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Binding performance of pepsin surface-imprinted polymer particles in protein mixtures

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Surface-imprinted polymer particles facilitate the accessibility of synthetic selective binding sites for proteins. Given their volume-to-surface ratio, submicron particles offer a potentially large surface area facilitating fast rebinding kinetics and high binding capacities, as investigated herein by batch rebinding experiments. Polymer particles were prepared with (3-acrylamidopropyl)trimethylammonium chloride as functional monomer, and ethylene glycol dimethacrylate as cross-linker in the presence of pepsin as template molecule via miniemulsion polymerization. The obtained polymer particles had an average particle diameter of 623 nm, and a specific surface area of 50 m^2 g^{-1}. The dissociation constant and maximum binding capacity were obtained by fitting the Langmuir equation to the corresponding binding isotherm. The dissociation constant was 7.94 µM, thereby indicating a high affinity; the binding capacity was 0.72 µmol m^{-2}. The binding process was remarkably fast, as equilibrium binding was observed after just 1 min of incubation. The previously determined selectivity of the molecularly imprinted polymer for pepsin was for the first time confirmed during competitive binding studies with pepsin, bovine serum albumin, and β-lactoglobulin. Since pepsin has an exceptionally high content in acidic amino acids enabling strong interactions with positively charged quaternary ammonium groups of the functional monomers, another competitive protein, i.e., α1-acid glycoprotein, was furthermore introduced. This protein has a similarly high content in acidic amino acids, and was used for demonstrating the implications of ionic interactions on the achieved selectivity.

1. Introduction

Molecular imprinting is a technique for introducing selective recognition sites into a polymer matrix. The synthesis includes the copolymerization of a functional monomer and a cross-linker in the presence of a template molecule.[1] Thus obtained recognition sites are ideally complementary in size and shape to the template molecule, and comprise functional groups that are sterically oriented towards complementary functional groups of the template molecule. The interaction between the molecularly imprinted polymer (MIP) and the template molecule is frequently considered to mimick the interaction between antibody and antigen.[2] MIPs have several advantages vs. antibodies such as superior chemical and physical stability, and easier and cost-efficient preparation. Imprinting of small molecules is nowadays a well-established method with a wide variety of applications in chromatography[3], solid phase extraction[4], pseudo-immunoassays[5], and sensors[6]. MIPs for small molecules are usually prepared with an organic solvent as porogen using bulk polymerization techniques.[7]

In recent years, interest in imprinting of biomacromolecules such as proteins has been significantly increasing for the generation of synthetic protein-selective receptor materials.[8] However, imprinting of proteins remains challenging due to the molecular dimensions, conformational flexibility, and limited solubility of proteins in organic solvents.[9] Consequently, more advanced imprinting strategies were therefore developed including epitope and surface imprinting. During surface imprinting, selective binding sites are formed at the polymer surface, and not within pores, as commonly the case for bulk MIPs. Hence, these binding sites remain accessible even for large (bio)molecules such as proteins.[10] Imprinted surfaces may be obtained by grafting a thin film onto a planar solid support[11] or onto polymer particles[12]. As the relative surface area increases with decreasing particle size, the preparation of surface-imprinted submicron sized polymers particles appears advantageous. Combining the largest possible surface area with unrestricted accessibility of binding sites results in high binding capacities and rapid binding kinetics.[13] Efficient routes for obtaining such materials are core-shell emulsion[14] or miniemulsion polymerization techniques[15]. In the latter, the polymerization occurs within the monomer droplets (i.e., the dispersed organic phase), while the template molecule (here, the protein) may remain in the continuous aqueous phase arranged at the phase boundary facilitating interactions with the functional monomers across this barrier. This one-step

synthesis eliminates additional steps, as required for core-shell approaches.[16]

In continuation of previous studies of our research group,[22] pepsin was used as well-characterized template protein.[17] Pepsin is an aspartyl protease, and catalyzes the cleavage of peptide bonds via hydrolysis.[18] Naturally, pepsin occurs in the stomach of living organisms, and exhibits its maximum activity at acidic conditions, but is active up to a pH value of 6.[19] Pepsin consists of a single peptide chain with 327 amino acids, and has a molecular weight of 35 kDa.[20] It has a remarkably low isoelectric point between 2.2 and 2.8[21], as it contains 43 acidic and only 4 basic amino acids[20].

