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Short communication

Polyallylamine-grafted cellulose gel as high-capacity anion-exchanger

Ung-Jin Kim*, Shigenori Kuga

Department of Biomaterials Science, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Yayoi 1-1-1, Bunkyo-ku, Tokyo 113-8657, Japan

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Abstract

A new cellulose-based anion-exchanger was prepared by grafting polyallylamine onto cellulose. The material was obtained by partial oxidation of a size-exclusion grade cellulose gel by aq. $NaIO_4$, forming dialdehyde cellulose, followed by Schiff base formation with a polyallylamine (PAA, molecular mass 5000) and subsequent reduction for stabilization. Three grades of PAA-cellulose gels, with amino group contents of 0.78, 1.01 and 1.28 mmol/g cellulose, were examined for their ionic interaction with mono- and divalent carboxylic acids at pH 2.5–5.5. While the retention factor for monovalent acids was nearly proportional to the amino group content of the gel, that for divalent acids was remarkably greater for the PAA-cellulose gel than for the conventional diethylaminoethyl (DEAE) cellulose gel bearing more amino groups (1.97 mmol/g cellulose). Such high capacity can be explained by the high local density of amino groups on grafted PAA, in contrast to the random and sparse charge distribution in conventional exchangers.

Keywords: Periodate oxidation; Anion-exchanger; Stationary phases, LC; Polyallylamine; Cellulose

1. Introduction

Porous cellulose gels are being increasingly used as column packing for liquid chromatography for size-exclusion as well as ion-exchange or affinity chromatography, because of their high chemical and mechanical stability [1–7]. As such, several cellulose-based anion-exchange materials having aminoethyl (AE), diethyaminoethyl (DEAE) [8] and triethylaminoethyl (TEAE) groups are commercially available [9]. These products are usually prepared by reacting halogenated amines with alkali-activated hydroxyls of cellulose. This procedure, however, has several drawbacks in industrial practice: (i) use of strong alkali; (ii) lengthy process for cleaning product; and (iii) limitation in cation content due to solubilization of highly substituted cellulose.

Instead of these alkaline derivatizations, there is a convenient reaction to introduce functional groups to cellulose under mild conditions. The periodate oxidation of cellulose can introduce aldehyde groups by cleaving the C2–C3 bond of glucopyranoside, resulting in the formation of two aldehyde groups per glucose unit (dialdehyde cellulose, DAC) [10–12]. The aldehyde groups can be further converted to carboxylic acid [13–18], hydroxyls [15,17], or Schiff

*Corresponding author. Tel.: +81-3-5841-7513; fax: +81-3-5684-0299.

E-mail address: u-kim@sbp.fp.a.u-tokyo.ac.jp (U.-J. Kim).

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base (imine) with primary amines [19–23]. All these steps proceed in aqueous solution at room temperature and are easy to control for varying the degree of substitution. Therefore the dialdehyde and dicarboxyl cellulose gel are potentially useful for aqueous chromatography as cellulose-based column packings [24–27].

In this study we attempted grafting of synthetic polyallylamine (PAA, $-[CH_2-CH(CH_2-NH_2)]_n-$), having a pendant primary amino group on every repeating unit [28–31], onto cellulose via Schiff base formation. The products were evaluated for their anion-exchange capabilities, with focus on the effect of the densely located amino groups in contrast to randomly distributed ones in conventional cellulose-based anion-exchangers.

2. Experimental

2.1. Materials

A commercial size exclusion-grade cellulose gel, Cellulofine GC-700sf (Chisso Co., Tokyo; particle size 25–44 μm; swollen and suspended in water) was used as starting material. This product is a highly porous, beaded cellulose gel, usually giving a packed column with [void volume]/[total volume] of around 0.5. Polyallylamine (PAA, M_w 5000, $M_w/M_n=3.58$) was provided by Nitto Boseki Co. (Tokyo) and used as received. Sodium metaperiodate ($NaIO_4$) and all other chemicals were of reagent grade (Wako Chemicals, Tokyo). Distilled water was used throughout the study.

