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Mechanism exploration of adsorption-immobilized enzymatic reactor using polymer-coated silica microbeads

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ABSTRACT

A verified mechanism of adsorption-immobilized enzymatic reactor for enhanced proteolysis is presented. Silica microbeads coated with poly (diallyldimethylammonium chloride) (PDDA) or poly (styrene sulfonate) (PSS) were used to trap trypsin and proteins on the surface through electrostatic interactions in order to improve digestion efficiency. Charge states measured by zeta-potentials showed their positively and negatively charged respectively. We found that high proteolytic efficiency could be achieved only if both proteases and proteins were adsorbed by materials. Once the proteins and proteases were confined together in a nanoscopic area, the enrichment of the substrate could lead to a high performance proteolytic effect. Electrostatic interactions were considered as the predominant adsorption factor rather than hydrophilic/hydrophobic interactions. In less than 5 min, in the presence of PSS-coated silica beads, 10 peptides digested from positively-charged cytochrome C were detected by matrix-assisted laser desorption/ionization mass spectrometry (MALDI-TOF), with the high sequence coverage up to 63%, while using PDDA-coated silica beads or conventional in-solution digestion yielded only 5 detectable peptides and 39% sequence coverage was obtained. Ovalbumin seemed incompatible with any kind of charged-material-aided tryptic digestion. The mechanism of adsorption-immobilized enzymatic processes has also been studied in detail. The adsorption equilibrium was proven to be attained in less than one minute, and the proteolytic procedure was regarded as the rate-determining step. This study provides a reasonable mechanism for an adsorption-material catalyzed proteolytic procedure and a promising guideline for designing the next generation of high-performance enzymatic reactors.

1. Introduction

A broad consensus has been achieved that mass spectrometry (MS) is one of the most powerful tools for detection and identification of proteins [1]. MS based bottom-up strategy is widely used due to its superior sensitivity and high resolution in the mass range of peptides [2,3]. This strategy involves the site-specific cleavage by protease (for example, trypsin), LC or gel-based separation and subsequent identification by peptides mass fingerprint (PMF) or tandem mass searched for in a theoretical digestion database [4,5].

As the critical step of bottom-up strategy, conventional enzymatic digestion procedure still limits the overall sensitivity and quality because of strong enzyme autolysis, low digestion efficiency, extended incubation time and tedious manual operation [6]. In the past few years, many immobilized enzymatic reactors have been developed to improve digestion efficiency [7–17]. A relatively higher concentration of enzymes was achieved when enzymes were confined in a narrow space. As a result, digestion could be accelerated and the autolysis of the enzyme would be reduced. Most research focuses on trypsin immobilization with emphasis on covalently binding trypsin onto surface modified beads [7–9], monolithic column [11–13] or trapping the enzymes into porous silicon [14,15], while little attention is paid to physically adsorbed carriers [16,17]. These adsorption-immobilized enzymatic reactors can also achieve high digestion efficiency with few preliminary treatment steps. Qiao and co-workers [18] reported that cyano-functionalized mesoporous silicate was a good support for the immobilization of trypsin and made a good effort to enhance digestion. However, the mechanism of physical adsorption-immobilized enzymatic reactor has not been systematically discussed. Qian [19] showed their opinion that the physical adsorption was achieved due to electrostatic interactions. Selective extraction and digestion of proteins with different isoelectric points can be achieved by adjusting the surface charge of the catalysts. However, the other groups

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represented hydrophilic/hydrophobic interactions between material and protein which also played an important role in the adsorption process [20].

Herein, we discuss the mechanism of physical adsorption for the enzymatic digestion reactor in detail. Both electrostatic and hydrophilic/hydrophobic interactions were taken into consideration. Silica microbeads (S-1) coated with different polymers have been synthesized to evaluate the catalytic efficiency during the proteolytic process. In the digestion buffer (e.g., 25 mM ammonium bicarbonate solution, pH 8), S-1 coated with poly diallyldimethylammonium chloride (S-PDDA) or poly styrene sulfonate (S-PSS) were either positively or negatively charged individually [21] while S-1 linked with hydrophobic C11 chain (S-M) possessed negative charges in addition to hydrophobic character. We used these materials for standard protein digestion and discovered that charge-carrying materials had biased catalysis efficiency against proteins with different isoelectric points. The electrostatic interactions have been demonstrated as the driving forces in the adsorption procedure rather than hydrophilic/hydrophobic interactions. Based on electrostatic interactions, proteins and proteases that carry the opposite charges against the material could be adsorbed onto the surface of the material together, thus making the local concentration of substrates much higher. The confinement of proteins and enzymes in a limited space would greatly enhance the proteolytic efficiency. On the other hand, once the proteins and enzymes carry different charges, they could not be adsorbed by materials simultaneously. As a result, no positive performance would be obtained compared with traditional in-solution digestion. Besides this dominant factor of proteolysis efficiency, adsorption process has also been explored. We found the adsorption equilibrium of a specific concentration of substrates could be reached in a very short time and the proteolytic procedure was regarded as the rate-determining step. We believe that the study is meaningful and helpful for the development of next generation adsorption-immobilized enzymatic reactors.

