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Ion-exchange separation of proteins by polyallylamine-grafted cellulose gel

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Abstract

A cellulose-based anion exchanger bearing water-soluble polycation was tested for separation of proteins. The exchanger was obtained by partial oxidation of cellulose gel by aq. $NaIO_4$ followed by Schiff base formation with polyallylamine (PAA, molecular mass 5000). The retention behavior of proteins for three grades of PAA-cellulose gels, with amino group contents of 0.35, 0.59 and 0.96 mmol/g cellulose, was examined at several pH values and compared with that for conventional DEAE-cellulose gel with amino group content of 1.07 mmol/g cellulose. The retention of proteins by PAA-cellulose gels was remarkably greater than that for the DEAE-cellulose gel. Pairs of proteins having close isoelectric points and molecular masses (human and bovine serum albumins; β-lactoglobulin A and B) could be separated by the PAA-cellulose gel columns. Such efficiency can be ascribed to high local density of grafted polyallylamine, in contrast to the random and sparse charge distribution in DEAE-cellulose.

Keywords: Stationary phases, LC; Anion exchangers; Proteins; Polyallylamine; Cellulose

1. Introduction

Ion-exchange chromatography is an important technique for the analysis and separation of proteins. The major advantage of the technique is that it provides mild separation conditions that maintain the native structure of proteins. Ion-exchange beads based on dextran, agarose and cellulose are commercially available [1–5]; the cellulose-based materials are especially advantageous due to their high chemical and mechanical stability.

We have found dialdehyde and dicarboxyl cellulose useful for aqueous chromatography as column packing [6,7]. We attempted grafting of synthetic polyallylamine {PAA, $-[CH_2-CH(CH_2-NH_2)]_n-$}, having pendant primary amino groups on every repeating unit [8–10], onto cellulose (Fig. 1) [11]. This material showed remarkably higher ion-exchange capacity for divalent acids than a DEAE-cellulose gel. This effect was considered to result from the high charge density and flexibility of grafted polyallylamine chains. From the results we expect that this material is especially suited for analysis and separation of multivalent anionic species. In this study we prepared several PAA-grafted gels from a commercial cellulose gel having

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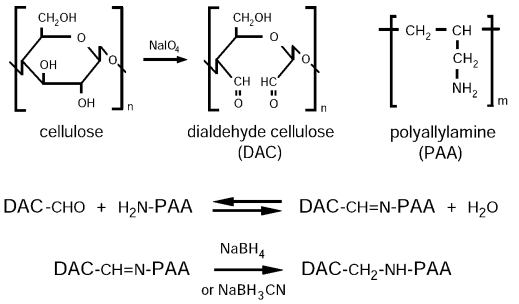

Fig. 1. Formation of imine (Schiff base) and its reduction to secondary amine.

large pore size and examined their anion-exchange capability for several proteins.

2. Experimental

2.1. Materials

A commercial size exclusion-grade cellulose gel, Cellulofine GCL-2000m (Chisso, Tokyo; particle size 53–125 μm; lightly crosslinked by polyethylene glycol; swollen and suspended in water) was used as starting material. Polyallylamine (PAA, weight-average molecular mass (M_W) 5000; $M_w/M_n=3.58$, where M_n=number-average molecular mass) was provided by Nitto Boseki (Tokyo, Japan) and used as received. Sodium metaperiodate ($NaIO_4$), bovine serum albumin and human serum albumin were obtained from Wako (Tokyo, Japan). β-Lactoglobulin (Lg) containing LgA and LgB fractions was obtained from Sigma (St. Louis, MO, USA). All other chemicals were of reagent grade (Wako). Distilled water was used throughout the study.

