactions are involved.

EXPERIMENTAL SECTION

Synthesis and Characterization of Material. The MOSF materials were synthesized according to our previous report,[30] and MgO-MOSF was synthesized by a simple wet impregnation according to the literature method.[32] The synthesis of MOSF was carried out at 35 °C in buffer solutions (pH 5.0) with the presence of tetramethyl orthosilicate as a silica source and poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) ($EO_{20}PO_{70}EO_{20}$) copolymer as a template. The final MOSF products were obtained by calcination at 550 °C for 5 h. In a typical impregnation process, 0.4 g of calcined MOSF was dispersed in 30 mL of saturated magnesium nitrate water solutions under stirring overnight, filtrated and then baked at 673 K for 3 h. Scanning electron microscopy (SEM) images were recorded on a JEOL 6400microscope operated at 10 kV, where the samples were coated with platinum. The Mg/Si molar ratio was measured by the energy dispersive X-ray (EDX) spectroscopy. Nitrogen sorption isotherms of samples were obtained by a Quantachrome's Quadrasorb SI analyzer at 77 K. Before measurements, samples were degassed at 473 K for 6 h in vacuum. A zeta-potential meter (Malvern Zetasizer Nano) was used to measure the zeta potentials of materials by dispersing the materials in ammonium bicarbonate buffer (25 mM, pH ~8.0) at 298 K.

Immobilization of Proteins or Enzymes into Materials. Kinetic experiments to determine the amount of trypsin (molecular diameter ~3.8 nm, molecular mass ~23.8 kDa) adsorbed into MOSF as a function of contact time were conducted by mixing 4 mL of 1.0 mgmL^{-1} protein solution (25 mM NH_4HCO_3 buffer at pH 8.0) with 5 mg of MOSF under stirring at 298 K. The immobilization capacity of proteins and enzymes in materials were evaluated by measuring saturated protein adsorption amounts. Typically, 1 mg of macroporous materials were added into 500 μL of different protein solutions with varied concentrations in the ammonium bicarbonate buffer (25 mM, pH ~ 8.0). The mixtures were stirred at 298 K for 2 h to reach the adsorption equilibrium. The adsorbed amount was measured using a difference method with protein concentrations determined before and after loading of substrate by UV absorption at 280 nm[33] on a V-550 UV/vis spectrophotometer.

Analysis of the Standard Proteins. In-solution digestion was performed according to a widely used published method.[20] Various amounts of proteins were dissolved in ammonium bicarbonate buffer (25 mM, pH ~8.0) and incubated at 37 °C with trypsin at an enzyme/substrate ratio of 1:30 (w/w). For porous materials catalyzed digestion, the catalyst was directly added into the above-mentioned system with a final concentration of 0.13 mg/mL (except that 0.33 mg/mL catalyst was used when the myoglobin concentration is 2 ng/μL). The digestion products were analyzed on an Applied Biosystems 4700 proteomics analyzer (matrix assisted laser desorption/ionization time-of-flight mass spectrometry, MALDI-TOF MS), using α-cyano-4-hydroxycinnamic acid (CHCA, 99%) as matrix. The instruments polynomial to correlate time-of-flight with m/z was precalibrated by an external calibration in accordance with the manufacturer's recommendations, using standard myoglobin digest. All mass spectra were obtained in the positive ion reflector mode with an accumulation of 2000 laser shots under a laser intensity of 4000 instrument units, where 20 different sites in each spots were detected and each site proceeded 100 times of laser shots. Two spots were deposited and detected for one sample each time, and repeated at least for three times. Mass spectrometric data analysis was performed on the GPS Explorer software from Applied Biosystems with Mascot as a search engine and NCBInr as a database. During database

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searching, at most one missed cleavage site about proteolysis was accepted.

Preparation of the Complex Biological Samples. The normal human liver cytoplasm sample was obtained from Liver Cancer Institute of Zhongshan Hospital, Fudan University. The protein extraction procedure has been previously reported.[20] After lyophilizing, appropriate amount of extracted proteins was dissolved in 300 μL reducing solution (6 M guanidine hydrochloride, 100 mM ammonium bicarbonate, pH 8.3) with the protein concentration adjusted to 3 mg/mL and mixed with 1 μL of 1 M dithiothreitol. The mixture was incubated at 37 °C for 2.5 h. Then 5 μL of 1 M iodoacetamide was added and incubated for another 30 min at ambient temperature in darkness. After these procedures, the sample was lyophilized and redissolved in 300 μL acidic buffer (0.1% trifluoroacetic acid (TFA, 99.8%), 5% acetonitrile (ACN, 99.9%), and 94.9% H_2O) for reverse phase liquid chromatography (RPLC) separation. The prepared solution was first injected and captured on a trap column, and then eluted and separated on an analytical LC column (Agilent ZORBAX SB-C18, 4.6 × 250 mm, 5 μm) for 60 min by using 0.05% TFA in water as mobile phase A and 0.05% TFA in acetonitrile as mobile phase B on a Shimadzu LC-20AD system capillary pumping system.