2. Experimental

2.1. Synthesis and characterization of surface-modified silica microbeads

Silicalite-1 (S-1) nanoparticals were hydrothermally synthesized as previously reported [22]. In the presence of microwave irradiation according to the gel composition of 1:0.195:19.60 SiO2:tetrapropylammonium (TPAOH):H2O, the precursor sols were stirred continuously at room temperature for 48 h before they were hydrothermally treated under microwave irritation. The microwave-assisted synthesis procedure was divided into two steps according to the hydrothermal-treated temperature. The synthesis solution was firstly treated at 80 °C for 90 min under microwave irritation, and then the synthesis temperature was increased to 120 °C to facilitate the crystal growth. The resulting nanocrystals were cooled to room temperature and then washed with distilled water and centrifuged 4–5 times at 15000 rpm (Anke TGL-16 G-A). The non-pulsed microwave irritation made the heating continuously and preciously (limit of accuracy: ± 1 °C) in the temperature range of 0-250 °C.

The S-1 particles were soaked into cationic PDDA (0.2 wt%) and anionic PSS (0.1 wt%) solutions sequentially to coat 3 or 4 layers of electrolytes in the order of PDDA/PSS/PDDA or PDDA/PSS/PDDA/PSS, which resulted in particles with either positive or negative charges via electrostatic attractions. All of these coating processes were accomplished at room temperature in 20 min. The S-M particles were prepared from the sols with methoxydimethyl–undecylsilane which was added into the sol, i.e. the low temperature process (80 °C for 120 min), and then the system was increased to 120 °C for 60 min to facilitate the crystal growth by microwave irradiation.

The size, morphology and dispersibility of the crystal particles were observed by scanning electron microscope (SEM, Philips XL 30). The ultimate size of the nanoparticles was determined by the average value of more than 100 nanoparticles according to the SEM images. To estimate the amount of protein adsorbed by material, we measured the UV absorbance of the proteins solution before and after the addition of the materials [23]. The material solution was used as the control sample for data calibration. UV wavelength was chosen to be 595 nm.

2.2. Standard protein digestion

All the protein solutions were firstly denatured at 100 °C for 5 min. For in-solution digestion, various amounts of cytochrome C and ovalbumin were dissolved in special buffer (e.g. 25 mM ammonium bicarbonate, pH 8) with the enzyme-to-substrate ratio at 1:40 (w/w), and incubated at 37 °C. For material catalyzed digestion, each kind of material was directly added into the same digestion system (without increasing the enzyme or protein concentration), and the solution was also incubated at 37 °C.

2.3. MALDI-TOF MS analysis

For standard proteins, proteolytic efficiency was evaluated by peptide mass fingerprinting (PMF). 0.5 μL of in-solution digest or material catalyzed digest was dropped onto a MALDI steel plate directly and dried at room temperature before the addition of 0.5 μL of 0.6 mg/mL α-cyano-4-hydroxycinnamic acid (CHCA) matrix (freshly prepared in 0.1% TFA, 50% acetonitrile). The tryptic digestion products were analyzed on an Applied Biosystems 5800 proteomics analyzer. Positive ion reflector mode was performed and the spectrum of each spot was obtained by accumulation of 2,000 laser shots. GPS Explorer software from Applied Biosystems with Mascot as a search engine against NCBI database was employed for protein identification. The protein was profiled according to the PMF combined with tandem mass spectra. Trypsin was chosen as the proteolytic enzyme and one miss cleavage was allowed. The mass tolerance of the precursor ion was set to 100 ppm and that of the fragment ions was set to 1.0 Da.

2.4. Measurement of proteins adsorption capacity of materials

The protein amount adsorbed on different surface-modified silica microbeads was determined by UV spectrometry (595 nm). Each protein was dissolved in buffer solution was mixed with 60 μg microbeads and incubated at 37 °C for over than 60 min. After centrifugation at 14,000 rpm for 1 min, the microbeads settled down to the bottom of the container. Then, the supernatant was collected to quantify the protein concentration that remained in the liquid phase via Bradford assay [24]. The solution without protein was chosen for background correction.