2.2. Preparation of dialdehyde cellulose gel

The cellulose gel suspension (5 g solid in 150 ml water) was mixed with 100 ml of 3.1, 7.7 or 15.4 m*M* aq. $NaIO_4$. The mixture was stirred gently at room temperature in the dark for 24 h. After the reaction was stopped by adding 1 ml of ethylene glycol, the oxidized gel was washed by five times of decantation using 1 l of water each. The products are denoted as DAC-02, DAC-05 and DAC-10, respectively; here the attached figures denote the intended level of oxidation, expressed as number of aldehyde groups per 100 glucose residues. The actual aldehyde content was determined by elemental analysis for nitrogen of oxime formed by the DAC and hydroxylamine hydrochloride [6].

2.3. Preparation of polyallylamine-grafted cellulose gel

The DAC gel suspension (2 g solid in 50 ml water) was mixed with polyallylamine solution (4 g solid in 50 ml, adjusted to pH 5 by HCl) and stirred gently at room temperature for 4 h. The resulting material was washed with water by repeated decantation and resuspended in 100 ml of water. To this mixture 5 mmol of $NaBH_4$ was added and stirred for 4 h at room temperature, to reduce the imine groups. The gel was washed by repeated decantation. The products are denoted as DAC-02-PAA, DAC-05-PAA and DAC-10-PAA, corresponding to the DACs described above. The nitrogen content was determined by elemental analysis, and converted to amino group per 1 g cellulose.

2.4. Column packing and evaluation of size-exclusion properties

The gel particles were packed in a stainless steel column (100×6 mm I.D.). Gel suspension (1 g solid in 30 ml water) was poured into a stainless steel reservoir attached to the column, and water was pumped in at flow-rate of 2.5 ml/min. The obtained packed column showed a pressure drop of 1.6–2.4 MPa at flow-rate of 1 ml/min. The dry mass of gel packed in the column was nearly the same (about 0.3 g) for all the gels tested.

The size-exclusion behavior of gels was determined by pullulan standards (Showa Denko, Tokyo, Japan) and small molecules (ethylene glycol, glucose and γ-cyclodextrin). Elution of solute was monitored by refractive index (RI-1530; Jasco, Tokyo, Japan) and optical rotation (OR-990; Jasco). The distribution coefficient for each solute was calculated by $K_{av}=(V_e-V_0)/(V_t-V_0)$, where V_e, V_0 and V_t are solute elution volume, void volume (pullulan P-800, molecular mass 788 000) and total volume (ethylene glycol), respectively.

2.5. Ion-exchange chromatography

Each protein solution (0.5%, w/w) dissolved in a starting buffer was injected from a 120-μl sample loop after being filtered through a 0.45-μm membrane filter. Ion-exchange chromatography was carried out at flow-rate of 1 ml/min with linear gradient of NaCl. The column was maintained at 25 °C. Elution of proteins was monitored by ultraviolet absorption at 280 nm. For comparison, the same test was carried out with a commercial anion exchanger, DEAE-Cellulofine A-800m (Chisso, particle size 53–125 μm).

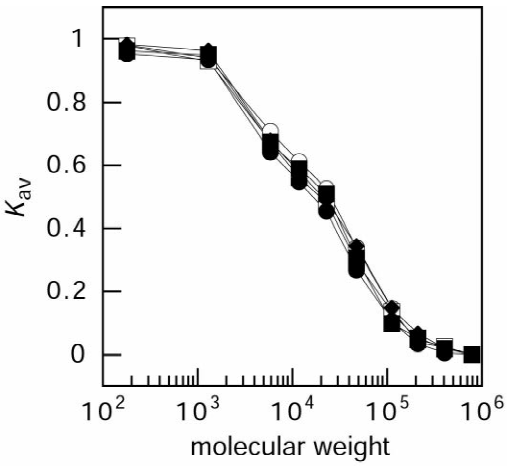

Fig. 2. Size exclusion calibration curves for cellulose gels. Column, 100×6 mm I.D.; eluent, water; flow-rate, 0.3 ml/min; detection, refractive index. Solutes were pullulan standards, γ-cyclodextrin, glucose and ethylene glycol. (□) Cellulofine GCL-2000m, (♦) DAC-02-PAA, (■) DAC-05-PAA, (●) DAC-10-PAA, (○) DEAE-Cellulofine A-800m.

