Synthesis of Porous Zwitterionic Sulfobetaine Monoliths and Characterization of Their Interaction with Proteins

Camilla Viklund and Knut Irgum*

Department of Chemistry, Umeå University, S-901 87 Umeå, Sweden

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ABSTRACT: New synthesis routes for incorporation of zwitterionic sulfobetaine groups into porous polymeric monoliths have been developed. The first approach involved photoinitiated copolymerization of *N*,*N*-dimethyl-*N*-methacryloxyethyl-*N*-(3-sulfopropyl)ammonium betaine (SPE) and ethylene dimethacrylate (EDMA) as cross-linker. Alternatively, the internal surfaces of porous poly(trimethylolpropane trimethacrylate) (poly-TRIM) monoliths were provided with zwitterionic "combs" by thermally initiated grafting of poly-SPE chains. The grafted monoliths exhibited an electrolyte responsive flow permeability, whereas the permeability of the copolymerized monoliths was unaffected by changes in ionic strength in the interval tested. Both synthesis routes yielded polymers capable of interacting reversibly with proteins in aqueous solutions, thus paving the way for novel selectivity dimensions in the field of bioseparations. This report also reveals the possibility of modulating the protein−polymer interaction strengths over a wide range on monolithic zwitterionic sorbents, by addition of low concentrations of a chaotropic ion to the aqueous phase.

Introduction

Sulfoalkylbetaine polymers constitute a class of zwitterionic polymers with permanent cationic and anionic charges incorporated in close proximity as pendant moieties attached to the polymer chain.[1] The synthesis of sulfoalkylbetaine polymers was first reported in 1958 by Hart and Timmerman,[2] who were also able to demonstrate that the solubility of the investigated linear sulfoalkylbetaine polymers has an "antipolyelectrolytic" dependence on the electrolyte concentration and that this salt dependency follows the Hofmeister lyotropic series[3] for anions capable of salting in macromolecules from aqueous solutions. This antipolyelectrolytic behavior manifests in viscosities and solubilities which increase with salt concentration, as opposed to conventional polyelectrolytes, which show the opposite electrolyte dependence. On the molecular level, addition of electrolyte induces a shielding of the closely spaced charges which reduces the self-association and causes an expansion of the polymer chains, an effect that also gives rise to a complex critical temperature of solution behavior.[1,4] These properties qualify polysulfoalkylbetaines as "smart" polymers,[5] i.e., macromolecules that undergo morphological transitions in response to chemical or physical stimuli. Accordingly, this class of polymers has received substantial attention in physical polymer chemistry, where studies have focused on solubility measurements of homo- and copolymers in aqueous solutions and organic solvents,[3,6] clustering,[7] hydrogel properties,[4,8−15] cloud points,[3,9] intrinsic viscosities,[3,9] static and dynamic light scattering,[1,9,14] dielectric measurements,[16] critical temperature of solution,[1] and head−head interactions,[17] using techniques such as solid state[7] and aqueous (deuterium)[16,18] NMR, small-angle X-ray scattering,[19,20] and laser Raman spectroscopy.[1]

What most of these studies are aiming at is investigating the competition of inorganic salts and small organic molecules with solution phase self-interactions of zwitterionic polymers of high and balanced charge density, using polysulfoalkylbetaines as model polymers. It is therefore curious why no attempts have been made to extend these interactions to amphoteric biological macromolecules, thereby exploiting the chromatographic potential inherent in the ability of controlling the interactions between antipolyelectrolytic zwitterionomers and biological macromolecules.[21] In this study we present the first findings of our investigations of porous monolithic sulfoalkylbetaine polymers, demonstrating that interactions between proteins and these polymers pave the way for a new mode of separating biomacromolecules using fully aqueous buffers.

Experimental Section

Materials. The zwitterionic monomer *N*,*N*-dimethyl-*N*-methacryloxyethyl-*N*-(3-sulfopropyl)ammonium betaine (SPE) was obtained from Raschig Chemie GmbH, Germany, and was used without further purification. Ethylene dimethacrylate (EDMA, 90%) was purchased from Fluka AG, Buchs, Switzerland, while trimethylolpropane trimethacrylate (TRIM, technical grade), benzoin methyl ether (99%), and 3-methacryloyloxypropyltrimethoxysilane (99%) were obtained from Aldrich, Steinheim, Germany. Polymerization inhibitors were removed by batch shaking with aluminum oxide for at least 24 h prior to use. Potassium peroxodisulfate and LC grade tetrahydrofuran (THF) were purchased from Merck (Darmstadt, Germany), while the methanol was of "analyzed HPLC grade" (J.T Baker, Deventer, Holland). Proteins used for determining the protein interactions were purchased from Sigma (St. Louis, MO).