3. Results and discussion

3.1. Preparation and characterization of surface-modified silica microbeads

Surface-functionalized silica microbeads were prepared in two steps. First, hydrothermal method had been used to synthesize the core silica microbeads. Then, different derivatives with uniform morphology and size were obtained to investigate the

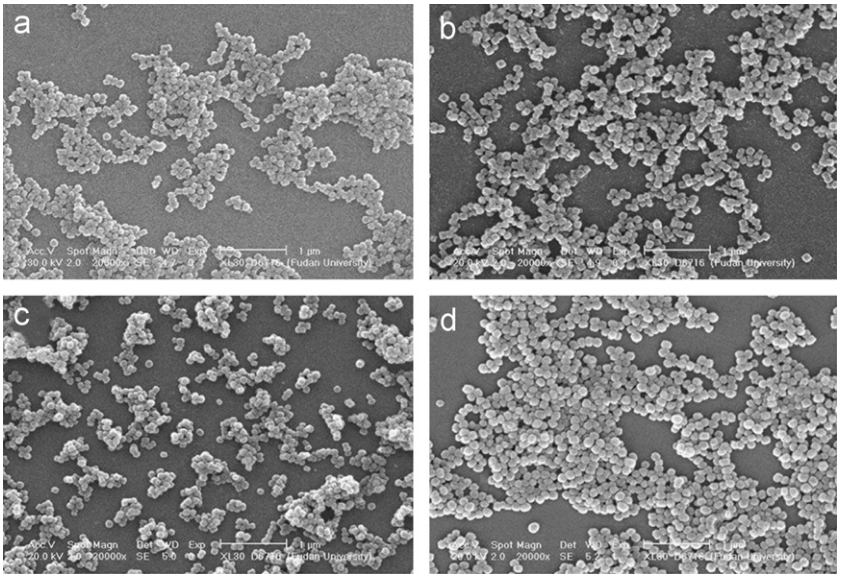

Fig. 1. SEM images of (a) S-1, (b) S-PDDA, (c) S-PSS, and (d) S-M.

proteolytic procedure from various aspects. The reason for choosing silica microbeads laid by thin polymers as the immobilization material was that they could minimize the influence on proteolysis caused by types of catalysts. Fig. 1 displayed the SEM images of both the original silica microbeads and the functionalized derivatives. As shown in Fig. 1a, the particle size of original silica microbeads (S-1) is very uniform with the average diameter of about 100 nm. These silica microbeads are well-dispersed in aqueous solution. S-1 carried negative charges in neutral aqueous solution due to an abundance of hydroxyl group [25], so the positively-charged polymer, PDDA, can easily be attached and laid over S-1 microbeads. Conversely, the negatively charged polymer PSS could be laid over S-PDDA tightly by relying on electrostatic interactions and thus making the surface charge reversed. Fig. 1 (b–d) revealed that S-1 with well-defined outward structures was successfully synthesized. The thin polymer layers PDDA (b), PSS (c) and long alkyl chain C11 (S-M, d) were coated on S-1 individually. The zeta potential was measured to be −40.0, +41.0 and -49.9 mV for S-1, S-PDDA and S-PSS in a 25 mM of ammonium bicarbonate (ABC) buffer respectively, illustrating that bare S-1 and S-PSS presented negative charges whereas S-PDDA presented positive ones in a buffer of pH 8. Benefiting from high charge density and well dispersibility, PDDA and PSS modified microbeads exhibited strongly stable charge properties in specific buffer solutions compared with S-1 microbeads. The zeta potential of S-M (alkyl chain modified silica microbeads) was −47.6 mV, and the contact angle was measure to be 104.6°, indicating that it is negative-charged and possesses hydrophobic character. Each of silica microbeads was further investigated as an enzymatic reactor.

3.2. Investigation of proteolytic efficiency based on material catalyzed digestion

Cytochrome C (CytoC) and ovalbumin (Ova) were chosen as model proteins to investigate material-catalyzed proteolytic efficiency. The theoretical isoelectric point (pI) values of cytochrome C and ovalbumin are 9.59 and 5.19 respectively, indicating these two proteins are typical basic and acidic proteins. It should be noted that trypsin has a pI value of 8.69, so it carries positive charges in digestion buffer (pH 8.0). CytoC was firstly taken as the model substrate to compare the proteolytic efficiency with different digestion methods. For conventional in-solution digestion, 100 μL of 20 ng/μL CytoC was dissolved in ABC buffer (25 mM, pH 8.0) and incubated at 37 °C with trypsin at an enzyme/substrate ratio of 1:40 (w/w). For material-catalyzed digestion, the original silica microbeads (S-1) and different surface-modified silica beads (S-PSS, S-PDDA, and S-M) were directly added into the same digestion system (without increasing the enzyme or protein concentration) to see if these materials could assist in accelerating the enzymatic digestion process. The amount of each material was optimized to a final concentration of 0.3 mg/mL. At time points of 5 min, 15 min, 60 min and 12 h, the digests were collected and analyzed by MALDI-TOF MS. Peptide mass fingerprinting (PMF), combined with tandem mass spectra results, were used to measure the proteolytic efficiency of different digestion method. As shown in Fig. 2, it could be found that in the presence of S-1 (b) or S-PSS (d), the digest efficiency for 5 min is much better than those with S-PDDA (c) or in-solution (a). With S-1 or S-PSS, 10 peptides originated for CytoC were obtained and confidently indentified with an amino acid sequence coverage of 63%, as effective as the same as in-solution digestion for 12 h (f), suggesting that complete digestion had been reached. A maximum absolute signal intensity of 3361.6 in S-1 and 6976.6 in S-PSS method showed a significant improvement of the proteolytic process, compared with the signal intensity of 407.1 in-solution digestion. Meanwhile, the digest solutions could be deposited directly onto the MALDI stainless steel plate for subsequent MS analysis. Additionally, no centrifugation process was needed to separate the material from the solution, making the entire procedure easy to handle and saving the operator time. However, we did not get the same results using S-PDDA. Only 5 peptides have been identified after adding S-PDDA into the digestion system for 5 min and the amino acid sequence coverage was only 39%. After more than 60 min of incubation, the S-PDDA digestion system showed comparable proteolytic efficiency to in-solution digestion: 10 peptides were matched with sequence coverage of 63% (Fig. S1 a–d). All of the above mentioned results demonstrate that electrostatic interactions are one of the essential factors for physical adsorption between materials and proteins. As basic proteins, CytoC and trypsin were positively charged in ABC buffer (pH 8.0) so they can easily be adsorbed simultaneously by negatively charged S-1 or S-PSS. During this process, both protease and to-be digested proteins were enriched and

