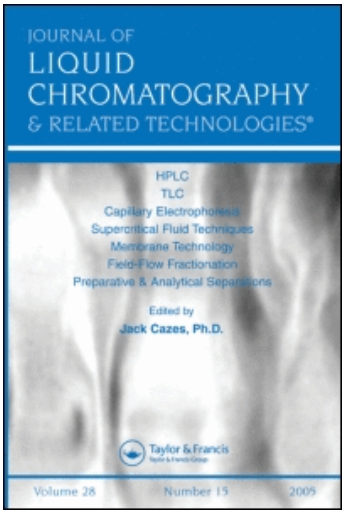

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Selective Removal of DNA from Bioproducts by Polycation-Immobilized Cellulose Beads

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Abstract: This paper describes a method for the selective removal of DNA from various cellular products using columns packed with polycation-immobilized cellulose beads. Polyethyleneimine (PEI), poly-*N,N*-dimethylaminopropylacrylamide (poly(DAPA)) and poly(ε-lysine) (PεL), all of which have cationic properties, were used as the ligands on the beads. Cellufine-GC15® and -CPC® were used as cellulose matrices. Adsorption of DNA by the beads was determined using a batchwise method or a column method. Each bead type showed high DNA adsorbing activity at pH 7.0 and ionic strengths of $\mu = 0.05–0.8$. The larger the pore size of the beads, the larger the DNA-adsorbing activity. The DNA adsorbing capacities per wet mL of PEI-, poly(DAPA)- and PεL immobilized Cellufine-CPC with large pore sizes, were 3.7, 3.2, and 1.8 mg, respectively. When a protein, such as bovine serum albumin (BSA) or γ-globulin, was present in solution with the DNA under physiological conditions (pH 7.0, $\mu = 0.2$), the DNA selectivity of the PEI immobilized Cellufine-CPC columns was unsatisfactory, because both the DNA and the protein were adsorbed into the column. In contrast, the poly(DAPA) immobilized Cellufine-CPC column selectively removed DNA from each protein solution contaminated with DNA under similar conditions: the DNA concentration in each treated protein solution was below 10 ng mL^{-1}, and high recovery of each protein (>92%) was obtained.

Keywords: Nucleic acids, DNA, *N,N*-Dimethylaminopropylacrylamide, Poly(ethyleneimine), Selective removal, pK_a, Amino group content, Bovine serum albumin, γ-Globulin

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INTRODUCTION

In recent years, with the increasing development of drugs using biotechnology, useful biologically important substances have been mass produced by recombinant genes. Drugs produced using recombinant gene technology often contain nucleic acids as impurities. The influence of such nucleic acids on the living body has not been fully clarified. For this reason, FDA and WHO have published guidelines on residual nucleic acids in biologicals. According to WHO guidelines,[1] it is desirable that the amount of residual nucleic acids in an adult dose should be not more than 10 ng.

In physiological solutions, DNA exists in a wide range of molecular sizes, with M_w from 1×10^4 to 1×10^6. On the other hand, molecular weights of proteins are generally about 1×10^4 to 5×10^5. Therefore, it is extremely difficult to separate DNA from proteins solely by size separation methods, including size exclusion chromatography and ultrafiltration. To remove DNA from protein solutions, selective adsorption has proven to be the most effective. It has already been reported that a cationic polymer adsorbent such as chitosan beads[2] is very useful as an adsorbent for anionic biorelated polymers such as lipopolysaccharides (LPS) and DNA. We have already found that aminated poly(γ-methyl L-glutamate) particles show superior DNA selectivity to a chitosan adsorbent.[3] However, a great disadvantage of this type of adsorbent is the low chemical stability of an ester bond originating from the side chain of poly(γ-methyl L-glutamate). Thus, regeneration of these at high and low pH values is ruled out. We have also found that crosslinked poly(ethyleneimine) (PEI) beads[4] and crosslinked *N,N*-dimethylaminopropylacrylamide (DAPA) beads[5] show superior DNA selectivity to commercial anion exchangers. Each bead type is expected to keep its entire structure in solutions with high and low pH values. The high DNA selectivity of each bead with small pores is due to the size exclusion effects on protein molecules. However, it is difficult to produce crosslinked DAPA and crosslinked PEI beads on a large scale because of low yields in their suspension crosslinking methods.

In this work, as novel DNA adsorbents, we developed polycation immobilized cellulose beads that can be produced on a large scale and can be easily regenerated many times. We previously reported[6] that poly(ε-lisine) immobilized cellulose beads have a high adsorbing activity for pyrogenic lipopolysaccharide. Lipopolysaccharide is an amphipathic substance having both anionic (phosphoric acid groups) and hydrophobic (lipophilic group) regions.[7] DNA molecules are also high molecular weight substances with phosphoric acid groups. Thus, it is expected that polycation immobilized cellulose beads may be used as selective adsorbents for DNA. As ligands having cationic properties, we chose PEI, poly(DAPA), and poly(ε-lysine). The cellulose beads (Cellufine-GC15® and -CPC®) are a suitable matrix because the column packings of these beads show a remarkably high flow rate resistance in liquid chromatography.