Polymerization Procedures: Copolymerized Monoliths. The general procedure used for preparing the poly(SPE-*co*-EDMA) zwitterionic monoliths was to dissolve the water-soluble SPE monomer and 1% (w/w) benzoin methyl ether in methanol, followed by addition of the water-insoluble cross-linker EDMA. The polymerization mixtures were prepared in glass vials and sonicated for 10 min in a Bransonic 221 (Branson Ultrasonics, Danbury, CT) ultrasonic bath. The mixtures were thereafter degassed by purging with helium for 5 min and filled into DURAN glass columns (150 mm long by 2.7 mm i.d. with wall thickness 1.65 mm; Kebo Lab, Stockholm, Sweden), whose inner surface had been treated by reaction with neat 3-methacryloyloxypropyltrimethoxysilane

* Corresponding author. e-mail: kim@chem.umu.se.

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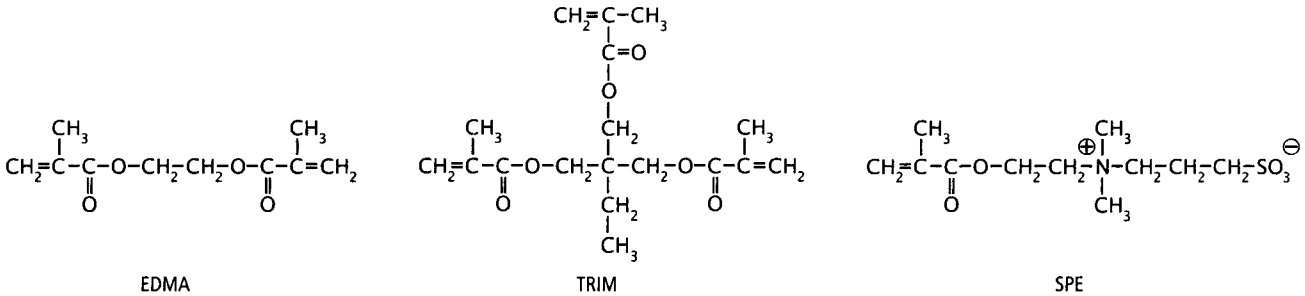

Figure 1. Structures of the monomers used for preparing the zwitterionic monoliths and grafting substrates.

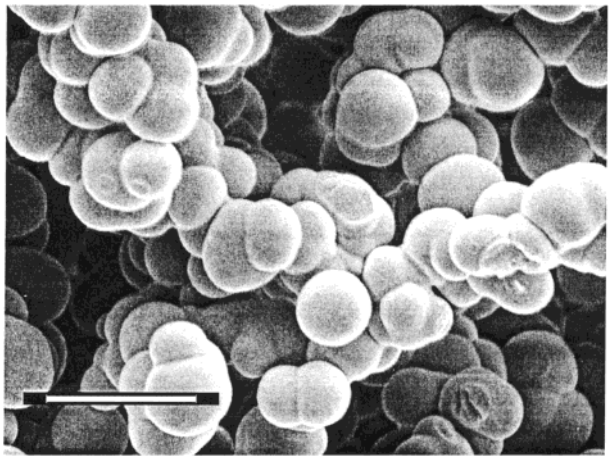

Figure 2. Scanning electron micrograph of a representative copolymerized monolith as described in Table 1, containing 43% SPE in the monomer mixture. The micrograph shows the fracture surface after snapping the monolith apart and treating it according to the procedure in ref 23. The bar represents 5 μm.