We have previously reported on the preparation of pepsin surface-imprinted polymer particles via miniemulsion polymerization,[22] whereby the influence of four different functional monomers, and the amount of the pepsin template on the imprinting effect were investigated. The highest imprinting effect was obtained using (3-acrylamidopropyl)-trimethylammonium chloride (APTMA) and a higher template amount. APTMA is water-soluble, and was therefore copolymerized with the cross-linker at the phase boundary after diffusion from the continuous water phase to droplet surface. The imprinting effect was evaluated by comparing the pepsin binding capacity of the MIP, and a non-imprinted control polymer (NIP). The NIP was prepared by exactly the same procedure, but in absence of the template. The binding capacity was obtained from batch rebinding experiments. The selectivity of the optimized MIP was investigated via individual selectivity studies. Batch rebinding experiments were therefore performed separately with different proteins. Thereafter, the binding capacities of the different proteins were compared to the binding capacity of pepsin. The obtained results revealed that an increased selectivity for pepsin was obtained after imprinting.

In the present study, we have significantly extended the performance analysis reported within the previous study.[22] Here, the maximum binding capacity and binding kinetics were investigated via batch rebinding experiments at varying initial pepsin concentrations, and at different time intervals. Furthermore, competitive selectivity was tested during batch rebinding experiments, where pepsin, bovine serum albumin (BSA), and β-lactoglobulin (LG) were present in the same incubation solution for MIP and NIP. After incubation, binding of the proteins was determined via sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Resulting, the selectivity of the MIP for pepsin at simulated real-world conditions, i.e., if different proteins compete for the binding sites of MIP and NIP, was confirmed. An additional individual selectivity study was carried out with α1-acid glycoprotein, which has a similar isoelectric point and molecular weight as pepsin. Thereby, the influence of ionic interactions and the isoelectric point of proteins on the selectivity of the MIP were investigated.

2. Experimental

2.1 Materials

(3-acrylamidopropyl)trimethylammonium chloride (APTMA) (75wt-% solution in water), ethylene glycol dimethacrylate (EGDMA), 2,2-dimethoxy-2-phenyl-acetophenone (DMPAP), pepsin, bovine serum albumin (BSA), β-lactoglobulin (LG) and α1-acid glycoprotein were obtained from Sigma-Aldrich. n-Hexadecane (HD) was from Alfa Aesar and Lutensol AT50, a poly(ethylene oxide)-hexadecyl ether with 50 units of ethylene oxide, from BASF. EGDMA was distilled under reduced pressure and stored at -20 °C before use. The other chemicals were used as received and the water was of double-demineralized Milli-Q grade.

2.2 Preparation of the polymer particles

The polymer particles were prepared by miniemulsion polymerization, as described in our previous report.[22] The water phase was prepared by dissolving pepsin (150 mg), APTMA (192 mg), and Lutensol AT50 (180 mg) in degassed water (15 g). A mixture of EGDMA (1.475 g), HD (75 mg) and DMPAP (51 mg) was added to the water phase, and was stirred for 1 h in order to obtain a pre-emulsion. The miniemulsion was prepared by ultrasonication of the pre-emulsion for 120 s with an amplitude of 60% (Branson sonifier W450 Digital, ½'' tip) at 0 °C in order to prevent polymerization. The polymerization was initiated at room temperature for 20 h (i.e., photo-initiation with a UV lamp with 366 nm). Non-imprinted polymer (NIP) particles were prepared in the same way in absence of pepsin.

2.3 Removal of pepsin

The water phase of the obtained MIP and NIP suspensions was replaced by pure water using 10 centrifugation/redispersion cycles. Water was used to remove pepsin, the surfactant, and any unreacted monomer. The centrifugation was executed at 13,000 rpm for 1 h, and the redispersion via a sonication bath for 20 min and a vortex for 40 min. The UV absorbance was analyzed in the absorbance range of 180-340 nm in order to control effective removal. The solid content of an aliquot of the polymer suspensions was determined 2 times by gravimetric analysis, and then averaged. Afterwards, pure water was added resulting in polymer suspensions with a solid content of 2wt-%.