This paper describes the preparation of the polycation immobilized cellulose beads and their characteristics, and then provides a method for the chromatographic removal of DNA from a protein solution, which is contaminated with DNA, using the beads packed columns.

EXPERIMENTAL

Materials

A 30 wt-% PEI aqueous solution (degree of polymerization: 1600, M_w: 7×10^4) was purchased from Wako Pure Chemical (Osaka, Japan). The DAPA monomer was kindly supplied by Kohjin Co., Ltd., (Tokyo, Japan), and was purified by vacuum distillation at 131°C $mmHg^{-1}$. A 25 wt-% poly(ε-lysine) (degree of polymerization: 30, pKa 7.6, M_w: 4×10^3)[8] aqueous solution, produced by *Streptomyces albulus*, and cellulose beads (Cellufine-GC15[9] and -CPC[10]) were obtained from Chisso Co. Ltd. (Tokyo, Japan). Chloromethyloxirane was purchased from Nacalai Tesque (Kyoto, Japan). Purified DNA (from salmon spermary, M_w: 3×10^5), BSA (M_w: 6.9×10^4, pI: 4.9), and γ-globulin (from human serum, M_w: 1.6×10^5, pI: 7.4) were purchased from Nacalai. DEAE-Sepharose CL-6B® (Pharmacia Biotech, Uppsala, Sweden) was used as a standard adsorbent.

Polymerization of DAPA

Ion exchange water (330 mL) and DAPA monomer (20 g) were mixed in a 1 L three necked flask equipped with a stirrer, thermometer, and nitrogen inlet tube. To this mixture was added 0.7 g of ammonium persulfate, and this was followed by stirring at room temperature for 16 hours to afford an aqueous solution of a polymer, under a nitrogen atmosphere. The weight average molecular weight of the poly(DAPA) obtained was measured by size exclusion chromatography (on the basis of polyethylene oxide) and found to be 3×10^5.

Preparation of DNA Adsorbent

In 150 mL of 3 wt-% sodium hydroxide solution, 10 mL of Cellufine-GC15 (Cellufine(S)), or -CPC (Cellufine(L)) was suspended by stirring at 30°C for 1 h, and 21 mL of chloromethyloxirane was then added to the suspension. The mixture was stirred at 30°C for 2 h and the resulting chloromethyloxirane activated cellulose particles were collected and washed with water, and then suspended in 22 mL of the 25 wt-% poly(ε-lysine) aqueous solution. The suspension was stirred at 45°C for 2 h. The resulting poly(ε-lysine) immobilized Cellufine-GC15 (PεL-Cellufine(S)) and poly(ε-lysine) immobilized

Cellufine-CPC (PεL-Cellufine(L)) were collected and washed with 200 mL of 2 M sodium chloride solution and water. PEI and poly(DAPA) immobilized cellulose beads were prepared by the method used for PεL-Cellufine using the 30 wt-% PEI solution and poly(DAPA) solution, respectively. These beads with diameters of 44 to 105 μm were used as DNA adsorbents. A schematic illustration of the structures of the various DNA adsorbents is shown in Fig. 1.

Crosslinked DAPA adsorbent was prepared in a suspension copolymerization using DAPA monomer and divinylbenzene monomer as previously reported.[5]

Determination of Pore Size, Amino Group Content and pK_a

The pore size of bead matrices was estimated as molecular mass exclusion (M_{lim}) using aqueous size exclusion chromatography. The M_{lim} value was determined as the molecular mass of the polysaccharide by extrapolating the linear part of the curve as described previously.[11] The amino group content of beads was quantified by pH titration and elemental analysis. The apparent pK_a ($pK_{a,app}$) of each bead's surface was quantified by pH titration using a modification of the method from the Henderson-Hasselbach Equation.[12]

DNA and Protein Assays

The DNA concentration in sample solutions was determined by fluorometric analysis[13] using a spectrofluorophotometer FP-6500 (JASCO, Japan) with