Analysis of the Biological Samples. A fraction of the abovementioned separated proteins (incubation time from 40 to 50 min) was selected, lyophilized and redissolved in the ammonium bicarbonate buffer (25 mM, pH ~ 8.0) with a final concentration of 1 mg/mL. For in-solution digestion, 20 μg proteins were incubated overnight with trypsin at an enzyme/substrate ratio of 1:30 (w/w). The porous materials catalyzed digestion was performed by adding an additional amount of 40 μg materials, and the digestion time was shortened to 2 h. The digested peptides were analyzed by RPLC/ESI-MS/MS using an Applied Biosystems QSTAR XL mass spectrometer. The analysis of the biocomplex sample was performed according to the general accepted protocols, which have also been adapted in recent literatures.[34] The parameters for database searching were set as follows: (1) mass tolerance at ±0.6 Da for MS and ±0.8 Da for MS/MS, (2) at most one miss cleavage site of tryptic enzyme specificity, (3) charge state 2, 3, and 4) no smoothing of spectra was applied. Peptides matched with significant homology ($p < 0.05$) were considered as identified peptides. To further increase the degree of confidence of proteins, the Mascot search program, with a database of IPI_HUMAN_3.35, was used for peptide sequence identification.

RESULTS AND DISCUSSION

Characterization of Macroporous Materials. Figure 1 displays the SEM images of MOSF and MgO-MOSF materials. For MOSF, foam-like structures can be observed and the pore size is estimated to be ~100 nm (Figure 1a). In the case of MgO-MOSF, Figure 1b shows that the macroporous structure of MOSF is successfully preserved after the impregnation of MgO. The EDX measurement reveals a final Mg/Si molar ratio of 0.75. Meanwhile, the zeta potential is measured to be −35.7 and +5.82 mV for MOSF and MgO-MOSF in a NH_4HCO_3 buffer respectively, showing that MOSF is negatively charged whereas MgO-MOSF is positively charged in a buffer condition at pH ~8.

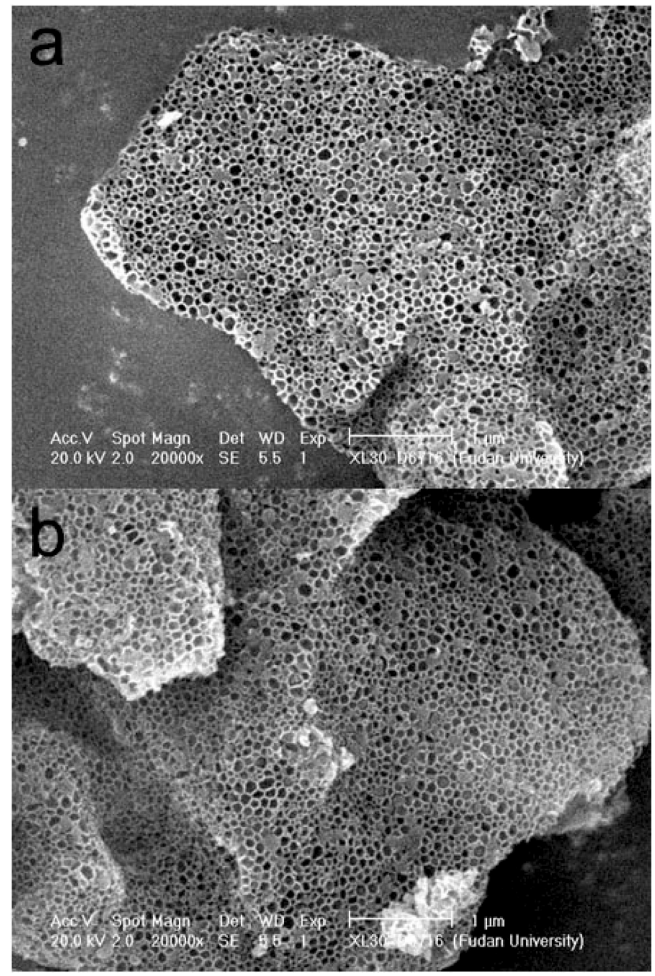

Figure 1. SEM images of (a) MOSF and (b) MgO-MOSF.