2.4 Characterization of the polymer particles

The hydrodynamic average particle diameter (z-average) and polydispersity index (PDI) were analyzed by dynamic light scattering (DLS) with a Zetasizer NanoZS (Malvern Instruments). The scattering angle was 173°, the wavelength 633 nm, and the temperature 25 °C. Three measurements were performed and averaged. The polymer suspensions were diluted to a solid content of 0.01wt-% in water prior to the measurements. For the zeta potential studies, the polymer suspensions were diluted to the same solid content, yet in a KCl solution (10^{-3} M). The zeta potential was measured at 25 °C by laser Doppler electrophoresis using the Zetasizer NanoZS (Malvern Instruments). Three measurements were executed and averaged. Transmission electron microscope (TEM) images were obtained with an EM10 microscope (Zeiss) operating at 80 kV. For that purpose, the polymer suspensions were diluted to a solid content of 0.1wt-%, and placed onto a 400-mesh hydrophilic copper grid. The suspensions on the grids were dried at room temperature, and coated with carbon. The polymer particles were dried under vacuum at 40 °C for the determination of the specific surface area. These studies were performed using nitrogen adsorption analysis via a QuadraSorb SI (Quantachrome Instruments) using the Brunauer-Emmett-Teller (BET) equation.

2.5 Batch rebinding experiments

For the rebinding studies, 250 µL of MIP or NIP suspension (2wt-%) were mixed with 250 µL of a protein solution, and incubated under shaking at room temperature. A blank sample was prepared in the same way in absence of any protein. Afterwards, the polymer particles including the bound protein were removed via centrifugal filters (modified PES, Nanosep, Pall) by centrifugation at 3,000 rpm for 5 min. Protein solutions of different concentrations were prepared for obtaining a calibration curve, and were treated similarly. The concentration of the protein in the supernatant was determined by measuring the absorbance at 277 nm in a 10 mm cuvette with a Nanodrop 2000C UV-Vis spectrophotometer (Thermo Scientific). Three measurements were averaged per sample. All experiments were performed in duplicates calculating the average and the spread. The binding capacity of MIP and NIP were calculated using the following equation:

$$Q = \frac{(\beta_{init} - \beta_{free})V_{sol}}{m_{polym}A_s} = \frac{m_{prot,ads}}{A_{polym}},$$

where Q is the binding capacity, β_{init} is the initial protein mass concentration, β_{free} is the protein mass concentration in the solution, V_{sol} is the volume of the solution, $m_{prot,ads}$ is the mass of protein adsorbed on MIP or NIP, m_{polym} is the mass of MIP or NIP, A_s is the specific surface area calculated from the average particle diameter obtained by DLS, and A_{polym} is the surface area of the polymer particles.

2.5.1 Binding isotherms

For obtaining binding isotherms, batch rebinding experiments were carried out with pepsin solutions of different initial concentrations ranging from 0.4 to 1.6 mg mL^{-1} (after mixing with the polymer suspension). The incubation time was 20 h, and a centrifugal filter with a molecular weight cut-off (MWCO) of 100 kDa was used for separating the supernatant.

2.5.2 Binding kinetics

For obtaining binding kinetics, batch rebinding experiments were performed using a pepsin solution with an initial concentration of 0.8 mg mL^{-1} (after mixing with the polymer suspension), and varying the incubation time from 1 min up to 20 h. Thereafter, a centrifugal filter of a MWCO of 100 kDa was used for separation of the supernatant.

2.5.3 Individual selectivity studies

For individual selectivity studies, batch rebinding experiments were carried out using either pepsin or α1-acid glycoprotein solutions with an initial concentration of 22.9 nmol mL^{-1} (after mixing with the polymer suspension). The incubation time was 20 h, and a centrifugal filter of a MWCO of 300 kDa was used for separating the supernatant. The comparison of pepsin and α1-acid glycoprotein was facilitated by dividing the binding capacity with the molecular weight of each protein.

2.6 Competitive selectivity studies

For competitive selectivity studies, 90 µL of MIP or NIP suspension (2wt-%) were mixed with 90 µL of a solution containing pepsin, BSA, and LG at a respective concentration of 22.9 nmol mL^{-1}. The same experiment was carried out with pepsin, which was pre-incubated with pepstatin (45.7 nmol mL^{-1}) under shaking at room temperature for 1 h. Then, the mixture was incubated again under shaking at room temperature for 20 h. Reference samples containing the protein mixture and the pure proteins respectively, yet without adding MIP or NIP were prepared in the same way. Afterwards, the polymer particles including the bound protein were precipitated by centrifugation at 13,000 rpm for 10 min. The supernatant was mixed with Laemmli sample buffer containing SDS, tris(hydroxymethyl)-aminomethane (tris, pH 6.8), glycerol, bromophenol blue, and 2-mercaptoethanol, and was heated at 95 °C for 5 min. Then, 16 µL of each mixture and 6 µL of a protein molecular weight marker (PageRuler prestained protein ladder, 10 to 170 kDa, Thermo Fisher Scientific) were loaded onto a gel (Any kD Mini-protean TGX precast gel, Bio-Rad Laboratories). After executing the SDS-PAGE in a Mini-protean Tetra cell (Bio-Rad Laboratories) containing an SDS-tris-glycine (pH 8.8) running buffer, the gel was stained with Coomassie Brilliant blue G-250.