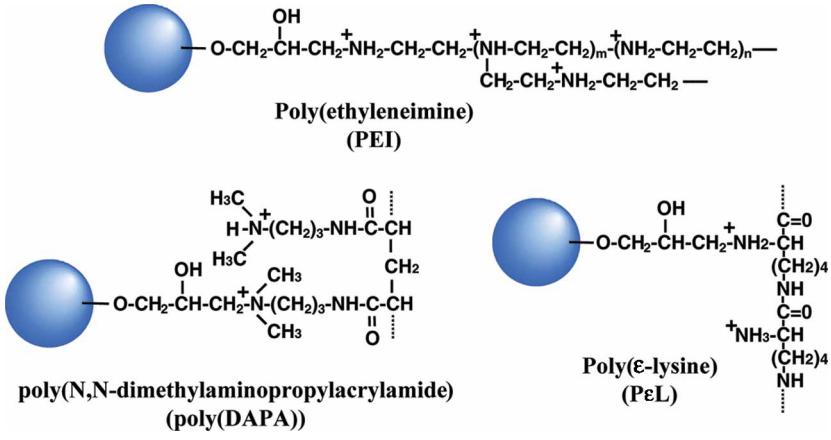

Figure 1. Structures of DNA selective ligands and their charge distribution. Cellulose beads are used as the matrix.

the fluorescent dye 4′,6-diamidino-2-phenylindole dihydrochloride hydrate (Nacalai). The protein concentration was measured using the bicinchoninic acid protein assay[14] with a BCA protein assay kit (Pierce Chemical, U.S.A.) at 580 nm using a microplate reader Fluostar Optima (Moritex, Japan).

Adsorption of DNA and Other Cellular Products

DNA and protein were dissolved in 0.02 M phosphate buffers (pH 7). The ionic strengths of the buffers were adjusted using sodium chloride.

For the batch method, adsorbent was washed before use in a glass Buchner funnel with a fritted disc (pore size: 30 μm) using 2 M sodium chloride followed by ultra pure water, and was finally equilibrated with various buffers. A 0.2 mL portion of wet adsorbent was suspended in 2 mL of DNA solution. The suspension was shaken for 2 h at 25°C, and filtered through a Millipore filter (0.8 μm) to remove the adsorbent. The DNA content of the filtrate was determined. Adsorption of proteins was investigated by a method similar to the DNA binding assay.

For the column method, adsorbent (1 or 1.7 mL) was packed into a sterile column (10 × 0.46 cm I.D.). The column was washed with 30 bead volumes of 2 M sodium chloride, 10 bead volumes of ultra pure water, and then equilibrated with 30 column volumes of 0.02 M phosphate buffer (pH 7, $\mu = 0.2$). A sample solution containing DNA was passed through the column at a flow rate of 0.1 or 0.2 mL min^{-1} at room temperature. Five or 6 mL fractions were collected, and concentrations of proteins and DNA were measured in each fraction. DNA saturated column packings were reused after washing with 20 column volumes of 0.2 M sodium hydroxide, 20 column volumes of 2 M sodium chloride, 20 column volumes of 0.02 M sodium acetate (pH 5) buffer, and 10 column volumes of ultra pure water. Columns were then equilibrated with appropriate buffers before reloading sample solutions.

RESULTS AND DISCUSSION

Properties of Various DNA Adsorbents

We previously found[3] that the adsorbing activity of DNA increased drastically with increasing pore size of the adsorbent matrix to sizes over the molecular weight of DNA. Therefore, as shown in Table 1, various polycation immobilized cellulose beads (polycation-Cellufine) with pore sizes of M_{lim} 1×10^3 to 5×10^4 and amino group contents of 0.12 to 1.20 meq wet-mL^{-1} were prepared as DNA adsorbents. In the column method, the larger the number of incorporated amino groups per column packing, the larger the DNA adsorption capacity of the packing. The amino-group content (meq wet-mL^{-1}) of the polycation-Cellufine (0.12–1.20) was larger than

Table 1. Characteristics of cationic polymer beads

Polymer beads		Ligand	Matrix	Pore size of matrix (M_{lim})[a]	pK_a[b]	Amino-group content (meq wet-mL $^{-1}$)	Degree of swelling in water (wet-mL dry-g $^{-1}$)
No.	Name						
1	PEI-Cellufine(S)	Polyethylenimine	Cellufine-GC15®[c]	1×10^3	8.6	0.35	6.0
2	PEI-Cellufine(L)		Cellufine-CPC®[d]	3.5×10^4	8.6	1.20	5.9
3	Poly(DAPA)-Cellufine(S)	Poly(dimethyl-amino-propylacryl-amide)	Cellufine-GC15	1×10^3	8.1	0.12	2.5
4	Poly(DAPA)-Cellufine(L)		Cellufine-CPC	3.5×10^4	8.1	0.35	7.0
5	PεL-Cellufine(S)	Poly(ε-lysine)	Cellufine-GC15	1×10^3	7.4	0.41	3.2
6	PεL-Cellufine(L)		Cellufine-CPC	5×10^4	7.5	0.34	11.5
7	Cross-linked DAPA[e]	Dimethylamino-propylacrylamide	DAPA/DVB	2×10^3	8.4	1.20	5.8
8	DEAE-Sepharose CL-6B®	Diethylamino-ethanol	Sepharose CL-6B	3×10^4	8.7	0.01	21.5