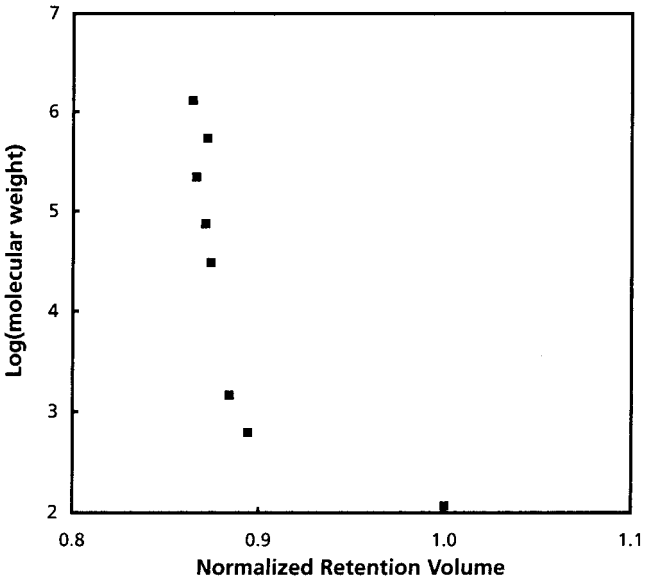

Figure 3. Size exclusion calibration curve of a (150 mm long by 2.7 mm i.d.) poly(TRIM) monolith using polystyrene molecular weight standards and tetrahydrofuran as eluent.

for 24 h or more at room temperature to produce methacrylic anchoring groups. The columns were sealed by pieces of silicone rubber tubing plugged with silicone stoppers and photopolymerized in the vertical position for 60 min using a Spectrolinker XL 1500 UV (Spectronics Corp., Westbury, NY) with eight 15 W fluorescent blacklight tubes (F15T8/BLB; GTE Sylvania), producing UV light of predominately 360 nm wavelength. After completion of the polymerization, each column was furnished with fittings, connected to an LC system, and soluble compounds still remaining in the monolith were washed out using water as the mobile phase.

Polymerization Procedures: Grafted Monoliths. The carrier monoliths were prepared by dissolving 40% (w/w) TRIM in a porogen mixture (80:20 isooctane:toluene by weight), to which benzoin methyl ether (1% w/w with respect to the TRIM monomer) had been added. Column preparation, degassing, and photopolymerization were carried out in an identical manner as for the copolymerized monoliths, using the same radiation parameters. After completion of the polymerization, the monolithic columns were provided with HPLC fittings and thereafter washed with methanol followed by equilibration with water at a flow rate of 0.3 mL/min in order to remove the porogenic solvent.

Grafting of the zwitterionic chains into the pore system of the monoliths was achieved by filling the column with a solution containing 10% zwitterionic monomer SPE in an aqueous solution containing 1% potassium peroxodisulfate with respect to the weight of the monomer. The grafting solution was delivered to the column using a 2.5 mL injection loop using a flow rate of 0.2 mL/min. The flow was stopped after 6−7 min, and after sealing the column, the grafting reaction was allowed to proceed for 12 h at 70 °C. After completion of the reaction, the column was connected to the HPLC system and washed with at least 100 mL of distilled water and 25 mL of 0.25 M NaCl in order to remove excess monomer and ungrafted homopolymer still remaining inside the monolith.

Chromatography. The gradient HPLC system consisted of two Bischoff (Leonberg, Germany) HPLC Compact Pumps, a Bischoff Central Processor, and a Bischoff Lambda 1010 UV detector. The pumps were operated at a combined flow rate of 0.5 mL/min. Injections aimed at determining retention behavior were made through a Rheodyne (Cotati, CA) model 9010 PEEK injector with a 20 μL PEEK injection loop. Protein uptake capacities were determined with the aid of a Thermo Separation Products (San Jose, CA) Spectra series AS100 autoinjector fitted with a 20 μL PEEK injection loop, through repeated injection of 4.2 mg/mL lysozyme. Breakthrough was estimated as the amount of protein injected when the baseline peak resulting from the protein injections was noticeably larger than the baseline disturbance caused by injection of pure water. The column was regenerated with 0.1 M NaCl between each capacity evaluation. All protein probes were dissolved in water shortly before injection and detected spectrophotometrically at 280 nm, both in this experiment and in the retention studies. The detector signals were recorded on a Hewlett-Packard (Palo Alto, CA) model 3396A integrator. The built-in pressure measurement facility of an LKB 2150 HPLC pump (LKB, Bromma, Sweden) was used to measure the back-pressures in Figure 4.