3. Results and discussion

3.1 Submicrometer polymer particles

Stable polymer suspensions with submicron-sized particles were obtained by miniemulsion polymerization, as previously reported.[22] After polymerization, the water phase of the polymer suspensions was exchanged by centrifugation and redispersion cycles in order to remove pepsin, the surfactant, and any residual monomer. This was repeated until no UV absorbance in the absorbance range of pepsin and APTMA (180-340 nm) was observable anymore.

The average hydrodynamic particle diameter and the PDI value were determined before and after the extraction of pepsin via DLS (Table 1). The diameter of the MIP was about twice as high as the diameter of the NIP, which indicates that the particle diameter was affected by the presence of pepsin during the polymerization. The PDI value of the MIP was also higher compared to the NIP, thus indicating a broader size distribution affected by the template. In turn, the zeta potential of the polymer particles was barely affected by the presence of pepsin, and was generally quite high due to the copolymerization of positively charged functional monomer at the particle surface.

Table 1 Average hydrodynamic diameter (d_z), polydispersity index (PDI), and zeta potential (ζ) of MIP and NIP before and after pepsin extraction.

Polymer	Extraction	d_z [nm]	PDI [-]	ζ [mV]
MIP	-	727	0.31	56
	+	623	0.24	43
NIP	-	344	0.19	59
	+	467	0.22	51

The particle properties of MIP and NIP slightly changed after template extraction due to potential aggregation or loss of smaller particles during centrifugation and/or incomplete redispersion. The TEM images in Fig. 1 exemplarily show MIP

and NIP particles after the extraction. They are in a size range between 50 and 600 nm, and are characterized by comparatively rough surfaces. Particles with a diameter up to approx. 600 nm were observed in other TEM images obtained at a lower magnification (data not shown here).

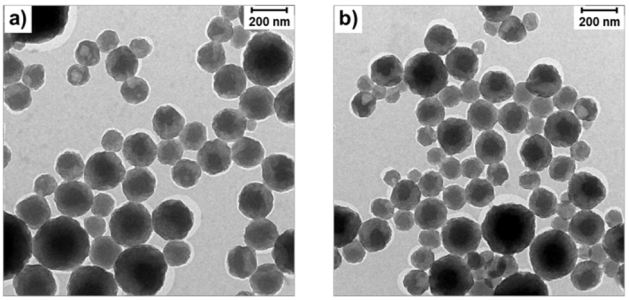

Fig. 1 Typical TEM images of a) MIP and b) NIP.

The specific surface area was determined by nitrogen adsorption measurements using the BET equation. The specific surface area of the MIP was approx. 50 m^2 g^{-1}, and of the NIP approx. 65 m^2 g^{-1} after the extraction. The observed difference between MIP and NIP may be attributed to the difference in the average particle diameter.

3.2 Binding isotherms

Batch rebinding experiments were performed with pepsin solutions of different initial concentrations ranging from 0.4 to 1.6 mg mL^{-1} for investigating the binding behavior of MIP and NIP. For that purpose, polymer suspensions were incubated with pepsin solutions for 20 h. The polymer particles were removed from the solution by centrifugal filtration, and the concentration of remaining pepsin in the supernatant was determined by UV photometry. The amount of bound pepsin was calculated by subtracting the remaining pepsin from the initially incubated amount of pepsin, and then normalized by the specific surface area. The latter was calculated from the average particle diameter obtained by DLS, as reported.[22] Thus obtained binding isotherms of MIP and NIP are shown in Fig. 2.