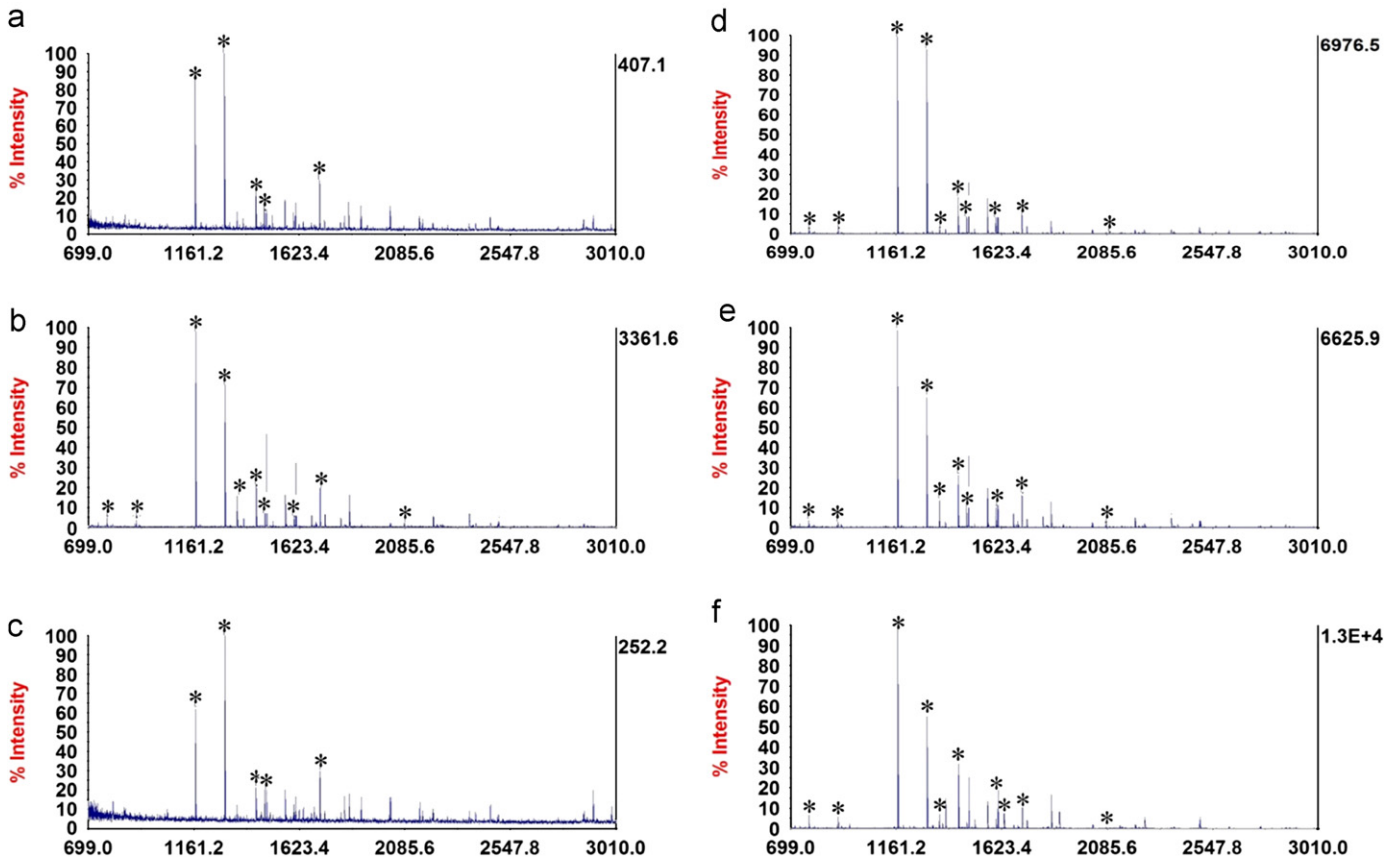

Fig. 2. PMF spectra of 20 ng/μL cytochrome C proteolytic products from in-solution digestion (a), S-1 catalyzed digestion (b), S-PDDA catalyzed digestion (c), S-PSS catalyzed digestion (d), S-M catalyzed digestion (e) at pH 8.0 for 5 min, and in-solution digestion at pH 8.0 for 12 h (f). The peaks marked with asterisks represent identified peptides of cytochrome C.