Size Exclusion Chromatography. The pore size distribution of the monolithic poly-TRIM grafting substrate was determined by size exclusion chromatography using polystyrene standards (1 mg/mL) having molecular weights ranging between 580 and 3 220 000 (Polymer Laboratories, Shropshire, UK). The mobile phase was THF at a flow rate of 0.5 mL/min, and the polystyrene probes were injected as 20 μL aliquots of 1 mg/mL concentration in THF. A UV spectrophotometric detector operated at 254 nm was used for detecting the eluted

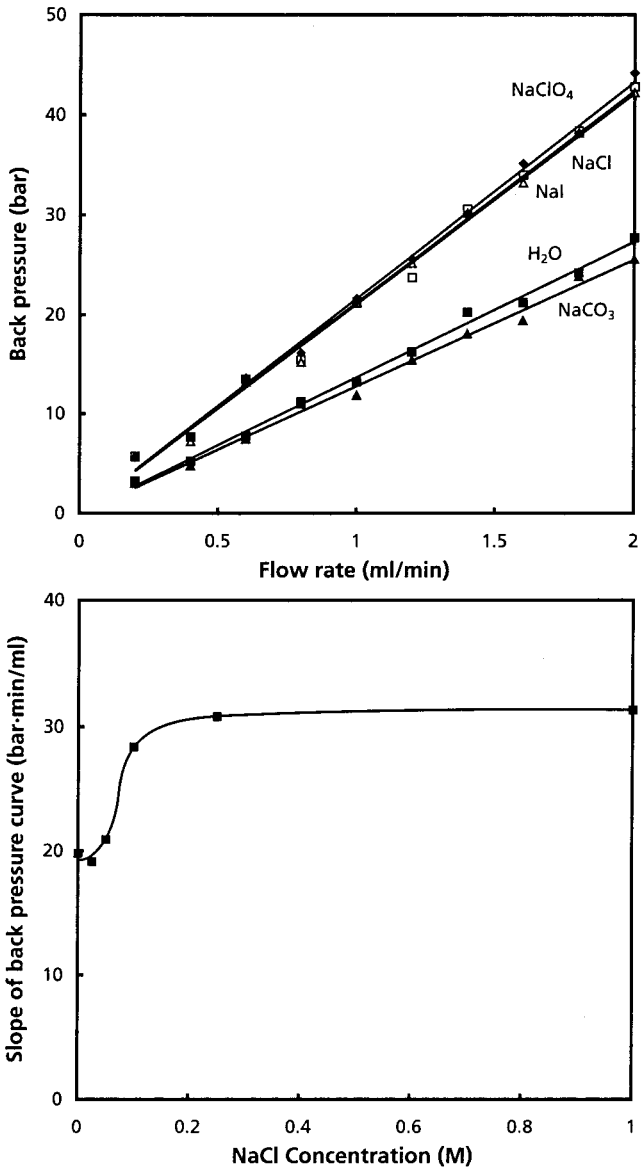

Figure 4. Back-pressure across a 250 mm long by 2.7 mm i.d. TRIM monolith grafted with SPE zwitterionic polymeric chains. The upper curve shows back-pressure curves for different salts at 0.25 M concentration, whereas the lower curve shows the slopes of the back-pressure vs flow rate curves at varying concentrations of NaCl. Curves are corrected for the differences in viscosities of the salt solutions.

polystyrenes. The retention volume for each polystyrene standard injected was normalized to the retention volume for toluene.

Circular Dichroism Measurements. Measurements of cytochrome *c* and lysozyme were carried out on a Jasco (Tokyo, Japan) model J-720 circular dichroism spectrophotometer in 10 mm quartz cuvettes over the wavelength range 300−650 nm on solutions of proteins at 1 mg/mL concentration in 10 mM sodium phosphate buffer at pH 7, with and without 10 mM $NaClO_4$ added. Difference spectra with and without sodium perchlorate were visually compared.

Results and Discussion

Copolymerized SPE Monoliths. To obtain monoliths carrying the zwitterionic functionality as pendant groups in the porous polymer matrix, a route was first developed for synthesizing monoliths[22] via direct photopolymerization of *N*,*N*-dimethyl-*N*-methacryloxyethyl-*N*-(3-sulfopropyl)ammonium betaine (SPE) and the

Table 1. Synthesis Parameters and Properties of Copolymerized and Grafted Zwitterionic Monoliths

SPE contents, % w/w	elemental analysis[a]	
	% S	% N
13[b]	1.24	0.59
23[b]	2.30	1.09
33[b]	3.35/3.45	1.59/1.58
43[b]	4.4	2.0
10[c]	0.11/0.11	0.072/0.083

[a]Each value corresponds to a mean of two measurements for each element. Where dual measurements are entered in the table, each value represents a duplicate analysis of monoliths polymerized at different occasions. [b]Percentage of SPE monomer with respect to the weight of the monomers in the original polymerization mixture consisting of 30% monomer mixed with 70% of methanol and 0.3% benzoin methyl ether. [c]Percentage of SPE monomer in the aqueous solution used for grafting on poly(TRIM) monoliths with a composition described in the legend of Figure 3.