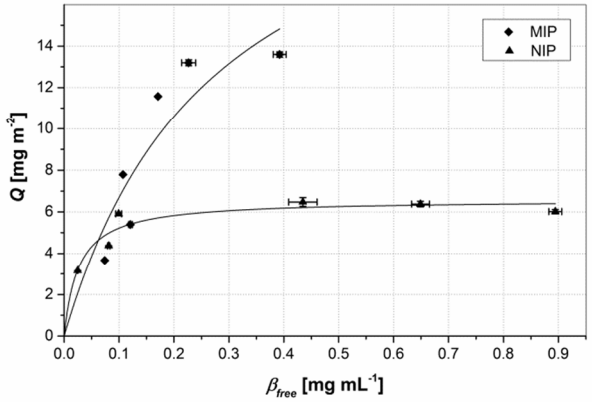

Fig. 2 Binding isotherms of MIP (◆), and NIP (▲) with pepsin. The curves were obtained by fitting the Langmuir equation to the experimental data points.

Evidently, the MIP bound more pepsin than the NIP across the entire concentration range. This implies that the imprinting procedure led to a distinct binding affinity for pepsin (i.e., imprinting effect). The difference in binding capability of pepsin between the MIP and NIP increases with increasing initial pepsin concentration. This indicates that there are not only selective, but also non-selective binding sites available at the MIP surface. Non-selective binding sites are inevitably generated due to random copolymerization of an excess in functional monomer, which again compares well to previously obtained results.[22]

The progression of the binding isotherms of MIP and NIP resembles the behavior of a Langmuir isotherm with saturation. The Langmuir equation was therefore fitted to the experimental data points, and the maximum binding capacity and dissociation constant were accordingly derived. The maximum binding capacity of the MIP (0.72 μmol m^{-2}) was significantly higher than that of the NIP (0.19 μmol m^{-2}), thus confirming that more binding sites were created via the imprinting process. The dissociation constant of the MIP was 7.94 μM, and was thus lower compared to the MIPs reported in previous studies,[22] thereby indicating a higher binding affinity. The obtained data is comparable to the dissociation constants derived by C. Wang et al, who prepared molecularly imprinted polymeric nanoparticles for atrial natriuretic peptide.[23] For comparison, the obtained dissociation constant is similar to the dissociation constant (2.5 10^{-7} M) of a monoclonal antibody specific for renin, which is another aspartyl protease.[24] While the dissociation constant of anti-pepsin antibodies is not available in literature, the dissociation constant of the complex formed between pepsin and its inhibitor pepstatin (9.7 10^{-11} M) has been reported, and is much lower than the one obtained for the MIP.[25] This indicates that further improvement of the affinity of such MIPs should be attainable, e.g., by using a mixture of suitable functional monomers. This may facilitate the formation of different types of non-ionic interactions between pepsin and the MIP comparable to the interaction between pepsin and pepstatin. However, the dissociation constant of the NIP (0.74 μM) was lower than of the corresponding MIP, and thus, a higher binding affinity would result. This result is surprising, and may be associated from the lower quality of the fit for the MIP (R^2=0.87) compared to the NIP (R^2=0.96). Future studies will focus on this effect by synthesizing significantly more material, which should enable more precise modeling of the binding behavior, and thus, more detailed insight.

3.3 Binding kinetics

Batch rebinding experiments were carried out with an initial pepsin concentration of 0.8 mg mL^{-1} at different time intervals ranging from 1 min to 20 h for studying binding kinetics. Otherwise, similar procedures as for obtaining the binding isotherms were applied. The resulting binding capacity of MIP and NIP was plotted versus time, as shown in Fig. 3.

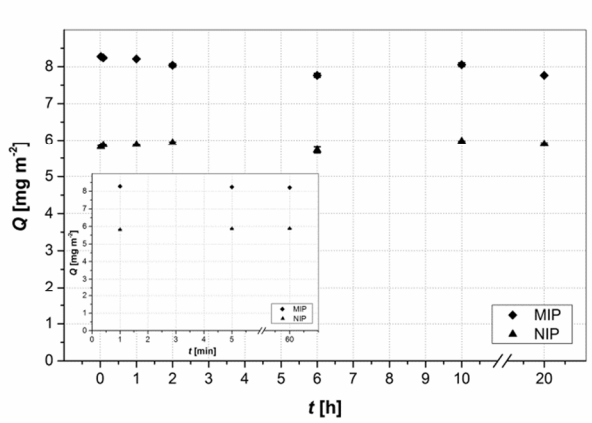

Fig. 3 Binding kinetics of MIP (◆), and NIP (▲) with pepsin.