confined in a limited region, thus greatly increasing the local concentration and making the digestion a very quick reaction. However, when positively charged S-PDDA was added into the solution, basic proteins would be widely dispersed in the solution because of charge repulsion. Similarly, this occurred with in-solution digestion. As for S-M, the silica microbeads modified with alkyl C11 long chains on the surface, the situation is a little bit different. Unlike polymer-coated silica microbeads, the proteins could be adsorbed onto S-M based on hydrophobic effect [20]. When S-M was added into the digestion system, 10 peptides were successfully identified and the sequence coverage increased to 63% in 5 min. Similar results were obtained from S-1, S-PSS and S-M experiment, whenever there was a hydrophilic or a hydrophobic surface. It is reasonable to assume that the electrostatic interactions played a more important part in adsorption than hydrophilic/hydrophobic interactions. Once proteins were adsorbed by materials through electrostatic interactions, the hydrophilic/hydrophobic interactions seemed less influential.

Then we tried to use an acidic protein, Ova, to test our hypothesis in another case. In this instance, 100 μL of 100 ng/μL of Ova with corresponding amounts of trypsin were employed. Our results were less than promising when S-1, S-PDDA, S-PSS or S-M was added. Table 1 shows the continuous proteolysis results lasting up to 12 h. In the first 5 min, only a few weak peaks could be detected with very low sequence coverage. As time went by, we still did not find any positive efforts on digestion using any of our charge-carrying materials, regardless if it was positively charged S-PDDA or negatively charged S-PSS. Furthermore, S-PDDA exhibited a severe suppression effect on digestion. Only 5 peptides and a corresponding 20% of sequence coverage were observed after 12 h of incubation, compared with 12 peptides and 48% of sequence coverage through in-solution digestion over the same time span. These findings supported the hypothesis we deduced from the digestion of the basic protein, CytoC. Electrostatic interactions were supposed to be the dominant factor when physical adsorption interactions occurred between proteins and

Table 1
Comparison of MS sequence coverage of tryptic ovalbumin digests by different surface-modified silica microbeads at pH 8.0[a].

	Incubation time	Peptides identified[b]	Sequence coverage
In-solution	5 min	4	19%
In-solution	15 min	5	21%
In-solution	60 min	9	35%
In-solution	12 h	12	48%
S-1	5 min	3	17%
S-1	15 min	7	28%
S-1	60 min	8	32%
S-1	12 h	13	50%
S-PDDA	5 min	2	10%
S-PDDA	15 min	3	17%
S-PDDA	60 min	3	17%
S-PDDA	12 h	5	20%
S-PSS	5 min	4	19%
S-PSS	15 min	4	19%
S-PSS	60 min	7	28%
S-PSS	12 h	12	47%
S-M	5 min	5	19%
S-M	15 min	6	28%
S-M	60 min	7	28%
S-M	12 h	13	47%

[a] 100 μL of 100 ng/μL ovalbumin were used to evaluated the proteolytic efficiency.
[b] One missed cleavage was allowed.

solid materials. Only in a situation that guarantees both proteins and enzymes are trapped within a confined space can the digestion process be accelerated. When S-1 or S-PSS was used as the immobilization material, trypsin would be entrapped to gather around the microbeads due to different charge types while Ova was still freely dispersed in the buffer. Because the substrates were in great excess relative to the amount of enzymes, in this situation, the protein/peptide-enzyme encounter probability is less influenced. Just as what happened in conventional in-solution digestion, proteolysis can be thought of as a succession of protein/peptide-enzyme encounter reactions that possess limited diffusion. Therefore, there was no obvious improvement or suppression effect compared to in-solution digestion. On the other hand, once S-PDDA trapped Ova through electrostatic interactions, Ova was thought to be confined in a limited space. As a result, the concentration of Ova in the solution would decrease drastically since the majority of trypsin would be retained in the solution; the encounter probability between substrates and proteases would also decrease sharply. This was consistent with what we found in comparison to the digestion efficiency between in-solution digestion and the added S-PDDA condition. As for S-M, a slight improvement was reached in the Ova digestion experiment: 5 peptides were identified with obtained sequence coverage of 21%. This may be due to the hydrophobic interactions that confined the substrates and the proteases within a limited space that promote digestion efficiency. However, these kinds of interactions were not strong enough, so the complete digestion would not be reached until overnight incubation (Shown in Table 1). Herein, we proposed that negative charges taken by S-M must be considered. In the presence of both electrostatic and hydrophilic/hydrophobic interactions effects, the former one took a predominant role in the adsorption process. The negative effects from the