Table 1. Adsorption Capacity of Proteins by Materials[a]

material	protein	MW (kDa)	pI	Cs (μM)
MOSF	cytochrome-c	12.4	10.6[35]	90
MOSF	trypsin	23.8	8.23[20]	26
MOSF	myoglobin	16.9	6.8[36]	7
MOSF	BSA	66.4	4.9[35]	0.8
MOSF	ovalbumin	45.0	4.6[35]	0.7
MgO-MOSF	ovalbumin	45.0	4.6[35]	16.9
MgO-MOSF	BSA	66.4	4.9[35]	2.8

[a] Note: MW is molecular weight, Cs denotes saturated adsorption amount.

The nitrogen sorption analysis shows that MOSF has a surface area of 430 m^2/g and pore volume of 1.3 cm^3/g, whereas the surface area and pore volume of MgO-MOSF decrease to 190 m^2/g and 0.5 cm^3/g, respectively, which can be attributed to the impregnation of magnesium oxide on MOSF surfaces.

Benefiting from the large pore size and high pore volume, MOSF materials show very fast adsorption kinetics and high immobilization capacity. Besides, selective adsorption of proteins can be achieved by adjusting the surface charge of materials. Various proteins with different pI were chosen to testify the immobilization capacities of MOSF and MgO-MOSF (Table 1). Supporting Information (SI) Figure SI-1 shows the adsorption amounts of trypsin in MOSF as a function of time. It is noted that

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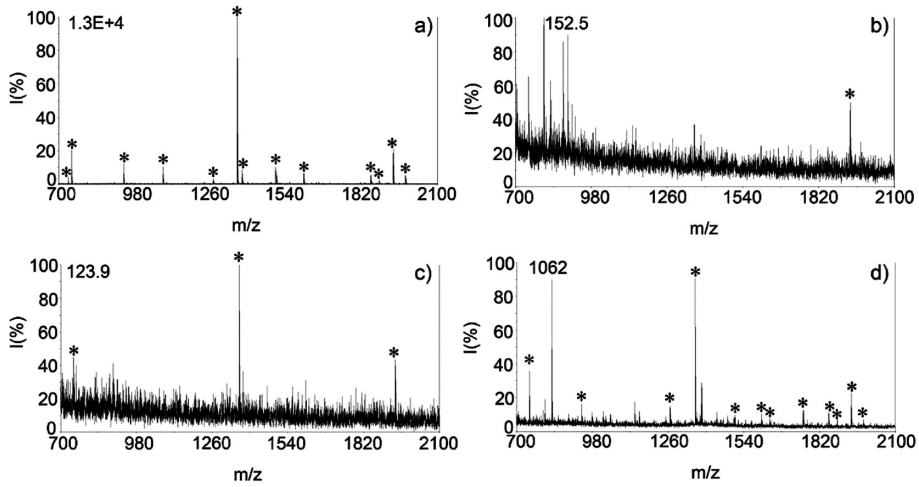

Figure 2. PMF spectra of proteolysis products from (a) 30 min MOSF catalyzed myoglobin (20 ng/μL) digestion, (b) 30 min in-solution myoglobin (20 ng/μL) digestion, (c) 12 h in-solution myoglobin (2 ng/μL) digestion, and (d) 30 min MOSF catalyzed myoglobin (2 ng/μL) digestion.

~95% of the maximum adsorption amount can be achieved in less than 1 min, illustrating a fast adsorption rate. As shown in Table 1, the maximum loading amounts for myoglobin (pI 6.8), trypsin and cytochrome-c (pI 10.6[35]) are 7, 26, and 90 μM (g MOSF)$^{-1}$ respectively, which is indeed large enough. In the case of ovalbumin (pI 4.6[35]) and bovine serum albumin (BSA, pI 4.9[35]), the maximum loading amounts in MOSF are much lower, which are 0.7 and 0.8 μM (g MOSF)$^{-1}$, respectively. In contrast, the immobilization capacities of MgO-MOSF toward these two proteins are significantly increased to 16.9 and 2.8 μM (g MgO-MOSF)$^{-1}$. To be mentioned, these adsorption experiments were all performed in a buffer at pH ~ 8.0. By analysis of the surface charge of proteins and materials, this kind of specificity can be explained with an electrostatic interaction mode. In the current adsorption condition, MOSF is negatively charged whereas MgO-MOSF is positively charged. Thereby, the negatively charged BSA and ovalbumin are of course preferentially extracted by the MgO-MOSF, whereas the positively charged cytochrome-c and approximately neutral myoglobin and trypsin can be easily adsorbed by the MOSF. The electrostatic interaction directed adsorption of proteins can be applied to perform selective nanospace confined proteolysis.