Fig. 3 illustrates that pepsin binding to the MIP was indeed exceptionally fast. After incubation for only 1 min, the binding equilibrium between pepsin and the MIP was obtained, and the amount of bound pepsin remained constant over 20 h. These binding kinetics demonstrate that there is a high mass transfer rate prevalent. This is particularly anticipated for submicron sized surface-imprinted polymer particles with binding sites present at the polymer surface, which are readily accessible for the target proteins. Similar binding behavior was observed for the NIP. While there was no difference in binding kinetics between MIP and NIP, significantly more pepsin was bound to the MIP. The binding kinetics observed herein are comparable to results obtained by G. Fu et al,[26] who determined the binding equilibrium between lysozyme and lysozyme surface-imprinted core-shell particles with an average diameter of 230 nm after incubation at approx. 5 min. In contrast, for other protein surface-imprinted polymers binding equilibria after around 60 min have been reported.[27,28]

3.4 Competitive selectivity studies

Batch rebinding experiments were executed using a mixture of pepsin - or pepsin pre-incubated with pepstatin - BSA (66 kDa, pI=5), and LG (18 kDa, pI=5). The concentration of each protein was 22.9 nmol mL^{-1}. These mixtures were incubated with MIP and NIP particles for 20 h. Then, the polymer particles were removed from the solution by centrifugation, and the proteins remaining in the supernatant were separated via SDS-PAGE and stained with Coomassie Blue. A typical image of the resulting gel is shown in Fig. 4.

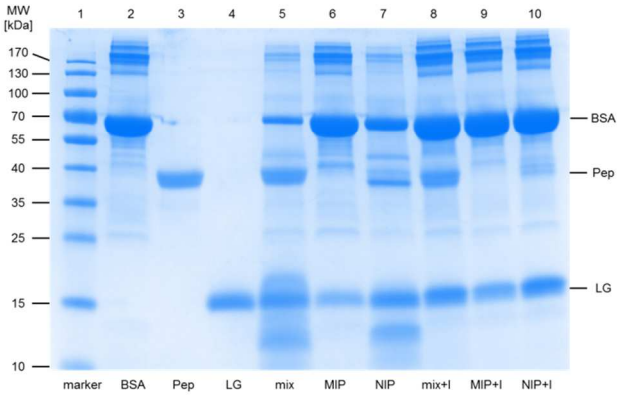

Fig. 4 SDS-PAGE gel obtained from the supernatant after competitive binding/selectivity study. Lane 1: molecular weight (MW) marker, lane 2: BSA, lane 3: pepsin (Pep), lane 4: LG, lane 5: mixture of BSA, Pep and LG, lane 6 and 7: protein mixture after incubation with MIP and NIP, lane 8: protein mixture after pre-incubation of Pep with inhibitor pepstatin (+I), and lane 9 and 10: protein mixture after pre-incubation of Pep with inhibitor pepstatin (+I) and incubation with MIP and NIP.

Lane 1 contains the protein molecular weight marker, while lane 2, 3, and 4 show the bands of neat BSA, pepsin, and LG, respectively as a reference. A neat protein mixture was also incubated for additional referencing, and is shown in lane 5. It is evident that the majority of BSA was degraded by pepsin during the incubation. The band of LG resembles the band of neat LG, except that it is convoluted with bands of BSA fragments, i.e., LG was evidently not degraded by pepsin. Lane 8 shows the protein mixture after the pre-incubation of pepsin with pepstatin. The band of each protein is comparable to the band of the respective neat protein, thereby confirming that the pepsin activity was effectively inhibited by the addition of the aspartyl protease inhibitor pepstatin[29]. Lane 9 and 10 show the protein mixture including the inhibited pepsin after the incubation with MIP and NIP. The entire pepsin, and a small amount of LG is absent in the protein mixture, which was apparently bound to the MIP. It is also clearly evident that the NIP did not bind the entire pepsin, and no LG.

Lane 6 and 7 show the protein mixture without pre-incubation of pepsin with pepstatin after the incubation with MIP and NIP. Notably, the pattern of lane 6 indicates that the pepsin activity was inhibited by binding to the MIP, which leads to two potential explanations: (i) pepsin was bound to the MIP via the active site or (ii) its active secondary structure was significantly affected by the binding event; to date this question remains open and is subject of further studies. The pattern given in lane 6 and 9 are comparable, thus indicating that pepstatin did not influence the binding efficiency between pepsin and MIP. Importantly, the NIP did not bind the entire pepsin, and pepsin remaining in solution was therefore able degrading BSA.