[a]Value deduced from the molecular weight of the polysaccharide by size-exclusion chromatography.[11]
[b]Apparent pK_a of the bead's surface.
[c]Cellulose beads (M_{lim}: 1×10^3, diameter: 44–105 μm) (Chisso, Japan).
[d]Cellulose beads (M_{lim}: 1×10^6, diameter: 44–105 μm) (Chisso, Japan).
[e]Copolymer prepared by cross-linking with dimethylaminopropylacrylamide (DAPA) and divinylbenzen (DVB) in our lab.[5]

that (0.01) of DEAE-Sepharose, which is a commercial anion exchanger. This advantageous property is due to the lower degree of swelling (wet-mL dry-g^{-1}) of the polycation-Cellufine (2.5–11.5) in comparison to that of DEAE-Sepharose (21.5).

Effect of Ionic Strength of Buffer on DNA Selectivity

For selective adsorption of DNA, it is necessary to decrease interactions between adsorbent and various bioproducts such as proteins. The effect of ionic strength (μ) on the selective adsorption of DNA from a BSA containing solution by various cationic beads was examined using a batchwise method. The cationic beads listed in Table 1 were used as adsorbents, and a BSA solution (1000 μg mL^{-1}) to which the purified DNA (salmon spermary, 10 μg mL^{-1}) had been added was used as a sample solution. The results are shown in Figs. 2a–f. Adsorbing activities for BSA decreased with increasing ionic strength of the buffer in all beads. Polycation immobilized Cellulose beads (PEI-Cellufine(S), PEI-Cellufine(L), poly(DAPA)-Cellufine(S), and

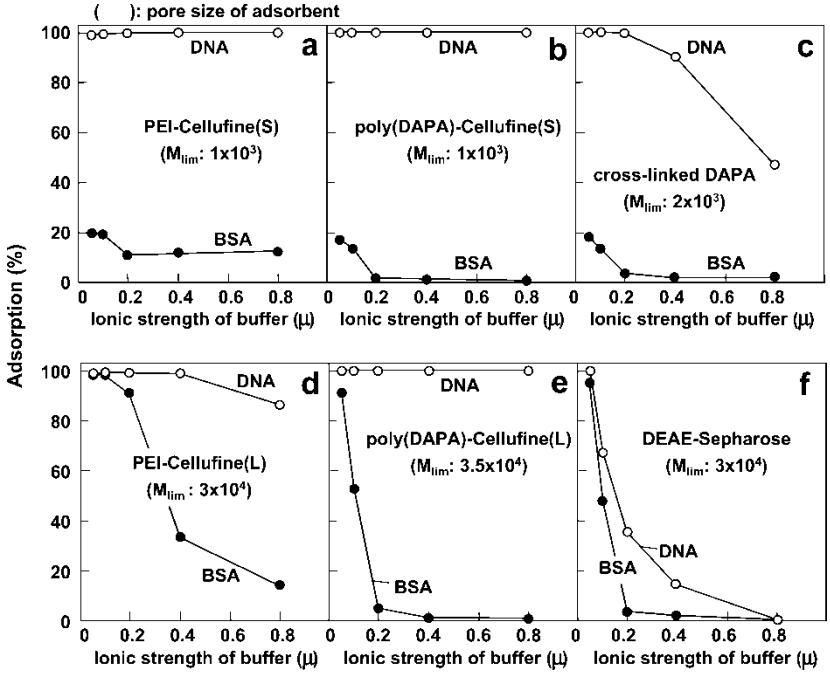

Figure 2. Effect of ionic strength of buffer on selective adsorption of DNA by various adsorbents from BSA solutions containing DNA. Adsorption of DNA was determined using a batch method with 0.2 mL of wet beads and a 2 mL sample solution (BSA: 1000 μg mL^{-1}, DNA: 10 μg mL^{-1}) at pH 7.0 and ionic strength $\mu = 0.05–0.8$.

poly(DAPA)-Cellufine(L)) showed a high DNA adsorbing activity (>86%) over a wide range of ionic strengths ($\mu = 0.05$ to 0.8). However, PEI-Cellufine(S) and -(L) adsorbed not only DNA but also BSA at any ionic strength, as shown in Figs. 2a and d, respectively. By contrast, poly(DAPA)-Cellufine(S) and -(L) selectively adsorbed DNA from a BSA solution at $\mu = 0.2$ to 0.8 (Fig. 2b and e). Crosslinked DAPA beads selectively adsorbed DNA without adsorption of BSA only at $\mu = 0.2$ (Fig. 4-c). DEAE-Sepharose had high adsorbing activities for both DNA and BSA at a low ionic strength, $\mu = 0.05$, and the adsorbing activities decreased remarkably with increasing ionic strength (Fig. 4-f). DEAE-Sepharose, therefore, could not selectively adsorb DNA at any ionic strength. Poly(DAPA)-Cellufine(S), poly(DAPA)-Cellufine(L), and crosslinked DAPA beads showed excellent selectivity for DNA under physiological conditions (pH 7.0, $\mu = 0.2$).