3. Results and discussion

3.1. Grafting of polyallylamine onto periodate-oxidized cellulose

Table 1 shows the aldehyde content of periodate-oxidized cellulose (DAC) and the amino group content of PAA-grafted cellulose gels. Since the average degree of polymerization (DPw) of PAA was 87, the amine/aldehyde ratio ([bound amine]/[original aldehyde]) should be 87, if one PAA molecule binds to cellulose by single Schiff base linkage and consumes all aldehyde groups. The actual amine/aldehyde ratio was 3.50, 3.10, and 2.74 for DAC-02, -05 and -10, respectively. The large discrepancy of these values and the expected value of 87 could be ascribed to two possible factors: (i) a single PAA molecule can react with many aldehyde groups, and (ii) aldehyde groups in small pores of the gel are not accessible for large polyallylamine molecules. These situations are similar to those for our previous material prepared from Cellulofine GC-700sf [11].

3.2. Size-exclusion properties of PAA-cellulose gel

The size-exclusion properties of Cellulofine GCL-2000m (starting material), PAA-cellulose gels, and DEAE-Cellulofine A-800m were determined by a series of nonionic solutes (Fig. 2). The distribution coefficient (K_{av}) of the PAA-cellulose gels was the same as that of the original gel. This is in contrast to the case of Cellulofine GC-700sf, which showed certain narrowing of pores upon PAA grafting [11]. This difference is probably due to larger pore size of

Table 1
Aldehyde and amino group content of dialdehyde cellulose (DAC) and PAA-cellulose gels

Dialdehyde cellulose			PAA-cellulose		
Sample	Number of aldehyde (/100 glucose unit)	Aldehyde (mmol/g cellulose)	Sample	Amino group (mmol/g cellulose)	Amino/aldehyde
DAC-02	1.55	0.10	DAC-02-PAA	0.35	3.50
DAC-05	3.14	0.19	DAC-05-PAA	0.59	3.10
DAC-10	5.57	0.35	DAC-10-PAA	0.96	2.74
			DEAE-Cellulofine A-800m	1.07	

Cellulofine GCL-2000m. The porosity of DEAE-Cellulofine A-800m is also the same as that of the present PAA-cellulose gels. Therefore the size-exclusion effect for protein molecules must be the same for all the gels prepared here. The packed columns also showed high mechanical and chemical stability similarly to the PAA-grafted Cellulofine GC-700sf.

3.3. Retention behavior of proteins on PAA-cellulose gel

Fig. 3 shows the elution curves of bovine serum albumin and human serum albumin by NaCl gradient on the PAA-cellulose gels at pH 5.5. While the amino group content of the gels was lower than that of DEAE-Cellulofine A-800m, the proteins are seen to have much stronger interaction with the former. The retention increased with the amino group content of the PAA-cellulose gels. Fig. 3c shows the elution curves of the human and bovine serum albumin mixture at pH 5.5. The two proteins have very close isoelectric points (p*I* values) (4.7–5.2 and 4.7–4.9) and molecular masses (66 241 and 66 296) and gave single peak by the DEAE material; in contrast, they could be moderately resolved by the PAA-cellulose gels.

Fig. 4 shows the elution curves of β-lactoglobulin (Lg) A and B at pH 5.5. The DAC-02-PAA gel gave a satisfactory separation of LgA and LgB, in contrast to the nearly single peak with DEAE-Cellulofine A-800m. LgA is known to have one more negative charge, which causes the slightly lower p*I* values (ca. 5.1) than LgB (ca. 5.2) although molecular masses (35 000) are essentially the same [5]. These results show high effectiveness of PAA-cellulose gel to the separation of very similar proteins from each other.