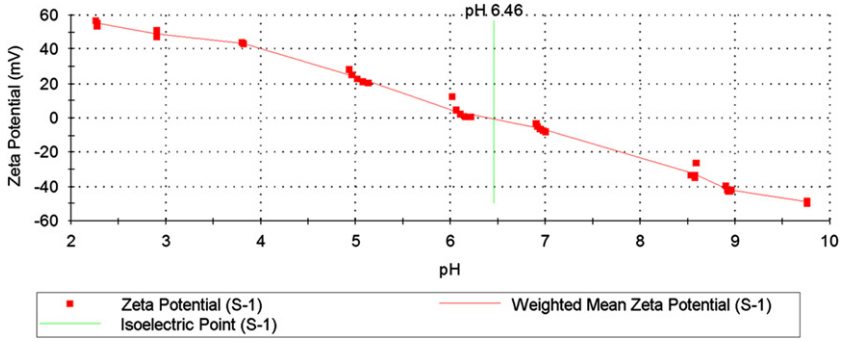

Fig. 3. The curve of zeta potential of S-1 in different pH conditions. The zeta potential describes the nature of electrostatic potential near the surface of a particle. As the pH value increased, the charge state around nanoparticals was changed from positive to negative. The graph showed the isoelectric point was pH 6.46

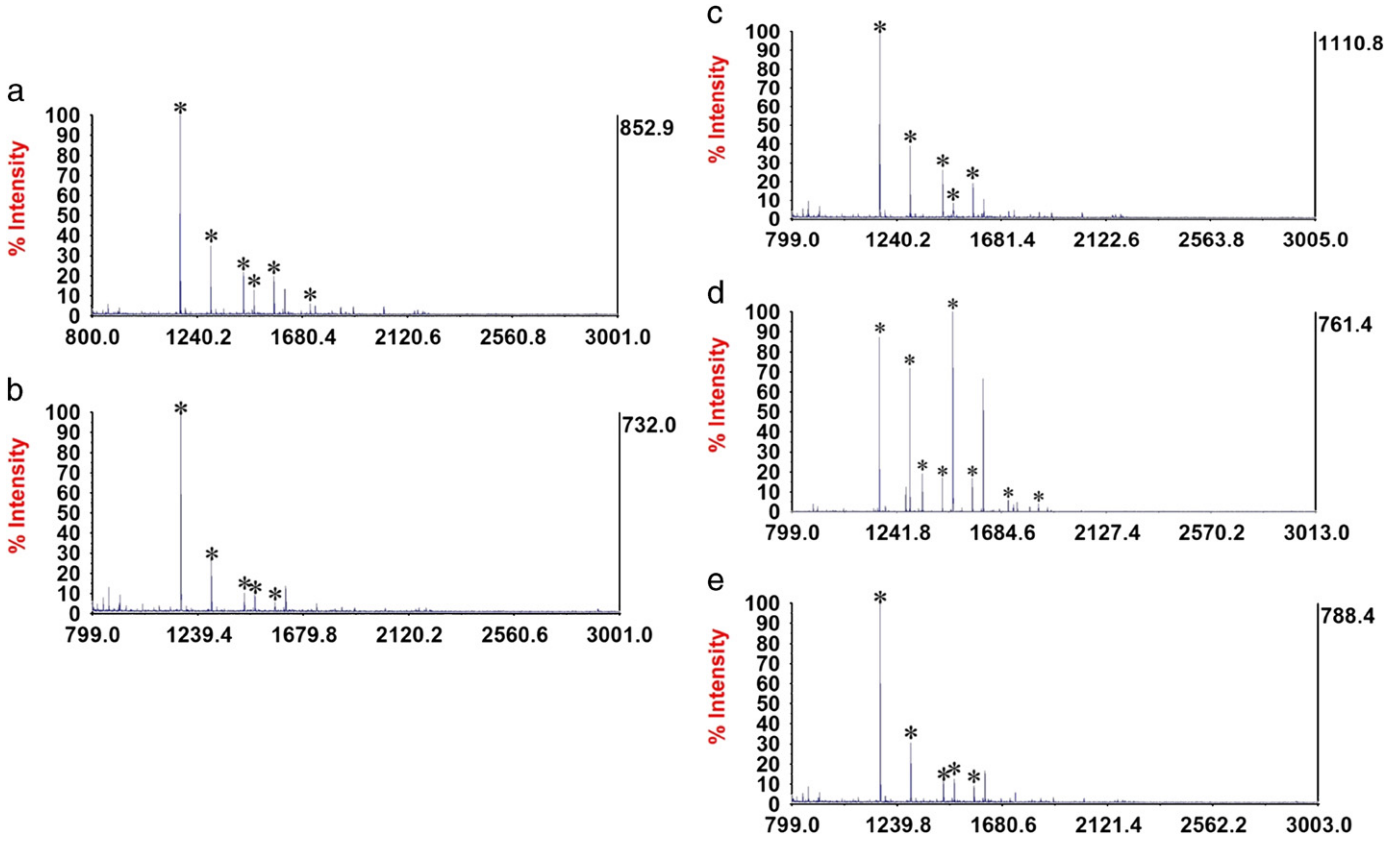

Fig. 4. PMF spectra of 100 ng/μL cytochrome C proteolytic products from (a) in-solution digestion or material catalyzed digestion by (b) S-1, (c) S-PDDA, (d) S-PSS, and (e) S-M at pH 6.11 for 5 min. The peaks marked with asterisk represent identified peptides of cytochrome C.

