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A novel polymer-grafted cation exchanger for high-capacity protein chromatography: The role of polymer architecture

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ABSTRACT

In this work, a *branched* polymer-grafted cation exchanger was synthesized on a Sepharose FF matrix via atom transfer radical polymerization using 3-sulfopropyl methacrylate potassium salt (SPM) as a functional monomer and 2-(2-bromo- isobutyryloxy)ethyl methacrylate as a branching monomer. The resulting branched poly(SPM)-grafted cation exchangers exhibit typical ionic exchange characteristics for lysozyme adsorption and the maximum adsorption capacity reach 450 mg/mL on Sep-BrS-S12B2 whereas γ-globulin adsorption is more dependent on polymer architecture. By adjusting the ratio of the monomers, very high adsorption capacity for lysozyme and γ-globulin can be achieved in the more branched Sep-BrL-S4B3 while γ-globulin has a much higher effective diffusivity in Sep-BrL-S4B3. Experimental evidences show that the performance of polymer-grafted cation exchanger can be improved by regulating polymer architecture. The calorimetric results further indicate that counter-ions are released from polymer and proteins during protein adsorption. With an increase of the adsorption density, adsorbed proteins experience a change of molecular orientation along poly(SPM) chain. In Sep-BrL-S4B3, dynamic binding capacities reached 145 mg/mL for lysozyme and 96 mg/mL for γ-globulin, demonstrating that Sep-BrL-S4B3 is a promising type of novel high-capacity cation exchangers. This research gives clue to the design of high-capacity cation exchangers and offers insights into protein adsorption on polymer-grafted cation exchangers.

1. Introduction

Since the advent of the Spherodex™ series ion-exchangers [1], polymer-grafted ion exchangers have gradually become an active subject of protein chromatography research [1–6]. In polymer-grafted ion exchangers, charged polymer chains affixed to the surface of porous materials brings about three-dimensional arrangement of binding sites for protein adsorption and higher ligand density, while simultaneously limiting pore accessibility for protein [7–14]. As a result, the properties of polymer-grafted ion exchangers are profoundly different from those of conventional ion exchangers, for which protein binding capacity depends primarily on the surface area of the particles [2,14]. Stone and Carta reported that the pore accessibility of dextran-grafted cation exchangers decreased dramatically with dextran grafting and the molecular weight of dextran, but the cation exchangers exhibited four to ten times higher pore diffusivities for lysozyme than its free solution diffusivity [14]. Such enhancement is also reflected in the binding of antibodies and other proteins in polymer-grafted ion exchangers [4,5,8,9,11,15]. In the last ten years, polymer-grafted ion exchangers have been investigated more widely as a promising alternative to Protein A chromatography to cope with the increasing titers of monoclonal antibodies [4,5,16].

A variety of commercial polymer-grafted ion exchangers for protein chromatography are listed in Table 1, and polymers are covalently attached in these ion exchangers by either grafting-to and grafting-from approaches. The grafting-to approach involves polymer chains reacting with the pore surface of porous materials or fibrous bundles of agarose [8,14–20]. Because of multiple-

Abbreviations: ATRP, atom transfer radical polymerization; ATR, attenuated total-reflection; Bpy, 2,2′-bipyridine; BIBB, α-bromoisobutyryl bromide; BIEM, 2-(2-bromoiso- butyryloxy) ethyl methacrylate; CuBr, copper(I) bromide; $CuBr_2$, copper(II) bromide; DB_{theo}, degree of branching; DMF, dimethylformamide; DBC, dynamic binding capacity; FTIR, fourier transform infrared; iSEC, inverse size exclusion chromatography; SPM, 3-sulfopropyl methacrylate; TEA, trimethylamine; Tris, tris(hydroxymethyl) aminomethane.

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Table 1
Properties of commercial polymer-grafted ion exchangers.[a]

Trade name	Basic matrix	Ionic capacity (mmol/L)	Lysozyme			γ-globulin		
			Adsorption capacity (mg/mL)	Binding capacity (mg/mL)	D_e/D_0 (–)	Adsorption capacity (mg/mL)	Binding capacity (mg/mL)	D_e/D_0 (–)
Fractogel® EMD SO_3^-	Methacrylate with "tentacle" polymer functional groups	70–110	178 [7]	90	–	128 [11]	60 [4,11]	0.07 [11]
Toyopearl® GigaCap S–650 M	Methacrylate with proprietary polymer functional groups	100–200	159 [10][b]	139 [11], 167	~0.47	–	100 [9]	–
Eshmuno™ S	Cross-linked hydrophilic vinylether with "tentacle" polymer functional groups	50–100	115–165[c]	100	–	–	80	–
SP Sepharose XL	Dextran-grafted agarose gel	180–250 [8]	400 [13]	–	0.8 [14]	301 [5], 330 [8]	–	0.27 [5]
Capto S	Agarose with dextran surface extender	110–140 [9]	310 [8]	140 [9]		330 [8]	100 [9]	
POROS 50 HS	polymeric particles with proprietary polymer functional groups	Not reported	158 [6]	–	0.1 [6]	–	–	–

[a] Unless otherwise marked, all data came from Ref. [4].
[b] Approximate order based on static HTS binding capacity measurements.
[c] Data from the manufacturer.

Table 2
Synthetic information and major properties of branched poly(SPM)-grafted cation exchangers.

Gel Name	SPM added	γ(–)	d_p(μm)	ρ_p (g/mL)	Ionic capacity (μmol/g)	M_n (g/mol)	M_w/M_n (–)	DB_{theo} (–)	α (–)	c_{NaCl} = 50 mmol/L		c_{NaCl} = 200 mmol/L	
										r_{pore} (nm)	Layer depth(nm)	r_{pore} (nm)	Layer depth(nm)
Sepharose FF	–	–	92 ± 1	1.033 ± 0.001	–	–	–	–	–	22.3	0.0	22.3	0.0
Sep-BrS-S1B2	1.47	7.33	95 ± 1	1.033 ± 0.001	119 ± 1	1.9×10^4	2.18	0.21	0.666	16.7 ± 0.8	5.6	17.1 ± 0.6	6.0
Sep-BrS-S4B2	4.00	20.0	95 ± 1	1.039 ± 0.001	166 ± 1	1.7×10^5	2.18	0.09	0.584	14.8 ± 1.4	7.5	15.5 ± 1.2	6.8
Sep-BrS-S12B2	12.0	60.0	108 ± 1	1.052 ± 0.001	335 ± 9	7.2×10^5	1.48	0.03	0.54	6.3 ± 0.7	16.0	8.1 ± 0.8	14.2
Sep-BrL-S1B2	1.47	7.33	95 ± 1	1.042 ± 0.001	231 ± 1	2.7×10^4	1.98	0.21	0.11	16.1 ± 0.9	6.2	16.6 ± 0.8	5.7
Sep-BrL-S4B3	4.00	12.0	97 ± 1	1.054 ± 0.001	356 ± 6	7.6×10^4	2.04	0.14	0.761	9.6 ± 0.5	12.7	10.3 ± 0.4	12.0
Sep-BrL-S4B2	4.00	20.0	104 ± 1	1.051 ± 0.001	389 ± 1	8.7×10^4	1.70	0.09	0.624	8.9 ± 0.6	13.4	9.6 ± 0.5	12.7
Sep-BrL-S12B2	12.0	60.0	107 ± 1	1.097 ± 0.001	689 ± 4	3.5×10^5	1.70	0.03	0.653	–	–	–	–