Efficient Protein Digestion by Macroporous Catalysts. The nanopore confined proteolysis was first investigated using standard protein samples. The results show that the method is not only simple, but also highly efficient and sensitive for the analysis of proteins, where the enzymatic reaction rate is greatly increased in the presence of MOSF. Horse heart myoglobin, a protein reported to be resistant to tryptic digestion, was first used to study the macroporous material catalyzed digestion.[36] The peptide mass fingerprinting (PMF) results of myoglobin digested (20 ng/μL) for 30 min in the presence of MOSF (Figure 2a) are much better than that obtained from standard in-solution digestion of 30 min (Figure 2b), and comparable to the results of standard overnight in-solution digestion (SI Figure SI-2a). With MOSF, 15 peptides originated from myoglobin are confidently identified with a MOlecular Weight SEarch (MOWSE) score of 160 and amino acid sequence coverage of 94% (Table 2). In contrast, the in-solution digestion yields only one peptide peak at a very low signal/noise (S/N) ratio in 30 min and 12 peptides (MOWSE score of 133, sequence coverage of 75%) for overnight proteolysis (Table 2). When the myoglobin concentration is decreased to 2 ng/μL, the standard in-solution digestion is not efficient even after 12 h incubation, which yields only three matched peptides (Figure 2c). However, in the presence of MOSF, the proteolysis is greatly accelerated, where 13 peptides are identified with the sequence coverage of 75% in the 2 ng/μL myoglobin sample after an incubation time of only 30 min (Figure 2d). The experiments were repeated for three times, and two spots were deposited and detected from one sample each time. For the MOSF catalyzed myoglobin digestion, the data analysis from six spots (SI Figure SI-3) reveals that the average sequence coverage is 94% with an average relative deviation of 2%, and the average database searching score is 159 with an average relative deviation of 5% (SI Table SI-1), indicating that the repeatability is well satisfied. Thus the overall variability can be well controlled in the analysis.

Different from the previous mesoporous material and/or microchip based enzyme reactors[20−22] where a preimmobilization of trypsin in the nanopores or microchannels is required, the present proposed strategy is based on a conventional in-solution digestion system, by simply adding a tiny amount of macroporous materials as catalysts into the digestion solution without any pretreatment. The digests together with trace amount of catalysts can be directly sent to mass spectrometer for analysis, thus providing a simple and economical protocol for fast proteolysis that can be universally applied to the high-throughput proteomic analysis. For the conventional in-solution digestion, the concentrations of enzyme and substrate largely affect the enzymatic reaction rate.[20] In our experiments, the concentration of MOSF in the proteolysis system is 0.13 mg/mL. Considering the equilibrium adsorption capacity of proteins in MOSF, it is estimated that the majority of enzyme and substrates can be quickly absorbed into MOSF, where a concentration increase of ~6000 times is anticipated (from 0.00067 mg/mL in solution to 3.9 mg/mL in macropores for enzyme, and from 0.02 mg/mL to 118 mg/mL for substrates). Thus, the enhanced efficiency of nanospace confined digestion is attributed to the in situ enrichment of both

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Table 2. In-Solution Digestion and MOSF or MgO-MOSF Catalyzed Proteolysis Results of Various Standard Proteins with Different Concentrations