2.2. Preparation of dialdehyde cellulose

The spherical cellulose gel suspension (150-ml containing 10 g solid) was mixed with 100-ml of aq. $NaIO_4$ containing 2.5, 5 or 10% molar equivalent to glucose residue of cellulose. The mixture was stirred gently at room temperature in the dark for 24 h. After the reaction was stopped by addition of ethylene glycol, the oxidized gel was washed with water by repeated decantation. The products are denoted as DAC-05, DAC-10 and DAC-20, respectively (the numbers are intended to indicate the mole % of aldehyde to glucose residue, i.e. twice of mole % of periodate added). The aldehyde content was determined by elemental analysis for nitrogen of oximes formed by the DAC and hydroxylamine.

2.3. Preparation of polyallylamine-cellulose gel

The DAC gel suspension (25-ml containing 1 g solid) was mixed with polyallylamine (25-ml containing 2 g), adjusted to pH 5 by HCl and stirred gently at room temperature for 4 h. The resulting Schiff base was reduced by adding 2.5 mmol of $NaBH_3CN$ dissolved in 5-ml water at room temperature for 4 h. The gel was washed by repeated decantation. The products are denoted as DAC-05-PAA, DAC-10-PAA and DAC-20-PAA, corresponding to DACs described above. The nitrogen content was determined by elemental analysis and converted to the amino group per weight of cellulose.

2.4. Column packing and evaluation of size-exclusion properties

The gel particles were packed in a stainless steel column (30×6 mm I.D.). Gel suspension (approx. 0.5 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 3 ml/min. The obtained column showed pressure drop of 1.2–1.6 MPa at flow-rate of 1 ml/min.

The size-exclusion property of gels was determined by pullulan standards (Showa Denko Co., Tokyo) and small molecules (ethylene glycol, glucose and γ-cyclodextrin). 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

Carboxylic acids were dissolved in a selected buffer to make 0.01–0.1 wt % solution, which was injected from a 120 μl sample loop and eluted by the same buffer solution. The tested range of pH was 2.5–5.5. Acetate buffer (0.1 *M*) was used for pH 4.0–5.5. The buffers of pH 2.5–3.5 were prepared by

adding 0.1 *M* HCl to the pH 4.0 acetate buffer. The flow-rate was 1 ml/min and the column was maintained at 25°C. Elution of solute was monitored by refractive index, optical rotation (OR-990; Jasco, Tokyo), or ultraviolet absorption. For comparison, the same test was carried out with a commercial anion-exchange cellulose, DEAE-Celluofine A-500m (Chisso Co., particle size 53–125 μm). This product has similar porosity as that of Celluofine GC-700sf used as starting material for PAA grafting described above.

Ethylene glycol was used as total volume marker. Retention factor was calculated using $k' = (V_e - V_t)/V_t$, where V_e is the elution volume of the compound and V_t is the elution volume of ethylene glycol. Tested solutes were mono-and divalent aromatic carboxylic acids (benzoic acid, *o*-, *m*-, and *p*-fluorobenzoic acids; phthalic, isophthalic and terephthalic acids), and several amino acids having one or two carboxyls (alanine, glutamic acid and aspartic acid).

3. Results and discussion

3.1. Grafting of polyallylamine onto cellulose via dialdehyde groups

Table 1 shows the aldehyde content of periodate-oxidized cellulose (DAC) and the amino group content of PAA-grafted cellulose. The aldehyde content was determined from nitrogen content (elemental analysis) of oxime derived from DAC samples. The aldehyde content expressed as number per 100 glucose residues (Table 1, 2nd row) should agree with the numbers in ''DAC-**'' if oxidation was achieved quantitatively; i.e. DAC-05 should have 5 aldehyde groups per 100 glucoside units. The actual aldehyde content corresponded to about 75% of the intended levels for all samples as seen in Table 1. We measured consumption of IO_4^- in the reaction mixture by absorption at 290 nm [13], and found that all IO_4^- was completely consumed after 24 h at room temperature. This means that about 25% of IO_4^- was lost by side reactions, but the cause of the loss is not clear at this moment.