The interaction of proteins with ion exchangers is naturally affected by pH. Protonation of amino groups of stationary phase is suppressed at higher pH; on the contrary, the degree of dissociation of protein's acidic groups increases. Also, the interaction can depend on the charge distribution of protein. Thus the overall interaction strength can depend on the pH in complicated ways. Fig. 5 shows the actual behavior of bovine serum albumin at pH 7.5 and 8.3. The protein was more strongly retained by the PAA-cellulose columns, not being eluted by NaCl gradient up to 0.3 *M* as in the case of pH 5.5,

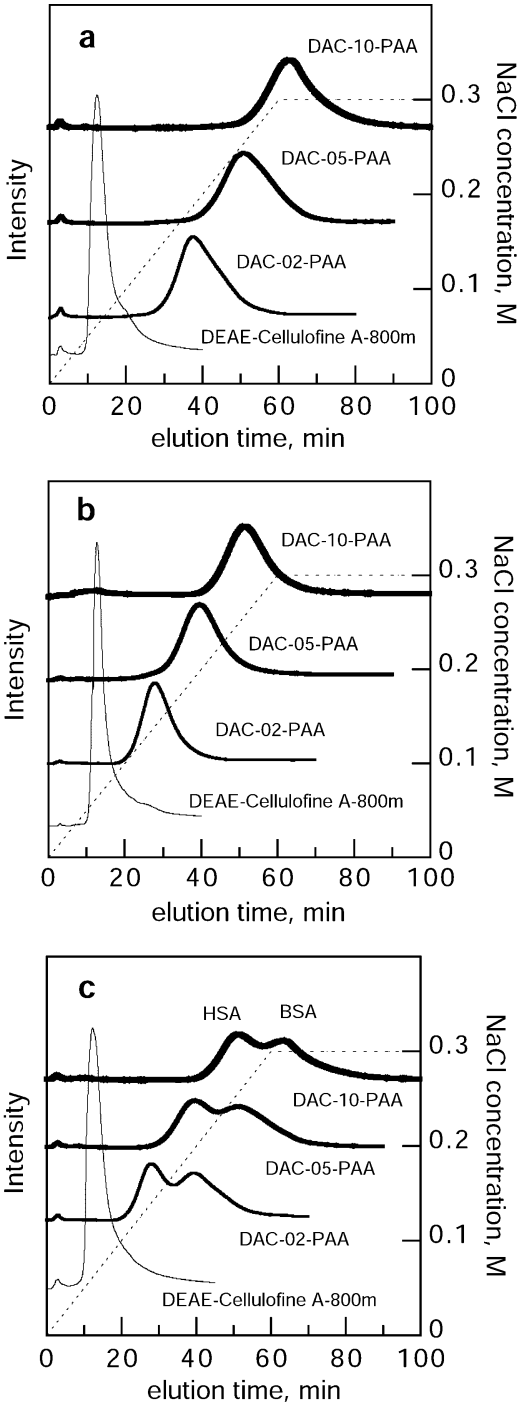

Fig. 3. NaCl-gradient elution curves of bovine serum albumin (a), human serum albumin (b) and their mixture (c) in 50 m*M* sodium acetate buffer, pH 5.5.

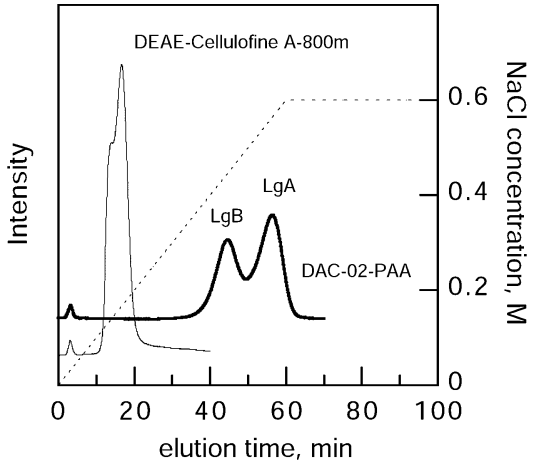

Fig. 4. NaCl gradient elution curves of β-lactoglobulin A and B (LgA and LgB) in 50 m*M* sodium acetate buffer, pH 5.5.

and a gradient to 1 *M* was necessary. Because of the many factors as described above, we cannot presently predict the pH dependence of the behavior of the present system.