Effects of Pore Size and pK_a on DNA Adsorbing Capacity

The effect of the pore size of beads on the adsorption capacity of DNA was examined by a column method. Solutions of the purified DNA were applied to columns packed with different polycation-Cellufine beads (given in Table 1). The DNA adsorbing activity of poly(DAPA)-Cellufine(L), which had a large pore size (M_{lim}: 3.5×10^4), was compared with that of poly(DAPA)-Cellufine(S), which had a small pore size (M_{lim}: 1×10^3). As shown in Fig. 3, each packing was gradually saturated with DNA. The poly(DAPA)-Cellufine(L) column exhibited high DNA adsorbing activity (adsorption rate: >99.9%) in effluent volumes from 6 to 48 mL: DNA concentrations in each fraction decreased from 50 $\mu g\ mL^{-1}$ to less than 10 $ng\ mL^{-1}$. In contrast, the poly(DAPA)-Cellufine(S) column showed relatively poor DNA adsorbing activity.

Figure 4 shows the effects of the pore size of beads and their pK_a on the DNA adsorbing capacity. For all beads, the DNA adsorbing activities depended strongly on the pore size (M_{lim}). The adsorbing capacity of PEI-Cellufine per mL column increased from 1.50 to 3.67 mg with an increase in the M_{lim} of the matrix from 1×10^3 to 3.5×10^4. In the polycation-Cellufine(S), which had a small pore size matrix, the DNA adsorbing capacity was independent of differences in the chemical structures of the ligand, the amino group content of the beads, and their pK_a values: PEI-, poly(DAPA)-, and PεL-Cellufine(S) beads showed similar adsorbing capacities (1.50, 1.40, and 1.37 mg mL $column^{-1}$, respectively). By contrast, in the polycation-Cellufine(L), which had a large pore size matrix, the DNA adsorbing capacity was more strongly dependent on the pK_a of bead's surface than its amino group content. The adsorption capacity increased from 1.81 to 3.67 mg mL $column^{-1}$ when the pK_a was increased from 7.5 to 8.6. As a result, PEI-Cellufine(L), which has a large

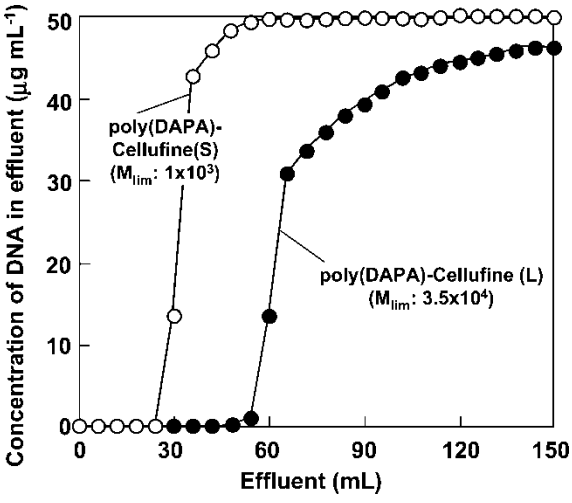

Figure 3. DNA adsorption capacity of poly(DAPA)-cellulose(S) column packing (M_{lim}: 1×10^3) and poly(DAPA)-cellulose(L) column packing (M_{lim}: 3.5×10^4). A 150 mL purified DNA solution (50 μg mL^{-1}, pH 7.0, $\mu = 0.2$) was passed through a 1.7 mL column (10×0.46 cm I.D.) at a flow rate of 0.1 mL min^{-1}. Six mL fractions were collected, and the DNA concentration in each fraction was measured.

pore size (M_{lim} 3×10^4) and the highest pK_a (8.6), showed the largest DNA adsorbing capacity. We suggest that the poor DNA adsorbing activity of the polycation-Cellufine(S), which has a small M_{lim}, probably results from an exclusion effect with respect to DNA molecules (M_{lim}: 1×10^5) when its pore size (M_{lim} as polysaccharides) is 1×10^3.