protein name	protein concentration (ng/μL)	catalyst	incubation time (h)	number of matched peptide	MOWSE score	sequence coverage (%)	absolute signal intensity
myoglobin	20	MOSF	0.5	15	160	94	1.3×10^4
myoglobin	20	none	0.5	1	failed	failed	0.2×10^3
myoglobin	20	none	12	12	133	75	3.7×10^3
myoglobin	2	MOSF	0.5	13	84	75	1.0×10^3
myoglobin	2	none	12	3	failed	failed	0.4×10^3
BSA	20	MOSF	0.5	22	64	31	1.3×10^3
BSA	20	MgO-MOSF	0.5	32	78	36	8.8×10^3
BSA	20	none	0.5	30	68	42	3.7×10^3
cytochrome-c	20	MOSF	0.5	13	106	77	5.3×10^3
cytochrome-c	20	MgO-MOSF	0.5	failed	failed	failed	0.2×10^3
cytochrome-c	20	none	0.5	8	79	69	2.3×10^3
ovalbumin	20	MOSF	0.5	9	98	38	0.6×10^3
ovalbumin	20	MgO-MOSF	0.5	15	126	36	3.1×10^3
ovalbumin	20	none	0.5	10	89	52	1.1×10^3
β-lactoglobulin	20	MOSF	0.5	5	failed	failed	0.3×10^3
β-lactoglobulin	20	MgO-MOSF	0.5	15	76	82	9.9×10^3
β-lactoglobulin	20	none	0.5	11	67	79	0.5×10^3

enzymes and substrates. As a consequence, the digestion time is reduced while the detection sensitivity is largely enhanced.

Electrostatic Interaction Controlled Protein Digestion. Based on the above-mentioned electrostatic interaction directed protein adsorption, this nanopore confined proteolysis can be applied to specifically accelerate digestion of proteins with varied isoelectric points. The basic proteins, such as cytochrome-c, are expected to be preferentially adsorbed and then digested in the presence of MOSF, while the acidic proteins, such as ovalbumin, BSA and β-lactoglobulin (pI 5.1[37]), are preferentially extracted and digested in the presence of MgO-MOSF.

For cytochrome-c, the in-solution digestion (30 min) yields eight matched peptides with a sequence coverage of 69%, a maximum absolute signal intensity of 2300 and a MOWSE score