The strong interaction of the PAA-cellulose gels with proteins is probably effected by dense distribution of amino groups instead of random and sparse distribution in the DEAE-cellulose. The cationic group content of DEAE-Cellulofine A-800m, 1.07 mmol/g cellulose determined by elemental analysis, roughly corresponds to one DEAE group per six glucopyranoside units. This means an average distance of 3 nm (=6×0.52 nm) between neighboring DEAE groups along the glucan chain. Though the actual average separation would be somewhat smaller than this due to aggregation of cellulose chains, it should be still significantly greater than 0.3 nm, the length of repeating unit of polyallylamine. The situation is depicted schematically in Fig. 6.

Since the PAA-cellulose gels prepared here interact strongly with anionic proteins as described above, irreversible adsorption onto the gels can take place. Our test for bovine serum albumin from the gel columns at pH 5.5, determined from the peak areas, gave satisfactory recoveries of 96–98% for DAC-02-PAA and DAC-05-PAA, comparable to 98% for DEAE-Cellulofine A-800m. The DAC-10-PAA column, on the other hand, gave a somewhat lower recovery of about 90%, though it is likely to become higher with stronger elution conditions, i.e., higher final concentration of NaCl. Thus the adsorption of proteins onto the PAA-cellulose gels is virtually reversible, making their application to quantitative analysis/separation possible.

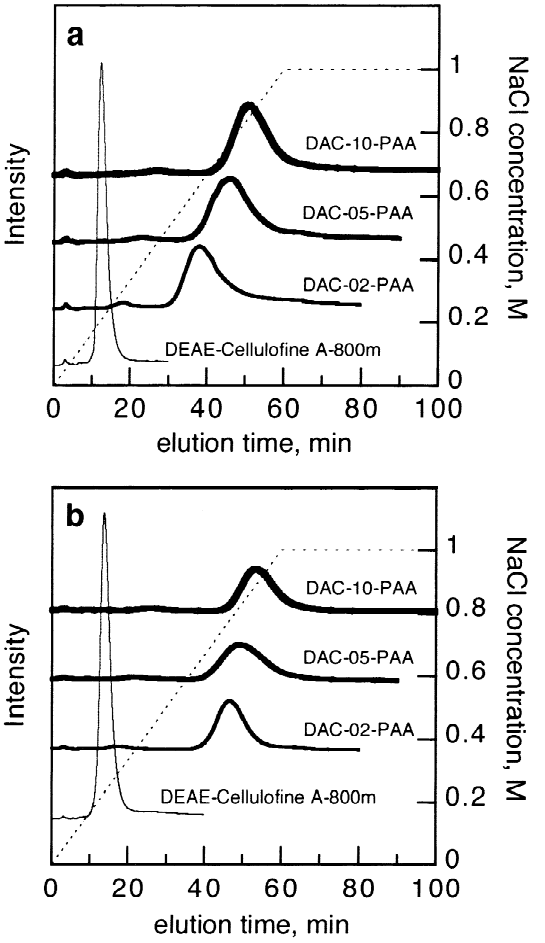

Fig. 5. NaCl gradient elution curves of bovine serum albumin in 50 m*M* Tris–HCl buffer, pH 7.5 (a) and pH 8.3 (b).

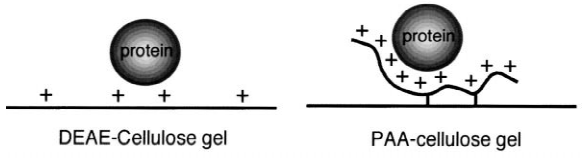

Fig. 6. Schematic model for binding of protein to PAA-cellulose gel or DEAE-cellulose gel.

4. Conclusion

A new class of cellulose-based anion exchanger was prepared by grafting polyallylamine to periodate-oxidized cellulose gel with large pore size. This material showed enhanced ion-exchange capacity and separation effects for proteins and would serve as a useful chromatographic material.

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