This study not only demonstrates that the developed pepsin-MIP indeed has a higher selectivity for pepsin compared to the NIP, but that even in the presence of other proteins, which compete for the binding sites of the polymer, pepsin is selectively recognized. Furthermore, the results obtained during the competitive study remain comparable to the results previously reported during the selectivity study using individual proteins.[22] Given that the NIP also revealed a certain preference for pepsin against BSA and LG, the polymer itself has an intrinsic selectivity for pepsin based on ionic interactions. BSA and LG have a higher isoelectric point (pI=5) compared to pepsin (pI=2.2-2.8), and are considered neutral in water,

whereas pepsin has a negative net charge and may thus be subject to additional non-selective attractive ionic interactions with MIP and NIP.

3.5 Individual selectivity studies

α1-Acid glycoprotein (pI=2.7, 41 kDa) has a similar isoelectric point and molecular weight as pepsin, and was therefore used as an additional competitive protein to investigate the influence of the ionic interactions on the selectivity of MIPs for pepsin. Batch rebinding experiments were performed with α1-acid glycoprotein similar to pepsin. The initial protein concentration was 22.9 nmol mL^{-1} using again an incubation time of 20 h. The resulting amount of bound α1-acid glycoprotein was then compared to the amount of bound pepsin (Fig. 5).

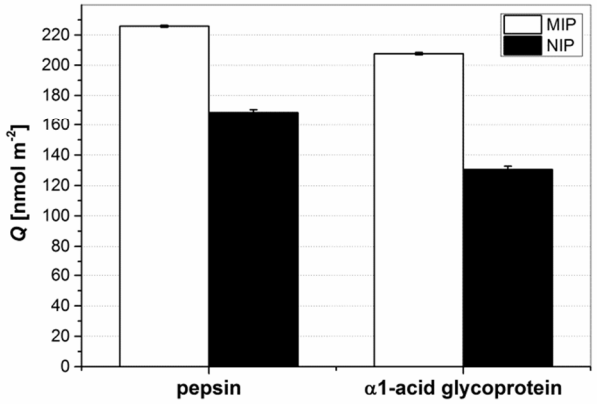

Fig. 5 Binding capacity (Q) of pepsin and α1-acid glycoprotein for MIP (white), and NIP (black).

Evidently, more α1-acid glycoprotein was bound to MIP and NIP compared to other proteins (e.g., thermolysin, lysozyme, and hemoglobin[22]). This indicates that there is indeed a significant effect of ionic interactions, and thus, of the isoelectric point of proteins on the binding characteristics. However, still more pepsin than α1-acid glycoprotein was bound to MIP and NIP confirming the selectivity for pepsin introduced by the imprinting process despite the electrostatic similarity of the proteins.

4. Conclusions

Positively charged polymer particles with submicron dimensions were synthesized via miniemulsion polymerization. The binding behavior of molecularly imprinted (MIP) and non-imprinted (NIP) particles was studied in detail according to the binding kinetics and binding isotherms revealing that equilibrium conditions were obtained after incubation periods of only 1 min. Hence, evidently the application of protein-MIPs e.g., as scavenging material should be preferably performed via short – yet, potentially multiple - incubation periods.
The observed binding isotherms resemble a Langmuir-type behavior. The maximum binding capacity and dissociation constant of the MIP were significantly higher compared to the NIP, thereby confirming that the MIP has significantly more selective binding sites. Of particular relevance was the finding that pepsin was still preferably bound to the MIP in a competitive binding scenario in presence of a protein mixture containing pepsin, BSA, and LG. An individual selectivity study with α1-acid glycoprotein finally revealed that ionic interactions, and thus, the isoelectric point of proteins play an important role during selective binding of protein-MIPs. As an outlook, more complex competitive selectivity studies are anticipated finally leading to the application of such synthetic receptors in e.g., cell lysates and similarly complex real-world samples.

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Binding performance of pepsin surface-imprinted polymer particles in protein mixtures

TOC graphic:

Surface-imprinted polymer particles were prepared via miniemulsion polymerization. They provide high binding capacities, fast rebinding kinetics and selective rebinding of the target protein investigated by batch rebinding experiments.