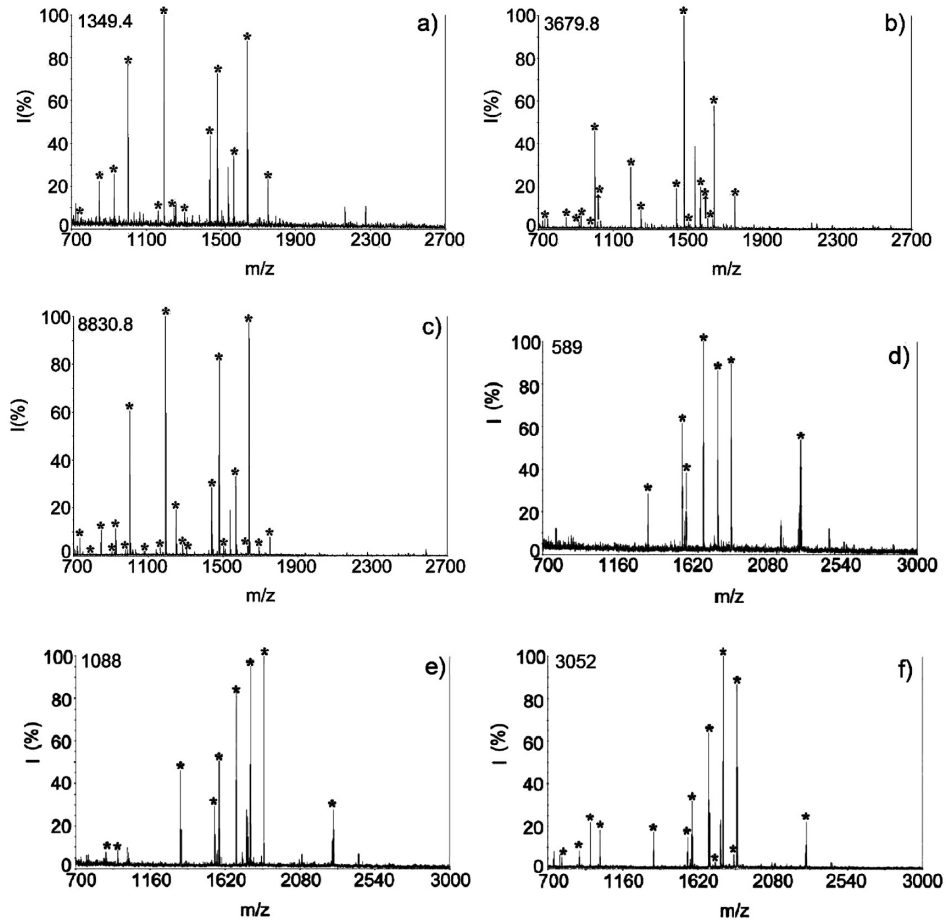

Figure 3. PMF spectra of proteolysis products from (a) 30 min MOSF catalyzed BSA (20 ng/μL) digestion, (b) 30 min in-solution BSA (20 ng/μL) digestion, (c) 30 min MgO-MOSF catalyzed BSA (20 ng/μL) digestion, (d) 30 min MOSF catalyzed Ova (20 ng/μL) digestion, (e) 30 min in-solution Ova (20 ng/μL) digestion, and (f) 30 min MgO-MOSF catalyzed Ova (20 ng/μL) digestion.

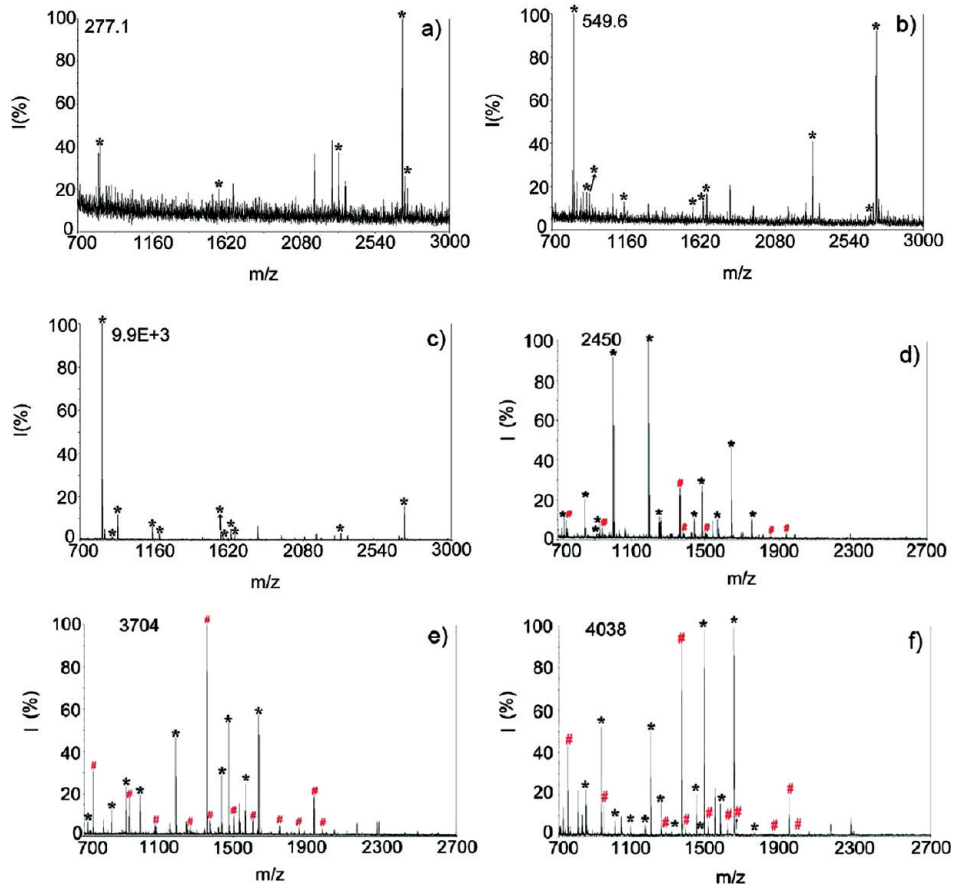

Figure 4. (a) 30 min MOSF catalyzed β-lactoglobulin (20 ng/μL) digestion, (b) 30 min in-solution β-lactoglobulin (20 ng/μL) digestion, (c) 30 min MgO-MOSF catalyzed β-lactoglobulin (20 ng/μL) digestion, (d) 30 min in-solution digestion of the protein mixture of myoglobin (20 ng/μL) and BSA (20 ng/μL), (e) 30 min MOSF catalyzed, and (f) 30 min mixture of MOSF and MgO-MOSF catalyzed tryptic digestion of the above protein mixture in (d). # peptides from myoglobin, * peptides from BSA.

of 68, whereas 13 matched peptides with a sequence coverage of 77%, a maximum absolute signal intensity of 5300 and a MOWSE score of 106 are observed in the presence of MOSF for the same digestion (SI Figure SI-2b and c, Table 2). However, the PMF spectrum suggests that the digestion efficiency is largely decreased in case of MgO-MOSF catalyzed system, where a maximum absolute signal intensity of only 200 is obtained and the database searching is failed to identify the protein (Figure SI-2d, Table 2).