The amino group content of the PAA-cellulose gels, shown in the right side of Table 1, was also determined from their nitrogen content. 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. This situation, however, is not likely to be the case because of two factors: (i) single PAA molecule can react with many aldehyde groups; (ii) some portion of aldehyde groups may be left unreacted after grafting reaction, especially due to size-exclusion effect of the gel (See below for size-exclusion effect of these gels). In fact, the actual amine/aldehyde ratio ranged from 3.25 to 1.38 (the rightmost column of Table 1), being much smaller than 87. This large discrepancy indicates that the factors stated above have significant influence on binding of PAA to the cellulose gel. Also, the ratio decreases sharply with increase in aldehyde content. This behavior can be ascribed to the effect of higher spatial density of aldehyde groups. Since we do not know the percentage of aldehyde groups consumed by the reaction with polyallylamine (i.e. Factor (ii) above), we presently cannot evaluate the actual

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

Dialdehyde cellulose			PAA-cellulose		Amino/Aldehyde
Sample	Number of aldehyde (/100 glucose unit)	Aldehyde (mmol/g cellulose)	Sample	Amino group (mmol/g cellulose)	
DAC-05	3.83	0.24	DAC-05-PAA	0.78	3.25
DAC-10	7.46	0.46	DAC-10-PAA	1.01	2.20
DAC-20	15.07	0.93	DAC-20-PAA	1.28	1.38
			DEAE-Cellulofine A-500m	1.97	

[bound amine]/[graft linkage] ratio, which must be somewhere between 3.25 (for DAC-05-PAA, e.g.) and 87.

3.2. Chemical and mechanical stability of PAA-cellulose gel

Liquid chromatography packings must be both chemically and mechanically stable for practical operation. Since periodate oxidation and PAA grafting may adversely affect these properties of cellulose gel, we conducted basic tests as follows: 0.10 g (dry weight) of the PAA-cellulose gels were suspended in 10-ml of 0.1 *M* buffers of pH 2.0 (HCl–KCl), 5.0 (acetate) and 9.0 (borate) and left to stand for 30 days at room temperature. After this treatment the gels showed no weight decrease, thus showing apparent chemical stability. Also the size and shape of dialdehyde cellulose and PAA-cellulose gel particles were the same as the original cellulose gel and did not change after the treatment above. The pressure drop of the packed columns was virtually constant for several months of chromatography operation including many exchanges to different buffers. These results show the satisfactory performance of the PAA-cellulose gels as a column packing.

3.3. Size exclusion properties of PAA-cellulose gel

The size-exclusion properties of PAA-cellulose gels, Cellulofine GC-700sf (starting material), and DEAE-Cellulofine A-500m were determined by using a series of nonionic solutes (Fig. 1). Distribution coefficient (K_{av}) vs. molecular mass curves show that the exclusion limit of PAA-cellulose gels lowered with increase in the amount of bound polyallylamine. This change apparently shows the influence of the random coil of bound polyallylamine, which causes additional size-exclusion effect to solutes. The pore size of DEAE-Cellulofine A-500m is slightly greater than that of the PAA-cellulose gels.

3.4. Ion exchange chromatography by PAA-cellulose gel

Several monovalent and divalent carboxylic acids (M_w 80–170) were tested for ionic interaction with the PAA-cellulose gels at pH 2.5–5.5. Since these probe molecules are small enough to penetrate these gels, they should be eluted at total volume if not for ionic interaction.

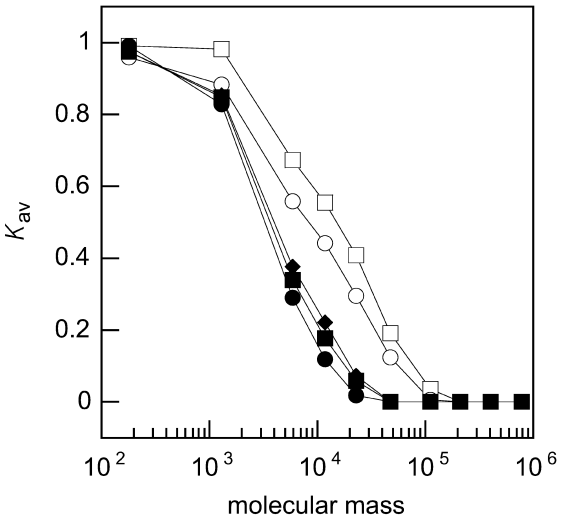

Fig. 1. Size exclusion calibration curves for cellulose gels. Column: 30×6 mm I.D.; Eluent: water; flow-rate: 0.2 ml/min; Detection: refractive index. Solutes were pullulan standards, γ-cyclodextrin, glucose and ethylene glycol. □: Cellulofine GC-700sf, ♦: DAC-05-PAA, ●: DAC-10-PAA, ○: DAC-20-PAA,: DEAE-Cellulofine A-500m.