methacrylic cross-linker 1,2-ethylene dimethacrylate (EDMA) (Figure 1), using benzoin methyl ether as radical precursor. A monolith photopolymerization process requires a porogenic solvent[23] in order to arrive at a porous structure, and methanol was selected since it could serve as a solvent for both the water-insoluble cross-linker and the water-soluble SPE, as well as yielding macroporosity in the final polymer. The content of sulfur and nitrogen incorporated in the final monolith increased linearly with the amount of SPE included in the polymerization solution mixture (Table 1). The scanning electron micrograph in Figure 2 shows a copolymerized monolith comprised of spherical units agglomerated into larger clusters transsected by large pore channels, characteristic of monolithic sorbents. This open structure allows liquid to be forced through the polymer without compression of the polymer using flow rates suitable for chromatographic purposes.

Grafted SPE Combs on Poly(TRIM). Our group has earlier reported that the hexafunctional monomer TRIM can been successfully used as a cross-linker together with 2,3-epoxypropyl methacrylate (GMA) in a mold photopolymerization process yielding porous monoliths suitable as supports for solid-phase chemiluminescence reactors.[23] TRIM (Figure 1) can also be preferably used for synthesis of polymers where pendant double bonds should be present at the surface after completion of the polymerization,[24] and in this study, the aim has been to prepare TRIM carrier monoliths with a porosity suitable for grafting of poly(SPE) in the pore structure, utilizing the remaining unsaturation. To be useful as grafting substrate, a sufficient number of macropores must be present in which graft polymerization can occur without clogging the pores by grafted chains or trapped homopolymer. Scouting experiments were done with a porogenic solvent consisting of varying amounts of isooctane and toluene, varying also the ratio between the monomer and the mixed porogenic solvent. The composition used in the experiment in Figure 3 was found suitable for establishing a graftable pore structure. As can be seen from the size exclusion calibration curve in Figure 3, the ungrafted poly-TRIM monolith contains mainly larger pore sizes and does thus not have any significant size exclusion ability since all molecular weights of injected polystyrene have virtually the same access to the internal monolith pore volume.

Recalling the solution properties of linear polysulfoalkylbetaine polymers, an electrolyte-dependent change of the apparent pore volume would be a convincing evidence of a successfully grafted monolith surface. The

responsive characteristics were thus monitored by flow permeability measurements in different electrolytes, since an electrolyte-responsive surface should be reversibly affected by the presence of ions in the surrounding media and cause the apparent pore volume to vary as a consequence of mobile phase type and concentration. The grafted monoliths did indeed exhibit a fast response to changes in electrolyte type and concentration, as visualized in Figure 4. The hydraulic permeability of the monolith decreased as the NaCl concentration in the external solution was increased, indicating salt-induced expansion the grafted polymer chains into the available flow passages. When the chaotropic salt sodium perchlorate was added instead, the flow permeability reduction took place at a lower concentration, whereas sodium carbonate (a "salting-out" salt) resulted in a permeability slightly higher than for pure water. The presence of a grafted electrolyte responsive layer was also verified by FT-IR spectroscopy, as sulfonic acid band shifts[1] could be monitored for grafted polymer samples suspended in NaCl (chloride is considered an intermediate anion in the Hofmeister series) compared to polymer suspended in water. The amounts of sulfur and nitrogen after grafting are shown in Table 1. As expected on the basis of the high cross-linking, no permeability variation induced by changes in electrolyte was seen for the copolymerized monoliths where the sulfobetaine functionality is incorporated into the methacrylate matrix.

Protein Interactions. Since the main target of this study was to investigate the possibility to obtain interactions between a cross-linked sulfobetaine polymer and proteins, a fast screening of the interactive characteristic of poly(SPE-*co*-EDMA) monoliths showed that the basic proteins lysozyme, chymotrypsinogen A, and cytochrome *c* were adsorbed when loaded from pure water, whereas acidic and neutral proteins eluted in the void volume. Elution of basic proteins was achieved by increasing the ionic strength, indicating an electrostatic interaction type. For example, a 150 mm long by 2.7 mm i.d. poly(SPE-*co*-EDMA) monolith synthesized from a polymerization solution containing 47% SPE and 53% EDMA in the monomeric phase required less than 10 mM sodium phosphate buffer (pH 7) in order to elute lysozyme. Because of the dual charge nature of the polysulfoalkylbetaines, it is reasonable to assume that interactive forces should not be exclusively reserved to biopolymers of net cationic charge. The positive charges introduced by the quaternary ammonium groups may therefore be sterically and electrically shielded from interaction with a large protein by the terminal sulfonic group arrangement. This assumption was supported by the expected retention order for small inorganic anions,[25] which followed the Hofmeister lyotropic series on the copolymerized monoliths ($Cl^- < Br^- < I^- < SCN^-$). It has furthermore been reported that the protein selectivity in conventional ion exchange protein chromatography is altered if the stationary phase charges are situated on flexible chains or "tentacles" reaching out from the pore surface, instead of being attached directly to the stationary phase surface.[26,27] Accordingly, by grafting poly(SPE) chains onto the pore structure of an uncharged monolithic substrate, we wanted to introduce a spatial charge dimension reaching out from the material surface, thus increasing the number of interaction sites available, as well as the flexibility of the interaction layer.