From these results (Figs. 2–4, and Table 1), we propose that the adsorbing activity of polycation-Cellufine for bioproducts was due to the simultaneous effects of their cationic properties and hydrophobic or other properties. Cationic adsorption is remarkably dependent on the pK_a of the adsorbent, pK_a of the bioproducts and ionic strength of the buffer.[15] Hydrophobic adsorption is independent of the ionic strength.[5,6] Nucleic acids (DNA and RNA) are polynucleosides with anionic regions (phosphate groups), pentoses, and purine and pyrimidine bases, and, thus, the DNA charge is anionic when the pH exceeds the pK_a (<2). The charge of BSA is also anionic at pH values greater than 4.9 (its pI). Adsorption of DNA or BSA by polycation-Cellufine decreased with increasing ionic strength of the buffer (Figs. 2a–f). This suggests that the beads adsorbed DNA and BSA via ionic interactions. As shown in Figs. 2a–f, all cationic adsorbents bound more strongly to DNA than proteins such as BSA under all the conditions tested. This is because DNA has a lower pK_a than BSA. On the other hand, even at high ionic strengths ($\mu = 0.4$–0.8), DNA was adsorbed by polycation immobilized Cellufine (Figs. 4a, b, d, and e). These findings suggest that the

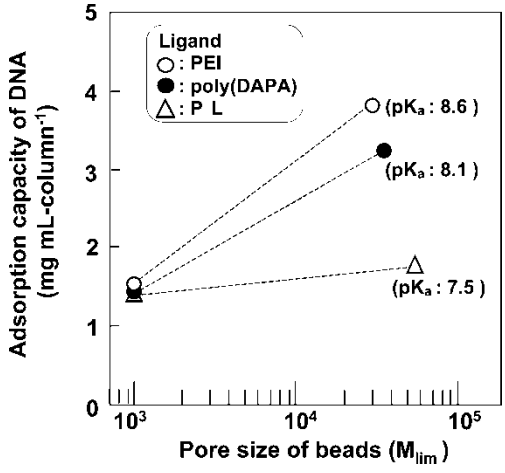

Figure 4. Effects of pore size of beads and their pK_a on adsorption capacity of DNA using column packings with polycation immobilized cellulose beads. The adsorption capacity of DNA was determined by a column method as described in Figure 3. The pore size of beads and their pK_a were derived from results in Table 1.

beads can adsorb DNA by hydrophobic binding. For selective adsorption of DNA from a bioproduct under physiological conditions (pH 7.0, $\mu = 0.2$), it was found that it is necessary to adjust not only the pK_a of ligand but also its hydrophobicity.

We previously reported that it is necessary to adjust the pore size (M_{lim}) of the beads to below 1×10^4 for selective adsorption of DNA without adsorption of protein.[3] It can also be seen from Figs. 2b and c that poly(DAPA)-Cellfine(S) and crosslinked DAPA beads having small M_{lim} values of $<2 \times 10^3$ showed the lowest BSA adsorption ($<5\%$) at an ionic strength (μ) over 0.1. However, when the pore size of the beads was decreased, the DNA adsorbing activity was decreased remarkably, as shown in Figs. 3 and 4. On the other hand, poly(DAPA)-, PEI-Cellfine(L), and DEAE-Sepharose, which have large pores, adsorbed not only DNA but also BSA at the low ionic strength of $\mu = 0.05$ (Figs. 2d, e, and f, respectively). The binding between BSA and each adsorbent probably results from entry of BSA into the large pores of the adsorbent's matrix. This undesirable adsorption of BSA decreased when the ionic strength (μ) of the buffer was increased to 0.2 or above: poly(DAPA)-Cellufine(L) showed a high DNA selectivity ($>99\%$) without adsorption of BSA ($<5\%$) at $\mu = 0.2$–0.8 and pH 7.0 (Fig. 2e).

These findings suggest that under physiological conditions (pH 7.0, $\mu = 0.2$), the DNA removing activity of polycation-Cellufine(L), which has large pores, is superior to that of polycation-Cellufine(S), which has small pores.

Removal of DNA from Protein Solution by the Column Method

DNA removing activity of the poly(DAPA)-Cellufine(L) column packing was compared with that of PEI-Cellufine(L) column packing under physiological conditions (pH 7.0, $\mu = 0.2$). A γ-globulin solution (1000 μg mL^{-1}) to which the purified DNA (10 μg mL^{-1}) had been added was used as the sample solution. The sample solution was passed through the column, and the effluent was collected in 5 mL fractions. The results are presented in Figs. 5a and b. The poly(DAPA)-Cellufine(L) column exhibited high DNA removing activity with a high recovery of γ-globulin (adsorption rate $<1\%$) in fractions 4 to 20. DNA concentrations in each fraction decreased from 10 μg mL^{-1} to less than 10 ng mL^{-1} (Fig. 5a). In contrast, the PEI-Cellufine(L) column showed relatively poor DNA selectivity: although its DNA removing activity is high (each fraction: <10 ng mL^{-1}), it showed undesirable adsorption of γ-globulin (Fig. 5b). The ionic interactions between the adsorbent and γ-globulin (pI 7.4) are not induced at pH 7.0 since proteins are cationic at pH values under their pI value. The γ-globulin is a weakly hydrophobic protein, and we previously reported[4] that hydrophobic bonds formed between γ-globulin and hydrophobic beads over a wide range of ionic strengths ($\mu = 0.05-1$). These findings suggest that the adsorption of γ-globulin to PEI-Cellufine(L) was mainly caused by hydrophobic binding.