Fig. 2 shows the pH dependence of retention factor k' of monovalent acids. The pH dependence is the same for all the gel samples, showing a large maximum at pH 4. This behavior is considered to result from superposition of the changes in dissociation states of acids and amino groups; i.e. low pH suppresses dissociation of acid, and high pH suppresses protonation of amino group. Since the dry weight of gel packed in the column was nearly the same for all the gels tested, the ion-exchange capacity of various gels can be compared among the gels by the k' value at pH 4. The k' was nearly proportional to the amino group content of the gel, including DEAE-Cellulofine A-500m. This is reasonable since the amino cation in stationary phase would interact with monovalent anions independently from the presence of other amino groups.

Fig. 3 shows the chromatogram and pH dependence of k' of of phthalic acid. Compared to the results in Fig. 2, the k' of the DEAE-cellulose gel for phthalic acid (159) is much greater than for mono-

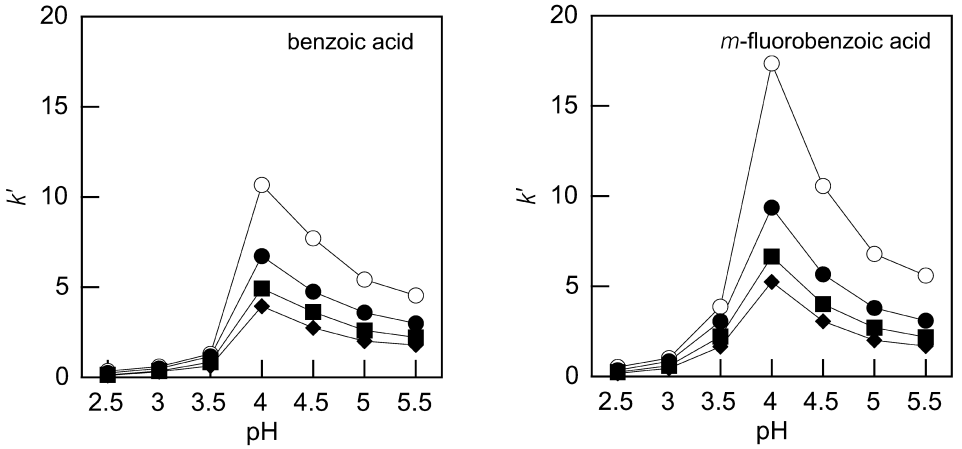

Fig. 2. Retention factor k' of cationic cellulose gels for benzoic acid and *m*-fluorobenzoic acid. ♦: DAC-05-PAA, ■: DAC-10-PAA, ●: DAC-20-PAA, ○: DEAE-Cellulofine A-500m.

valent acids (10.7 and 17.4). Since isophthalic and terephthalic acids gave similar results, this behavior is considered to be the same for divalent acids in general. This difference is considered to result from synergistic action of neighboring cations to divalent anion. Notably here, the order of k' is inverted between the DEAE-cellulose gel and PAA-cellulose gels; the capacity of the latter is nearly three times of that of the former. This strong interaction of PAA-cellulose gel with divalent anion is probably effected by dense distribution of amino groups instead of random and sparse distribution in DEAE-cellulose. The flexibility of PAA chain is also expected to enhance the chelate-like interaction.

We also examined amino acids. Fig. 4 shows the results for alanine, glutamic acid and aspartic acid. Alanine has no ionic interaction with the cationic gels since it is positively charged at this pH range (isoelectric point, p*I* 6.07). Dicarboxylic amino acids, glutamic acid (p*I* 3.08) and aspartic acid (p*I* 2.98), in contrast, show certain interaction with the cationic cellulose gels. The k' for these amino acids is much smaller than for phthalic acid and of the same level as for monovalent acids. This can be