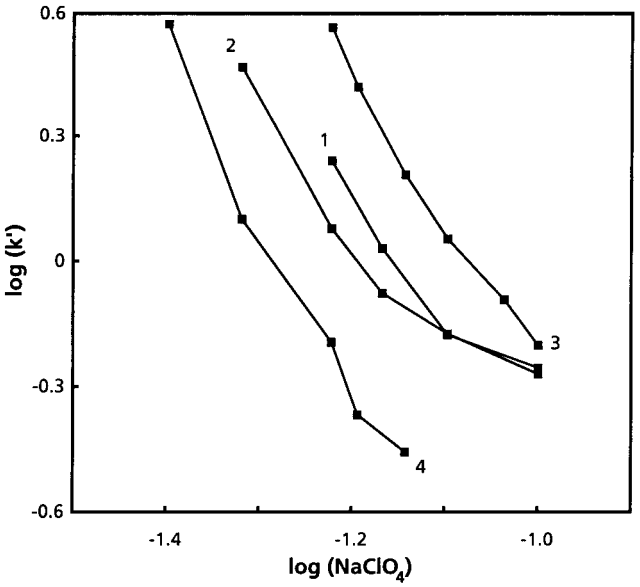

Figure 5. Capacity factor (k') as a function of [$NaClO_4$] at pH 6.9 under isocratic elution conditions for cytochrome *c* (1 and 3) and chymotrypsinogen A (2 and 4) on a poly(SPE-*co*-EDMA) monolith (1 and 2) and a poly(TRIM) monolith grafted with SPE (3 and 4). Three or more injections were made at each concentration and protein combination.

Table 2. Slopes of the log (k') vs log [$NaClO_4$] Curves[a]

monolith polymerization technique	cytochrome *c*	chymotrypsinogen A
copolymerization of SPE with EDMA	-2.21 ± 0.50	-2.26 ± 0.44
grafting of SPE onto TRIM monolith	-3.37 ± 0.22	-4.02 ± 0.39

[a] Values are given as slopes ±standard error of the slopes. All the retention data from Figure 5 are included in the regression analyses, although some of the curves appear to be subject to saturation with perchlorate (see the text).

Before grafting, no proteins injected were adsorbed on the poly-TRIM carrier monolith due to electrostatic interactions, while the poly(TRIM-*graft*-SPE) monolith showed interaction properties closely resembling those of the copolymerized resin. Figure 5 shows a comparison between the protein retention of poly(SPE-*co*-EDMA) and poly(TRIM-*graft*-SPE) using chymotrypsinogen A and cytochrome *c* as model proteins. Despite the different approaches taken for incorporating the zwitterionic groups, the amounts of sodium perchlorate needed for elution of the proteins from the copolymerized and grafted polymers were in the same concentration range, and the slopes of the retention curves were remarkably similar, although the grafted monolith had higher *Z* values[28] when all data points were used in the linear regression analysis of the log(k') vs log[$NaClO_4$] curves (Table 2). The log k' data were also plotted against the square root of the ionic strength, according to the theory of Ståhlberg et al.[29] (data not shown), but the fits were not better than the "conventional" *Z*-plot curves shown in Figure 5. The numerical difference appears to be higher than the initial parts of the slopes and may been due to saturation of the material with perchlorate ion[25] (see below), evident from the cytochrome *c* curves leveling off at higher perchlorate concentrations. Upon screening the protein interaction properties of the poly(TRIM-*graft*-SPE) monolith, it was also clear that an improved tenacity toward acidic and neutral proteins

had been obtained. Myoglobin and conalbumin were retained from purely aqueous solutions and eluted by increasing ionic strength (data not shown). We attribute this retentive effect to the comb arrangement of sulfoalkylbetaine groups on the "tentacles", including an increased accessibility of the quaternary ammonium groups and an overall increase in contact area between the polymer and the biomolecule, enabling simultaneous interactions with oppositely charged "patches" due to the charge anisotropy of protein surfaces.[30]