Chromatographic separation of BSA and DNA was investigated using poly(DAPA)-Cellufine(L) column packing. The column was washed with 0.02 M phosphate buffer (pH 7.0, $\mu = 0.2$) after the application of a BSA

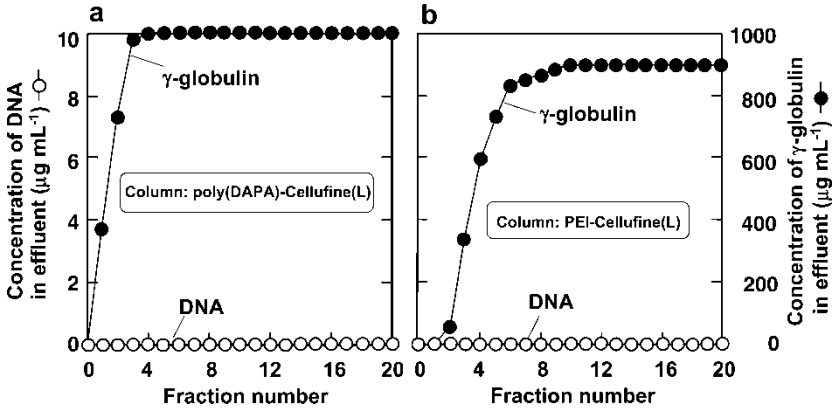

Figure 5. Selective adsorption of DNA from a γ-globulin solution containing DNA using (a) poly(DAPA)-cellulose(L) and (b) PEI-cellulose(L) columns. A 100 mL γ-globulin solution (1000 μg mL^{-1}, pH 7.0, $\mu = 0.2$), to which was added DNA (salmon spermary: 10 μg mL^{-1}), was passed through a 1.7 mL column at 0.2 mL min^{-1}. Five mL fractions were collected, and the concentrations of DNA and γ-globulin in each fraction were measured.

solution containing DNA. The results obtained are shown in Fig. 6. The packing showed excellent DNA removal activity: the DNA concentration in each fraction (Nos. 1–18) was less than 10 ng mL^{-1} under physiological conditions (pH 7.0, $\mu = 0.2$), and the BSA recovery was 98%. Furthermore, when the DNA-saturated poly(DAPA)-Cellufine(L) column was then treated with 2 M sodium chloride solution, only the DNA was eluted (DNA recovery: 95%). This result indicates that poly(DAPA)-Cellufine(L) allows not only selective removal of DNA, but also specific separation of DNA from proteins. We assume that the 5% of remaining DNA, which was not eluted, was strongly adsorbed onto the poly(DAPA) chain of the beads by hydrophobic binding and a multipoint attachment.

For practical applications, ease of regeneration is important, and poly(DAPA)-Cellufine(L) can easily be regenerated by frontal chromatography with 0.2 M sodium hydroxide, followed by 2.0 M sodium chloride. It seems that this favorable property will enable us to reuse the packing several times. The stable structure of this packing that is able to withstand extreme pH values is due to -CONH- bonds originating from poly(DAPA).

CONCLUSIONS

The results presented here suggest that poly(DAPA)-Cellufine(L) can reduce the concentration of DNA in bioproducts used for injection to 10 ng mL^{-1} or

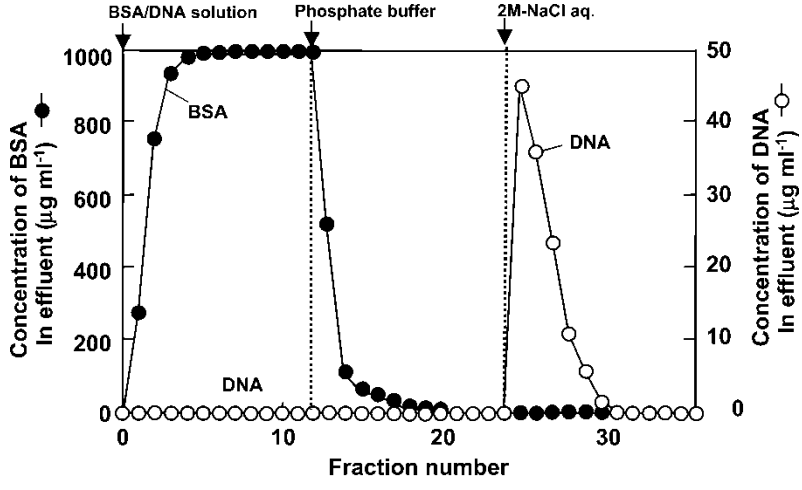

Figure 6. Chromatographic separation of BSA and DNA using a poly(DAPA)-cellulose(L) column. A 60 mL BSA solution to which DNA was added (BSA: 1000 μg mL^{-1}, DNA: 10 μg mL^{-1}, pH 7.0, $\mu = 0.2$) was passed through a 1.7 mL column at 0.2 mL min^{-1}. The DNA saturated column was washed with 60 mL buffer (pH 7.0, $\mu = 0.2$), and then treated with 60 mL of 2 M sodium chloride solution.