For BSA, the 30 min MgO-MOSF catalyzed digestion yields 32 matched peptides with a maximum absolute signal intensity of 8800 and MOWSE score of 78, whereas in-solution digestion yields 30 matched peptides with a maximum absolute signal intensity of 3700 and a MOWSE score of 68 in 30 min. Meanwhile, only 22 matched peptides with a maximum absolute signal intensity of 1300 and a MOWSE score of 64 are observed in the presence of MOSF in 30 min (Figure 3a−c). Same as BSA, the enzymatic reaction of ovalbumin is slowed down in the presence of MOSF (Figure 3d) compared to the in-solution digestion (Figure 3e). In-solution proteolysis yields 10 matched peptides with the sequence coverage of 52% and a maximum absolute signal intensity of 1089, whereas nine matched peptides with the sequence coverage of 38% and a maximum absolute signal intensity of 590 are observed in the presence of MOSF in 30 min. On the other hand, in the presence of MgO-MOSF, the PMF result shows an excellent catalysis effect which is much better than that obtained by in-solution digestion. An identification of 15 peptide peaks with a MOWSE score of 126 and an absolute signal intensity of 3052 was observed (Figure 3f, Table 2).

A similar catalysis effect is also observed in β-lactoglobulin (pI 5.1[37]) digestion. The database searching is failed in the presence of MOSF with a maximum absolute signal intensity of 300, whereas 15 matched peptides with a maximum absolute signal intensity of 9,900 and a MOWSE score of 76 are observed in the MgO-MOSF catalyzed proteolysis, and 11 matched peptides with a maximum absolute signal intensity of 500 and a MOWSE score of 67 are observed for in-solution proteolysis (Figure 4a−c). These conclusions can be directly obtained by comparing the matched peptides, the score and the sequence coverage obtained by MOWSE of the proteins as shown in Table 2. It is noted that for the protein with relatively low pI, an increased reaction rate is detected in the presence of MgO-MOSF, whereas a decreased reaction rate is found in the presence of MOSF. Accordingly, both protein adsorption and proteolysis results show that the surface charge of catalysts is another factor to affect the interaction between catalyst and biomolecules in addition to their large macroporous structure on the effect of the catalyzed digestion reaction. Therefore, selective enzymatic reactions can be achieved in our approach by controlling the surface charges of macroporous materials.

Selective Catalysis of Protein Mixture. A protein mixture of BSA (20 ng/μL) and myoglobin (20 ng/μL) was used to further

Table 3. MOSF or MgO-MOSF Catalyzed Proteolysis Results of Protein Mixture of Myoglobin (20 ng/μL) and BSA (20 ng/μL)

catalyst	identified protein	number of matched peptide	MOWSE score	sequence coverage (%)
none	both failed	failed	failed	failed
MOSF	myoglobin	13	118	81%
mixture of MgO-MOSF and MOSF	both BSA and myoglobin	15 for BSA; 10 for myoglobin	128 for BSA; 116 for myoglobin	21% for BSA; 75% for myoglobin

prove the selective acceleration of tryptic reaction by using the macroporous catalyst. The database searching result of the PMF spectra is summarized in Table 3. After in-solution digestion for 30 min, both BSA and myoglobin are failed to be identified (Figure 4d). While in the presence of MOSF as a catalyst, only myoglobin is successfully identified with a MOWSE score of 118 and amino acid sequence coverage of 81% (Figure 4e). However, when using the mixture of MOSF and MgO-MOSF as cocatalysts, both myoglobin and BSA are identified at the same time. The MOWSE score of BSA and myoglobin are 128 and 116, the sequence coverage of BSA and myoglobin are 21% and 75%, and the number of matched peptides of BSA and myoglobin are 15 and 10, respectively (Figure 4f).