Influence of Chaotropic Ions on Retention. The salt-dependent permeability variations, which we interpret as chain expansion induced by interaction of perchlorate ions with the zwitterionic groups on the grafted monoliths, suggest that the copolymerized monoliths might also be affected by the presence of perchlorate ions, although swelling-induced permeability changes were inhibited by dense cross-linking. If so, the strength of the interaction between the copolymerized monolith and dissolved proteins should be affected by salts analogous to the mechanisms responsible for the chain expansion seen with the grafted monoliths. Indeed, whereas less than 10 mM sodium phosphate buffer was needed to achieve complete elution of even the most strongly retained protein (lysozyme) from a poly(SPE-*co*-EDMA) monolith, the salt concentration had to be increased to approximately 200 mM to elute lysozyme when sodium perchlorate was substituted for phosphate buffer. Perchlorate is a chaotropic anion where the monovalent charge is delocalized over all four oxygen atoms facilitating interactions with basic compounds, and its strong interaction with quaternary ammonium type anion exchangers is well-known.[31] Consequently, to investigate the effect of perchlorate ions on the polymer−protein interaction more closely, a series of phosphate buffers of different strength at pH 7 were prepared, to which was added from 0 to 50 mM sodium perchlorate. The retention for lysozyme and cytochrome *c* was thereafter determined under isocratic conditions by varying the phosphate buffer concentration at each perchlorate concentration level. The capacity factor (or retention factor) was calculated according to

$$k' = (t_r - t_m)/t_m \quad (1)$$

where t_r is the time required for the protein to diffuse through the monolith and t_m is the monolith void volume.

Perchlorate ions strengthened the interactive forces between the proteins and the poly(SPE-*co*-EDMA) monolith substantially (Figure 6). The increase in retention was pronounced even at millimolar concentration, and by adding 10 mM of sodium perchlorate to the eluent, the phosphate buffer concentration required to elute lysozyme at unit capacity factor was increased by a factor of 10. Judging from the curve for cytochrome *c*, the monolith appeared to become saturated with perchlorate ion, an observation we have made also in zwitterionic chromatography of inorganic ions.[25] When the sodium perchlorate concentration was increased further, the displacing power of this salt alone became sufficient to accomplish elution, evident from 70 mM of sodium perchlorate adjusted to pH 7 being sufficient to elute cytochrome *c* at unit k' without addition of phosphate buffer, whereas for lysozyme, a $NaClO_4$ concentration of nearly 200 mM was needed, as mentioned above. There were furthermore no significant differences in the circular dichroism spectra of cytochrome *c* and lysozyme with and without perchlorate added, indicating that the perchlorate concentration that caused increased retention did not do so by causing conformational changes of the proteins.

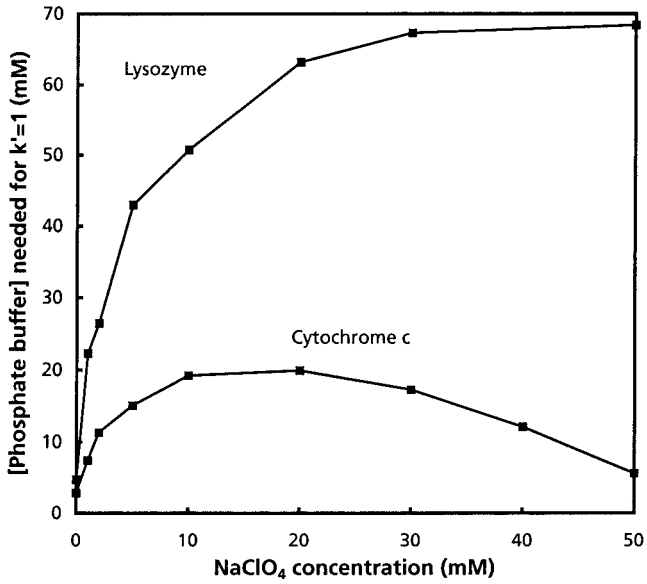

Figure 6. Concentration of sodium phosphate buffer at pH 7 required to obtain unity protein capacity factors for lysozyme and cytochrome *c* under isocratic elution conditions, as a function of [$NaClO_4$] added to the phosphate buffer. The monolithic column had a composition identical to that used in Figure 3. The values plotted are estimated by interpolation to log $k' = 0$ linear regression lines ($r^2 \geq 0.91$) of log k' vs log-[phosphate buffer] measured with a series of phosphate buffers at each sodium perchlorate concentration, yielding k' above and below 1. Three or more injections were made at each concentration combination.