lower under physiological conditions (pH 7.0, $\mu = 0.2$). The process does not affect the recovery of bioproducts, such as BSA and γ-globulin. This high DNA selectivity of poly(DAPA)-Cellufine(L) may be due to the simultaneous effects of the cationic properties of poly(DAPA) and its suitable hydrophobic properties, which arise when the pK_a values and M_{lim} values are adjusted to 8.1 and 3×10^4, respectively. In addition, we believe that the poly(DAPA)-Cellufine(L), which has a cellulose bead matrix, is a better column packing material than conventional polysaccharide beads for DNA removal or separation, because of its higher flow rate resistance.

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REFERENCES

1. Report on the WHO Expert Committee on Biological Standardization. WHO Weekly Epidemiological Record; 1977, *72*; 141–145.
2. Adachi, T.; Ida, J.; Wakita, M.; Hashimoto, M.; Ihara, H.; Hirayama, C. Preparation of spherical and porous chitosan particles by suspension evaporation with O/W/O multiple emulsions. Polym. J. **1999**, *31* (4), 319–323.
3. Hirayama, C.; Sakata, M.; Moriguchi, K.; Mizokami, H.; Ihara, H.; Ohkuma, K. Pore-size controlled and aminated poly(γ-methyl L-glutamate) particles for selective removal of nucleic acids. Polym. J. **1998**, *30* (8), 616–621.
4. Sakata, M.; Matsumoto, K.; Obaru, N.; Kunitake, M.; Mizokami, H.; Hirayama, C. Removal of DNA from a protein solution with crosslinked poly(ethyleneimine) spherical particles. J. Liq. Chromatogr. & Rel. Technol. **2003**, *26* (2), 231–246.
5. Sakata, M.; Nakayama, M.; Kamada, T.; Kunitake, M.; Hirayama, C. Selective removal of DNA from protein solution with copolymer particles derived from *N,N*-dimethylaminopropylacrylamide. J. Chromatogr. A. **2004**, *1030*, 117–122.
6. Todokoro, M.; Sakata, M.; Matama, S.; Kunitake, M.; Ohkuma, K.; Hirayama, C. Pore-size controlled and poly(ε-lysine) immobilized cellulose spherical particles for removal of lipopolysaccharides. J. Liq. Chromatogr. & Rel. Technol. **2002**, *25* (4), 601–614.
7. Seydel, U.; Labischinski, H.; Kastowsky, M.; Brandeburg, K. Phase behaviour, supermolecular structure, and molecular conformation of lipopolysaccharide. Immunobiology **1993**, *187*, 191–211.
8. Shima, S.; Sakai, H. Poly-L-lysine produced by streptomyces. Part III. Chemical Studies. Agric. Biol. Chem. **1981**, *45* (11), 2503–2508.
9. Matsumoto, K.; Hirayama, C.; Motozato, Y. Preparation of bead shaped cellulosic ion exchangers. J. Chem. Soc. Jpn. **1981**, *12*, 1890–1898.
10. Kuga, S. The porous structure of cellulose gel regenerated from calcium thiocyanate solution. J. Colloid Interface Sci. **1981**, *77*, 413–417.

11. Hirayama, C.; Ihara, H.; Nagaoka, S.; Furusawa, H.; Tsuruta, S. Regulation of pore-size distribution of poly(γ-methyl L-glutamate) spheres as a gel permeation chromatography packing. Poly. J. **1990**, *22*, 614–619.
12. Po, H.N.; Senozan, N.M. The Henderson Hasselbach Equation: Its History and Limitations. J. Chem. Educ. **2001**, *78* (11), 1499–1503.
13. Brunk, C.F.; Jones, K.C.; James, T.W. Assay for nanogram quantities of DNA in cellular homogenates. Anal. Biochem. **1979**, *92*, 497–500.
14. Wiechelman, K.J.; Braun, R.D.; Fitzpatrick, J.D. Investigation of the bicinchoninic acid protein assay: Identification of the groups responsible for color formation. Anal. Biochem. **1988**, *175*, 231–237.
15. Sakata, M.; Todokoro, M.; Kai, T.; Kunitake, M.; Hirayama, C. Effect of cationic polymer adsorbent pK_a on the selective removal of endotoxin from an albumin solution. Chromatographia **2001**, *53*, 619–623.

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