The results validate that the selective catalysis of protein mixture can be realized. Taking the protein mixture of BSA and myoglobin for instance, in-solution digestion is quite limited in efficiency and failed to identify neither of the proteins (Figure 4d). For comparison, the relatively basic protein, myoglobin, could be successfully identified from efficient proteolysis catalyzed by MOSF (Figure 4e). Furthermore, by utilizing the mixed catalysts, MgO-MOSF and MOSF, it is feasible to digest both the acidic protein, BSA, and the neutral protein, myoglobin, simultaneously. Based on the electrostatic interaction between biomolecules and catalysts, BSA and myoglobin can be enriched into the nanopores of MgO-MOSF and MOSF separately, thus both of them can be easily digested and identified (see Figure 4f and Table 3). This strategy is promising to be used for selectively enhancing or inhibiting the tryptic reactions toward different target proteins with specific pI, and also for the application of the protein mixture analysis through choosing the catalysts with controlled surface charge.

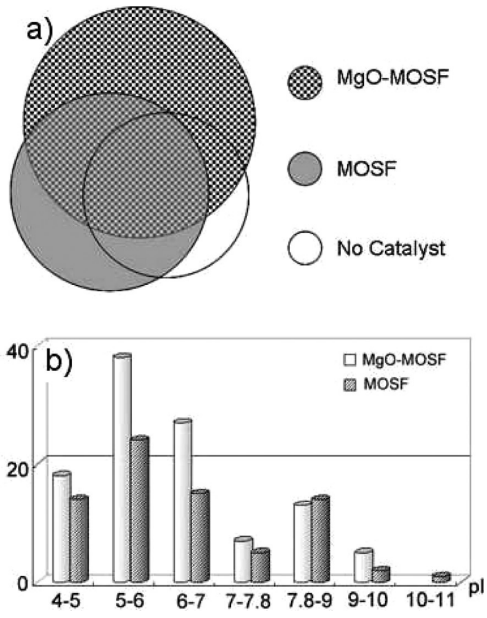

Figure 5. (a) Comparison of the identified proteins by three methods based on the standard overnight in-solution proteolysis technique with no catalyst, with MgO-MOSF and MOSF as catalysts for 2 h. (b) Comparison of identified proteins between digestions catalyzed by MOSF and MgO-MOSF, ignoring the overlap of the identified proteins between the methods.

Application to the Complex Biological Samples. Finally, the macroporous materials are applied to the analysis of a complex biological sample, a fraction of the proteins extracted from the cytoplasm of human liver tissue. As shown in Figure 5a, the standard overnight in-solution proteolysis method gives 100 proteins, while the MOSF and MgO-MOSF based digestion systems identify 165 and 198 proteins, respectively, for only 2 h (Figure 5a and SI Table SI-2,3,4). Taking the overlapped proteins into account, a total of 293 proteins are identified, where the overnight in-solution digestion contributes only 18 specific proteins. Moreover, the number of peptides identified using MOSF and MgO-MOSF as catalysts is significantly higher (237 and 173%) than that obtained by the conventional overnight in-solution digestion. Comparing with the results of MOSF system, 97% (32 of 33) excessive proteins identified in MgO-MOSF system are located in the pI range of 4−7.8 (Figure 5b), consistent with the proposed electrostatic interaction controlled digestion model. The results clearly indicate that the macroporous materials can be used as catalysts to make the tryptic digestion more efficient and sensitive, and for the successful application to a complex biological sample analysis.

CONCLUSION

In summary, we have demonstrated a concept of using macroporous materials as catalysts for highly efficient and controllable digestion. This new method is very simple, efficient and can be easily coupled with commercially applicable apparatus in proteomic research. Moreover, by adjusting the surface charge of catalysts to match the electrostatic interaction between catalyst and proteins/enzymes, selective acceleration of digestions can be achieved. Using the mixed materials as cocatalysts, we can extract the complete information from an unknown biological sample. It is anticipated that the nanopore based digestion strategy will lead to promising advances in the general biochemical applications where enzymatic reactions are involved by further optimizing the pore structure and surface properties.

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SUPPORTING INFORMATION AVAILABLE

Figure describing the adsorption of trypsin into MOSF as a function of time. Tables listing the MOSF catalyzed proteolysis results of myoglobin from six various spots, proteins identified from a fraction of proteins extracted from the cytoplasm of human liver tissue via in-solution digestion, MOSF catalyzed digestion and MgO-MOSF catalyzed digestion. Mass specrtra proteolysis products from 12 h in-solution myoglobin (20 ng/μL) digestion, 30 min MOSF catalyzed cytochrome-c digestion, 30 min in-solution cytochrome-c digestion, 30 min MgO-MOSF catalyzed cytochrome-c digestion, and 30 min MOSF catalyzed myoglobin (20 ng/μL) digestion from six various spots. This material is available free of charge via the Internet at http://pubs.acs.org.

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