Table 3. Dynamic Protein Uptake Capacity of a Poly(SPE-*co*-EDMA) Zwitterionic Monolith

$NaClO_4$ in buffer[a] mM	protein uptake capacity[b] mg/g[c]
0	0.3
5	2.0
10	3.7
15	4.3

[a] Concentration of sodium perchlorate added to 5 mM sodium phosphate buffer, pH 7. [b] Amount of lysozyme adsorbed by repeated injection of the protein in water into a poly(SPE-*co*-EDMA) monolith. [c] The dry monolith weighted approximately 0.25 g. The protein uptake capacity is reported as the amount of protein that could be injected before the baseline disturbance from the injection monitored at 280 nm became different from injection of pure water. Three breakthrough measurements were made at each $NaClO_4$ concentration.

The dynamic protein uptake in a continuous flow system was evaluated in phosphate buffer solutions of low ionic strength (5 mM, pH 7) to which was added varying amounts of sodium perchlorate. The protein uptake capacity was also substantially increased when the sodium perchlorate concentration was increased (Table 3), which supports the previously discussed chaotropic anion induced changes of the sulfobetaine polymer surface. This unique surface chemistry thus provides means for altering the protein uptake capacity of the polymer with maintained polymerization conditions. This is a great advantage when dealing with functional porous polymers, since even small changes in the composition of the polymerization solution might cause serious changes of physical properties such as

pore size distribution, surface area, and flow permeability.[23]

To assess whether other recognized chaotropic (water structure breaking) compounds could be used to increase the polymer−protein interaction, urea, guanidinium hydrochloride, and sodium thiocyanate were all tested as eluent modifiers. With eluents containing 10 mM urea or guanidinium hydrochloride added to 10 mM phosphate buffers (pH 7), both cytochrome *c* and lysozyme eluted in the void volume, i.e., the same lack of retention as with 10 mM phosphate buffer alone. Increasing the urea concentration to 90 mM did not increase the protein retention. It should be mentioned that urea itself did not act as a competing eluent in absence of phosphate buffer. However, the retention of the protein probes was increased when 10 mM sodium thiocyanate was added to the phosphate buffer, although the effect was not as pronounced as with sodium perchlorate addition.

Hydrophobic interactions between polymer and protein could be suspected and were tested by injecting the model proteins on a poly(SPE-*co*-EDMA) monolith using an eluent known as a strong hydrophobic interaction promoter (2 M ammonium sulfate buffer, pH 7). None of the model proteins were adsorbed on the monolith. Hydrophobic interactions do therefore not contribute to the peculiar interaction behavior of the sulfobetaine-based polymers. We provisionally ascribe the observed retention as being solely due to Coulombic interactions, which is expected on these highly charged surfaces. The capability of controlling and tuning the apparent net surface charge of these sulfoalkylbetaine polymers thus provides not only a new interaction mode for protein separation but also an intriguing tool for studies of reversible weak interactions between highly polar surfaces and proteins. The porous properties and the zwitterionic character of these novel sulfobetaine monoliths make them suitable as separation media for biopolymers, but these chromatographic properties will be reported on elsewhere.

Conclusions

Porous zwitterionic monoliths have been prepared by photoinitiated copolymerization of SPE and methacrylic cross-linkers, as well as by surface grafting of electrolyte responsive poly(SPE) on a rigid carrier poly(TRIM) monolith. The porosity of these polymers provides flow characteristics suitable for chromatographic flow systems. The flow passages of the grafted monoliths could be modulated by changing the electrolyte type or concentration, a characteristic that could be useful in other chromatographic modes. The interactions between proteins and the sulfobetaine type monoliths seem to be of electrostatic nature and thus provide a novel type of separation medium for bioseparations. This report also shows that the interaction strength between the polymer and proteins can be actively controlled over a broad range by addition of low concentrations of chaotropic ions in a process that is not driven by solvophobic forces. As a consequence, the dynamic protein uptake capability can be expanded by increasing the amount of chaotropic ion in the aqueous media. This unique feature is under further investigation and will be extended to other application areas.

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