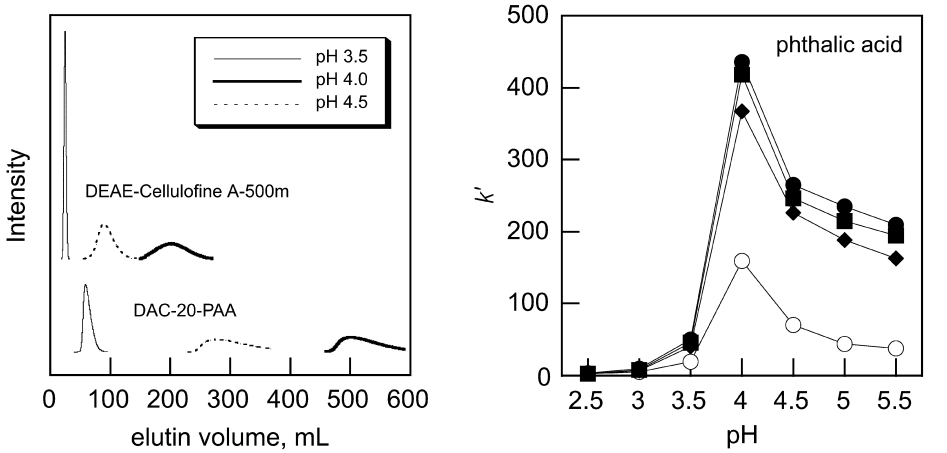

Fig. 3. Chromatogram and retention factor k' of cationic cellulose gels for phthalic acid. ♦: DAC-05-PAA, ■: DAC-10-PAA, ●: DAC-20-PAA, ○: DEAE-Cellulofine A-500m.

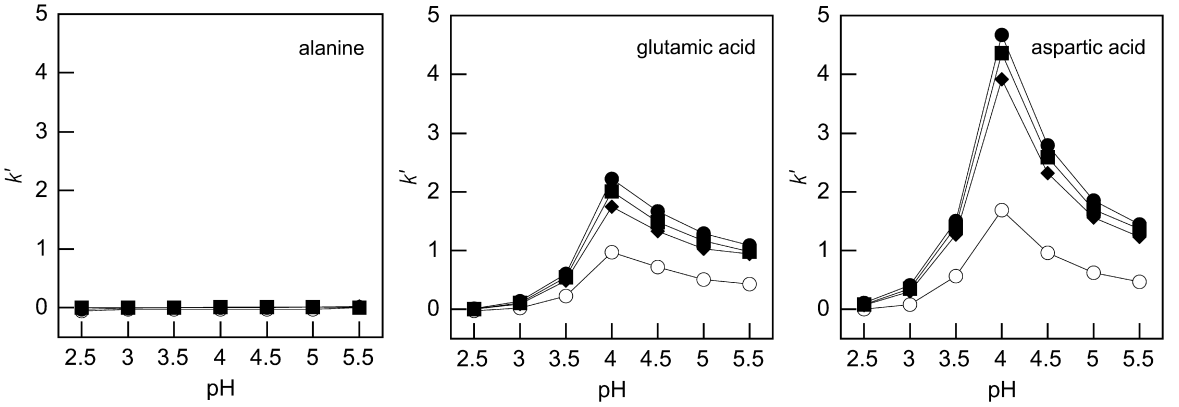

Fig. 4. Retention factor k' of cationic cellulose gels for amino acids. ♦: DAC-05-PAA, ■: DAC-10-PAA, ●: DAC-20-PAA, ○: DEAE-Cellulofine A-500m.

understood by the presence of dissociated amino group within the same molecule. In this case again, the ionic interaction of PAA-cellulose gel with glutamic and aspartic acids is about twice stronger than the DEAE-cellulose gel, suggesting involvement of similar action for phthalic acid. Meanwhile the k' value of aspartic acid was more than 2 times of that of glutamic acid. This suggests high sensitivity of ionic interaction of PAA-cellulose gel to differences in geometrical arrangements of ionic groups of analytes. This feature can give additional advantage as chromatographic separation by PAA-cellulose gel.

As stated earlier, the ion-exchange capacity of PAA-cellulose gels was proportional to the amino group content for monovalent acid, but nearly independent of it for divalent acids and dicarboxylic amino acids. This anomaly may result from the difference in flexibility of polyallylamine chains; i.e. the grafted PAA molecule on the gel prepared from DAC of lower degree of oxidation may be more flexible, thus providing more effective chelate-like association.

4. Conclusion

The spherical cellulose gel grafted with polyallylamine was found to be highly efficient anion-exchanger. This material, while maintaining the high chemical and mechanical stability of the original cellulose gel, showed remarkably higher ion-exchange capacity for divalent acids than a conventional DEAE-cellulose gel. This effect is considered to result from the high charge density and flexibility of grafted polyallylamine chains. This material is expected to be useful for ion-exchange analysis and separation of multivalent anionic species including many biologically active molecules such as proteins.

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