electric repulsion between Ova and S-M was overwhelming. Several immobilized enzymatic reactor and peptide enrichment strategies [26] were developed based on hydrophobic interactions, but only in the circumstance of none-charged materials. The same conclusion cannot be reached in the presence of charge-carrying materials.

To further investigate if the hydrophilic/hydrophobic interactions are a high influential factor in catalytically enzymatic systems, we evaluated the proteolytic process in an acidic environment. Fig. 3 displays the curve of zeta-potential values under different pH conditions for S-1 microbeads. In high pH conditions, S-1 presented negative value, indicating that it is negatively charged. However, in the lower pH condition, the reverse charge was presented on the surface of S-1. The isoelectronic point is pH 6.46. Therefore, 0.01 M of phosphate buffer (PB) with pH value 6.1 was chosen to be the acidic digestion buffer. The zeta potential was measured to be +2.71, +30.2, −43.3 and −0.9 mV for S-1, S-PDDA, S-PSS and S-M in PB buffer respectively. Under low pH condition, S-1 showed positive-charges but the hydrophilic character remained due to the presence of hydroxyl groups on its surface. Because of lower enzymatic efficiency of trypsin in acidic solution, herein, 100 μL of 100 ng/μL of CytoC with corresponding amount of trypsin with fixed enzyme-to-protein ratio (1:40) was employed. Fig. 4 shows spectra of proteolytic products from (a) in-solution digestion and (b–e) material catalyzed digestion. The proteolytic efficiency of S-1 or S-PDDA catalyzed digestion seems comparable to that of in-solution digestion: five or more peptides were successfully identified with 39% of sequence coverage. S-1 did not present as good effect as that in 25 mM ABC buffer because it was no longer negatively charged, though the hydrophilic property was less influenced by pH value. S-PSS exhibited better catalytic efficiency because it kept its negatively charged surface in the acidic condition. As a polymer with high charge density, PSS has a strong ability to remain negatively charged in a wide pH range. Therefore, it is an ideal enzymatic catalyst for basic proteins, and 8 peptides digested from CytoC were successfully identified in only 5 min. In this way, the electrostatic interactions were proven to be the mostly predominant factor for adsorption that should be considered in adsorption-immobilized enzymatic reactor.

3.3. *Proteins adsorption capacity of materials and the mechanism of adsorption processes*

To evaluate the protein amount adsorbed on different surface-modified silica microbeads, we calculated the UV adsorption of supernatant since proteins had been trapped. Briefly, when 200 μL of 20 ng/μL CytoC dissolved in ABC buffer (25 mM, pH 8.0) was mixed with 60 μg microbeads, 50 μL of the supernatant was collected to quantify the protein concentration that remained in the liquid phase. For Ova, 200 μL of 100 ng/μL Ova and 10 μL supernatant after centrifugation were used. As shown in Fig. 5, the *y*-axis represents the percentage of protein adsorbed on the material, and the adsorption efficiency of different surface-modified silica microbeads could be compared very clearly. In accordance with the microbead-catalyzed digestion result, we found that proteins can be readily adsorbed by opposite charged materials. As shown in Fig. 5a, more than 60% of CytoC was trapped around S-1 or S-PSS in 10 min while 36% of CytoC could be adsorbed by S-PDDA during the same time frame. For Ova, the difference was even bigger: 83% of Ova was trapped around S-PDDA in 10 min whereas less than 10% were adsorbed by S-1 or S-PSS (Fig. 5(b)). As for S-M, as much as 65% of CytoC was adsorbed while only 10% of Ova was adsorbed by S-M, which was in accordance with our electrostatic adsorption hypothesis. It is to be noticed that the adsorbance efficiency of trypsin on the beads

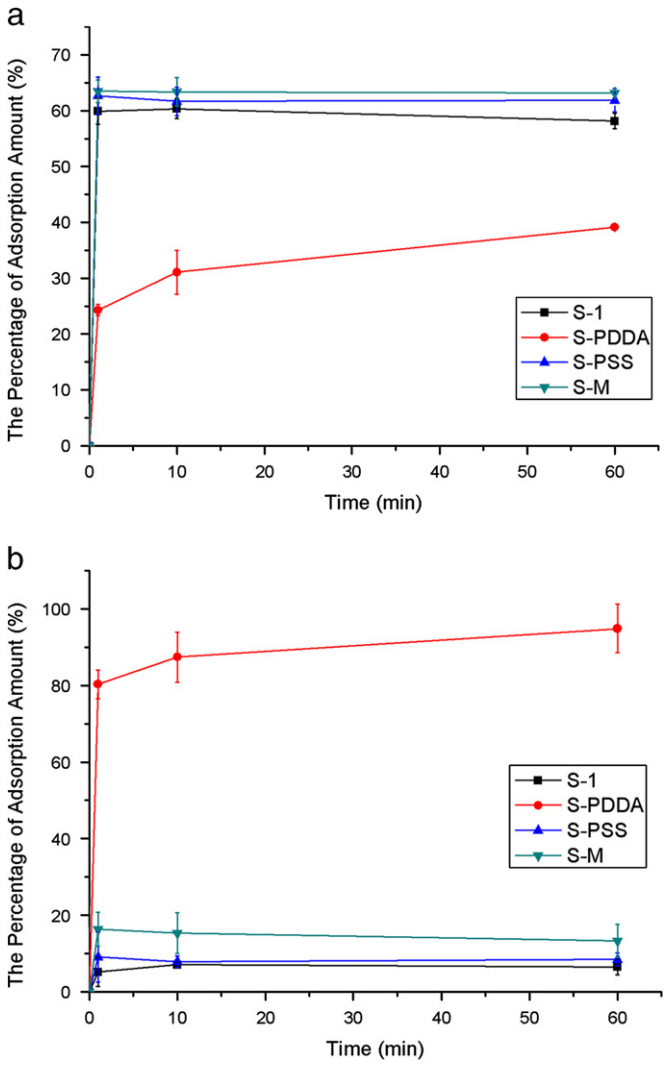

Fig. 5. Adsorption efficiency of different surface-modified silica microbeads for (a) cytochrome C (20 ng/μl) and (b) ovalbumin (100 ng/μl) in a solution of pH 8.

Table 2
Adsorption efficiency of different surface-modified silica microbeads for cytochrome C and ovalbumin in a solution of pH 6.1 in 10 min

	CytoC	Ova
S-1	17.1%	68.2%
S-PDDA	23.6%	86.1%
S-PSS	69.4%	31.2%
S-M	22.2%	63.0%

was calculated. As the same condition as the management for CytoC, 80.1%, 37.2%, 87.1% and 91.9% of trypsin were adsorbed by S-1, S-PDDA, S-PSS and S-M individually. The positive charged trypsin takes a charge-bias to different materials. And the loading amount could lead to proteolytic efficiency.

Adsorption efficiency of each material in acidic environment was also evaluated. 100 ng/μL of either CytoC or Ova was dissolved in PB buffer (pH 6.1). In this case, S-1 was reversed charged while S-PDDA and S-PSS still remained the same charge status due to their higher charge densities. After 10 min of incubation, the amount of CytoC adsorbed by S-1 was only reached to 17% compared with that in ABC buffer (59%). For Ova adsorption, the amount increased from 9% in ABC buffer to 68% in

PB buffer. However, the data of adsorption capacity of S-PDDA and S-PSS showed no obvious change (Table 2). The results supported the hypothesis that with regard to charge-carrying materials, electrostatic interactions rather than hydrophilic/hydrophobic interactions has a significantly greater influence on protein adsorption.

Moreover, the adsorption process was discussed to further explain the mechanism of physically immobilized enzymatic reactors. Fig. 5 clearly illuminates that the maximum adsorption amount can be achieved in a couple of minutes. In a physically immobilized enzymatic reactor system, the adsorption that occurred between proteins and materials were accompanied by proteolytic procedure [20]. Since the entrapping happens so quickly and efficiently, we may infer that the proteins around the materials are concentrated, and the proteolysis mainly occurs at local microenvironment. The material-catalyzed proteolysis consisted of two steps: the proteins and proteases were adsorbed onto microbeads, followed by digestion that relied on proteins-proteases encountering nearby microbeads. We also verified that proteolysis in the system was the rate determining step. It is known that the reaction rate is positively correlated with reagent concentrations, so enrichment of proteins and proteases simultaneously leading to increased concentrations will enhance proteolytic efficiency.

4. Conclusion

In summary, a deep and detailed investigation for the mechanism of adsorption-catalyzed proteolytic reactors has been presented. A series of surface-charged microbeads have been synthesized and employed to adsorb proteins and trypsin within a confined space to improve proteolytic efficiency. Zeta-potential of each microbeads were measured individually to reveal the charge character and monitor the change of charge state in different buffer solutions, as the MALDI-TOF was applied to evaluate the efficiency of proteolytic process. We verified that electrostatic interactions was the predominant factor for adsorption, and only in the condition where both proteins and trypsin were trapped simultaneously can positive results be achieved. Moreover, the process can be separated into two steps, and the enzymatic proteolysis was regarded as the rate determining step, which brought a theoretical guideline to further enzymatic digestion reactor research.

Acknowledgments

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2013.02.021.

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